

**CUMMINGS SCHOOL
OF VETERINARY MEDICINE AT TUFTS UNIVERSITY**

**SUMMER RESEARCH
PROGRAM AWARDS
2021**

- **Anivive**
- **AVMA/AVMF**
- **The Barkley Fund**
- **Blue Buffalo**
- **Boehringer Ingelheim**
- **Center for Animals**
- **Clinical Trials Office**
- **Cummings School of Veterinary Medicine at Tufts University**
- **Elizabeth A. Lawrence Endowed Fund**
- **Morris Animal Foundation**
- **Pet Smart Charities**
- **USDA Formula Funds**
- **Private Funding**
- **Elanco**

2021 Summer Research

Name	Class Year	Mentor	Title	Funding
Acquaviva, Mariana	V'24	Dr. F. Nutter	Anticoagulant Rodenticide Residue Levels in Liver Samples from a Maine Canada Lynx Population	Cummings School of Veterinary Medicine at Tufts University
Awad, Alexandra	V'24	Dr. E. McCobb and Dr. F. Nutter	Attitudes Towards Companion Animals in Egypt	Elizabeth A Lawrence Endowed Fund
Bixby, Ryan	V'24	Dr. M. Mueller	Behavioral and Physiological Assessment of Adolescent and Therapy Dog Interactions	Cummings School of Veterinary Medicine at Tufts University
Cashel, Abigail	V'24	Dr. E. McCobb	Demographics of Community Cats Presented to a Veterinary Clinic Over a Ten-Year Span	Cummings School of Veterinary Medicine at Tufts University
Choi, Diana	V'23	Dr. G. Beamer	Creation and Validation of Mycobacterium Tuberculosis Granuloma Recognition Software	Boehringer Ingelheim
Celatka, Jillian	V'24	Dr. E. Byrnes	Prenatal Oxycodone Exposure and Pup Vocalizations	Cummings School of Veterinary Medicine at Tufts University
Coughlin, Laura	V'23	Dr. G. Beamer and Dr. F. Nutter	Establishing serum biomarkers of Mycobacterium tuberculosis infection in African and Asian elephants	Cummings School of Veterinary Medicine at Tufts University
Dharmasena, Vidharshi	V'24	Dr. Ian Destefano	Likelihood of Infection in Veterinary Patients Treated for Sepsis in the Emergency Department	Elanco
Dolan, Samantha	V'23	Dr. J. Rush	Retrospective Evaluation of the Indication for and Outcome Following Sildenafil Administration in Cats with Heart Disease	Cummings School of Veterinary Medicine at Tufts University

Name	Class Year	Mentor	Title	Funding
Elwood, Stella	V'23	Dr. E. McCobb	Client Perceptions of an Educational Partnership to Provide Low Cost Basic Veterinary Care to an Underserved Community	Pet Smart Charities
Hojsak, Jillian	V'24	Dr. M. Pokras	Erosion of Paint-Coated Lead Fishing Gear in a Simulated Loon Gizzard	Private Donor Funds
Huang, Callista	V'24	Dr. J. Runstadler	Assessing the Prevalence of SARS-CoV-2 in Livestock Populations	USDA Formula Funds
Indeglia, Vanessa	V'23	Dr. A. Robbins and Dr. M. Gatlin	Assessing Bat Diversity and Abundance in Massachusetts Using Acoustic Monitoring	Cummings School of Veterinary Medicine at Tufts University
Karp, Shelby	V'23	Dr. L. Freeman and Dr. J. Rush	Comparison of Echocardiography and Cardiac Biomarkers in Cats Eating Different Types of Cat Foods	The Barkley Fund
Li, Jingyi (Jenny)	V'24	Dr. M. Pokras	An Exploratory Research of the Common Loon Microbiome	Private Donor Funds
Maguire, Maria	V'24	Dr. H. Gardner	Interrogating the Role of β -dystroglycan in DMD-Mutant Osteosarcoma	Boehringer Ingelheim
McDaniel, Kylie	V'23	Dr. F. Nutter and Dr. S. Pumphrey	Identifying, Classifying, and Cataloging Retinal Lesions in Asiatic Black Bears (<i>Ursus thibetanus</i>)	Cummings School of Veterinary Medicine at Tufts University
Munroe, Cassandra	V'24	Dr. C. Fellman	Short Course Amoxicillin for Treatment of Sporadic Cystitis in Dogs	Blue Buffalo
Nazzaro, Jullian	V'24	Dr. S. Borns-Weil	The Effect of COVID-19 on Canine Separation Anxiety	Cummings School of Veterinary Medicine at Tufts University

Name	Class Year	Mentor	Title	Funding
O'Malley, Brianna		Dr. S. Ayres and Dr. J. Graham	Non-Surgical Approach to Sterilizing Avian Species Using a Domestic Chicken Model	USDA Formula Funds
Orrico, Bill	V'23	Dr. M. Rosenbaum and Dr. H. Frank	The Effect of Landscape Disruption on Herpesvirus Prevalence in Bat Species of Northwestern Ecuador	Cummings School of Veterinary Medicine at Tufts University
Richards, Laura	V'23	Dr. C. Schonhoff	Nitric Oxide (NO) Signaling in the Female Developing Striatum: Investigating Oxycodone's Effects on NO levels, and investigating the effects of adding exogenous and inhibiting endogenous NO on Development of Dendritic Complexity	Cummings School of Veterinary Medicine at Tufts University
Scott, Samantha	V'23	Dr. V. Yang	Role of Canine Hemangiosarcoma-Associated Extracellular Vesicles in Promoting Tumor Angiogenesis	Morris Animal Foundation
Shaffert, Katie	V'23	Dr. H. Gardner	Investigating the Role of SETD2 in Canine Osteosarcoma	Anivive
Sherinian, Rachel	V'23	Dr. M. Bercier	Bat Activity and Diversity Across Land Types on the Cummings School of Veterinary Medicine Campus, North Grafton, MA, from June to August 2021.	Cummings School of Veterinary Medicine at Tufts University
Sistig, Lorena	V'23	Dr. A. Sheoran	Development of Anti-TNF α VHHs for Immunotherapy of Chronic Inflammatory Diseases in Humans and Canines	Cummings School of Veterinary Medicine at Tufts University
Specht, Aubrey	V'23	Dr. M. Rosenbaum	A Metagenomic Analysis of Antimicrobial Resistance Genes in the Boston Urban Rat Population	AVMA/AVMF

Name	Class Year	Mentor	Title	Funding
Wells, Jamie	V'24	Dr. E. Rozanski	A Novel Surgical Approach to Laryngeal Collapse in Brachycephalic Canines	Cummings School of Veterinary Medicine at Tufts University
Wunderlich, Emma	V'24	Dr. A. Rutberg	An Evaluation of Behavioral Changes (Maternal Aggression & Social Group Size, Composition, and Affiliation) of White-Tailed Deer (<i>Odocoileus virginianus</i>) Treated with Immunocontraceptive PZP	Internal Funds from CAP
Zavota, Gianna	V'23	S. Dowling-Guyer	An exploratory study of stress behavior patterns in dogs	Cummings School of Veterinary Medicine at Tufts University

Student Name:	Mariana Acquaviva V'24	Mentor:	Dr. Felicia Nutter
Project Title:	Anticoagulant Rodenticide Residue Levels in Liver Samples from a Maine Canada Lynx Population		
Funding Source:	Cummings School of Veterinary Medicine at Tufts University		

Summary:

Significance

Anticoagulant rodenticides (ARs) are the most commonly used chemical worldwide for lethal rodent control (van den Brink et al., 2018). For predatory species that consume prey targeted with ARs, both acute and chronic secondary exposures may occur (Hindmarch and Elliott, 2018). Sublethal effects have been documented in wildlife with AR bioaccumulation, which can lead to other causes of mortality (Elmeros et al., 2019). Canada lynx (*Lynx canadensis*) occupy areas of Maine where they could have access to exposed prey. However, lynx populations live in insulated forests, away from humans. In fishers, a similar carnivore behaviorally, AR exposure has been documented (Gabriel et al 2015). Therefore, I intend to analyze the exposure levels for lynx to counter assumptions that ARs are not of significant worry for population health in this species. For my proposed research, around 67 Canada lynx liver samples will be analyzed for presence of first and second generation ARs to understand the exposure prevalence in this previously unstudied species. This novel research can lead to further studies of ARs in lynx to understand the magnitude of threat to population health.

Aims

I aim to evaluate the presence and quantify levels of exposure to first- and second-generation anticoagulant rodenticides (ARs) in Canada lynx from Maine, to contribute to better understanding anthropogenic threats to Canada lynx conservation. I hypothesize that 1) the majority of Canada lynx samples tested will contain residues of at least one anticoagulant rodenticide, and 2) there will be more exposure to second generation anticoagulant rodenticides than to first generation anticoagulant rodenticides.

Methods

Canada lynx carcasses will be thawed, liver samples collected, then re-frozen and shipped to the Pennsylvania Animal Diagnostic Laboratory System (PADLS) Toxicology Laboratory to test for a panel of ARs from both first and second generations. I will report the prevalence by specific compound and by AR generation group. I will test for association between lynx life history data (sex, age class) and AR prevalence (by compound and generation group) by using chi-squared tests.

Conclusions

I expect to see that at least 50% of Canada lynx samples are positive for at least one AR subtype. In previous studies on carnivores with secondary exposure, positive identification of at least one rodenticide has exceeded 80-90% (Serieys, 2018). I also expect that there may be higher prevalence of second generation ARs due to the longer lasting effects of that generation.

Student Name:	Alexandra Awad V'24	Mentor:	Dr. Emily McCobb and Dr. Felicia Nutter
Project Title:	Attitudes Towards Companion Animals in Egypt		
Funding Source:	Elizabeth A Lawrence Endowed Fund		

Summary:

Specific Aims

Specific Aim 1: To explore the attitudes of Egyptians towards companion animals.

Hypothesis 1: Egyptian attitudes towards companion animals are more positive than the Middle Eastern countries surveyed to date.

Specific Aim 2: To add to recent research studies focusing on the human-animal bond in the Middle East.

Hypothesis 2: More studies that examine cultural impacts on the human-animal bond will assist in further understanding of the relationship.

Specific Aim 3: To investigate if these attitudes are influenced by demographics, culture, religion, and/or Western influence.

Hypothesis 3: Egyptian attitudes towards companion animals will correlate with these variables, such as age, educationlevel, and religion.

Student Name:	Ryan Bixby V'24	Mentor:	Dr. Megan Mueller
Project Title:	Behavioral and Physiological Assessment of Adolescent and Therapy Dog Interactions		
Funding Source:	Cummings School of Veterinary Medicine at Tufts University		

Summary:

Animal-assisted interventions (AAs) are increasingly used as a therapeutic technique for reducing social anxiety. There is evidence that these interventions are effective in mitigating the physiological consequences resulting from increased stress. However, there is a lack of research on the specific mechanism of action of AAs, the temporal aspects of the interaction, and the relative effects of specific human and animal behaviors, making it difficult to pinpoint the anxiolytic effects on individuals in these interventions. Trained therapy animals, which have been found to be highly attuned to human presence and emotion, may help reduce acute anxiety through proactive behaviors. However, it is also possible that actions of the therapy animal may have negative effects. Animals experiencing stress exhibit a variety of behaviors that may be interpreted by the participants and contribute to anxiety. The objective of this study is to use previously collected video data from this experimental protocol to assess how specific interactive behaviors of participants and dogs correspond with changes in adolescents' anxiety and physiological reactivity. This study will leverage existing video data collected as part of an experimental study that tested the mechanisms by which interacting with a therapy dog may affect anxiety. Using a well-validated laboratory-based social evaluative stressor (the Trier Social Stress Task for Children [TSST-C])³, 75 participants ages 13 to 17 years were randomized to one of three conditions: 1) interaction with a stuffed toy dog (active control); 2) social interaction (no physical contact) with a real dog; and 3) social + physical interaction with a real dog. Self-reported anxiety as well as psychophysiological data (electrodermal activity) were collected.

To analyze and code the video data, the Noldus observer XT 14 software will be used. Researchers blinded to the purpose of the study will use an ethogram to code social, physical, and verbal contact between participants and therapy dogs. Dogs will be coded based on affiliative and stress linked behaviors while human participants will be coded in terms of touch and vocalizations. The videos will be assessed by two raters and inter-rater reliability will be expected to be above 0.80 to be considered valid. The electrodermal data will be considered in conjunction with the temporal aspects of the interactions being coded, as well as the self-response questionnaires. This approach will allow for analysis rapid physiological antecedents and consequences of specific interactions between the participants and the animals. It will also provide a method of monitoring therapy dogs during animal assisted interactions with adolescents.

Student Name:	Abigail Cashel V'24	Mentor:	Dr. Emily McCobb
Project Title:	Demographics of Community Cats Presented to a Veterinary Clinic Over a Ten-Year Span		
Funding Source:	Cummings School of Veterinary Medicine at Tufts University		

Summary:

Overpopulation of unowned, free-roaming community cats detrimentally impacts the environment, animal welfare, and public health, sparking a need for human intervention. Trap-Neuter-Return (TNR), a method where community cats are trapped, sterilized, and then returned to their trapping site, currently serves as the most humane and effective method of community cat population control. Animal welfare advocates largely agree that TNR effectively decreases community cat overpopulation (Levy & Crawford, 2004; Levy, D. Gale, & L. Gale, 2003; Mendes-de-Almeida et al., 2011; Spehar & Wolf, 2019) and increases overall animal welfare (Kreisler, Cornell, & Levy, 2019; Levy & Crawford, 2004), but the current research methods carry intrinsic opportunities for error. TNR programs are often run at the grassroots level and studied via citizen science, which hinders standardization of data assessment and collection by researchers. For these reasons, it is difficult to accurately extrapolate community cat demographic data to different communities across the United States.

Additionally, most studies view TNR through the lens of community cat colonies, failing to consider impacts on the clinics themselves. Wallace and Levy (2006) were one of the first groups to follow demographics of community cats presented to TNR clinics over an eleven-year span, highlighting that admitted cats displayed similar profiles in clinical parameters in the northwestern, southwestern, and southern United States. Understanding community cat population demographics would allow individual TNR programs to better allocate limited resources, thereby decreasing levels of overpopulation and increasing welfare. Despite these convincing findings, these profiles are over fifteen years outdated and did not explicitly describe characteristics of community cats presenting to clinics in the northeastern United States.

Before a standardized model of community cat population demographics in the United States can be developed using empirical evidence, it is important to develop a primary model of characteristics of community cats presenting for TNR throughout the northeastern United States. This exploratory study will examine relevant patient-level clinical parameters in community cats presented to the Tufts Luke and Lily Lerner Spay/Neuter Clinic in Worcester County, Massachusetts between 2008 and 2018. Specifically, we plan to extract existing data from the clinic's electronic database and analyze trends in factors that could affect the time, expertise, and medical supplies required for spay and neuter in TNR programs. These clinical parameters include sex, estimated age, previous sterilization, preexisting health conditions (including injury, disease, and emaciation), complications requiring euthanasia on intake, pregnancy rates and seasonality, prenatal litter size, cryptorchidism, time from induction to recovery, and intraoperative complications. We hypothesize that these values will display variation throughout the ten-year period and that their averages will differ from previously reported values from other United States regions.

Student Name: Jillian Celatka V'24 **Mentor:** Dr. Elizabeth Byrnes

Project Title: Prenatal Oxycodone Exposure and Pup Vocalizations

Funding Source: Cummings School of Veterinary Medicine at Tufts University

Summary:

The use of prescription opioids, like oxycodone, has increased over the past decade, resulting in a dramatic rise in the number of infants exposed to prescription opioids *in utero*, many of whom experience neonatal opioid withdrawal syndrome (NOWS) (Raffaelli et al., 2017). Factors influencing the severity of NOWS, including the role of maternal withdrawal, remain unknown. While translational animal models of prenatal opioids are limited, several studies suggest that ultrasonic vocalizations (USVs) emitted by neonatal rodent pups can serve as a measure of NOWS (Byrnes and Vassoler, 2017). The aim of the current study is to test the hypothesis that neonatal USVs increase with direct exposure to oxycodone *in utero*, that these effects on USVs are not due to oxycodone withdrawal in the dam, but that they can influence maternal behavior. This hypothesis will be tested by comparing the quantity and quality of USVs in neonates exposed to differing levels of oxycodone *in utero* in litters reared by dams experiencing oxycodone withdrawal or reared by drug naïve foster mothers. Noldus Ultravox XT will be used to capture and analyze USVs, including metrics of both call quantity and call type. Noldus Ethovision will be used to analyze maternal behavior in response to calls. Both analyses of variance and regression analyses will be used to determine the effects of oxycodone dose and maternal withdrawal on USV measures and maternal behavior.

Student Name:	Diana Choi V'23	Mentor:	Dr. Gillian Beamer
Project Title:	Creation and Validation of Mycobacterium Tuberculosis Granuloma Recognition Software		
Funding Source:	Boehringer Ingelheim		

Summary:

Specific Aims

We have two main aims in this project: 1) To gain foundational knowledge about digital pathology and artificial intelligence, and develop the required skills and knowledge for practical application; and 2) the practical application of AI to digital pathology to validate and test computer algorithms. This includes accurate detection of individual cells within *Mycobacterium tuberculosis* granulomas, specifically lymphocytes, Acid-Fast stained bacteria, and plasma cells.

Background and Significance

Tuberculosis (TB) is diagnosed in 9–10 million human patients and causes 1–2 million deaths each year, remaining a global health threat. Most human patients develop pulmonary TB, with disease restricted to the lungs following airborne infection with *Mycobacterium tuberculosis*. Granuloma and cellular necrosis with neutrophil influx in lung tissue are hallmarks of pulmonary TB in susceptible humans infected with *M. tuberculosis*. However, the pathogenesis of tuberculosis also incites host response to lymphocytes and plasma cells. Acid-Fast stained *M. tuberculosis* bacteria can also be observed on a histopathology slide of an infected lung tissue. Recent publications demonstrate the role of AI tools on TB research to detect different features on a digitalized slide.

Artificial Intelligence (AI) tools are useful in extracting information from experimental histopathology samples, such as recognizing the minute patterns of microscopic change or delineating overlapping features as distinct lesions. It also allows the inspection of hundreds of slides to evaluate for very specific pathology-defined features of *M. tuberculosis* in the lung tissue in a time efficient and unbiased manner. AI analysis can detect pathology patterns that are difficult and cumbersome to manually identify. Also, with subsequent advent of machine learning and AI, the capacity to create pattern-recognition software with diagnostic capacity has ensued. Application of the software allows efficient and objective detection of pathologic lesions in the context of *M. tuberculosis* infection.

Previous studies [4,5,6] have already established the methods to successfully identify neutrophil clusters, granulomas, macrophage foci, lymphocyte foci, and pyknotic debris in TB infected Diversity Outbred (DO) mice lung images. The Diversity Outbred (DO) mice is a genetically heterogeneous mouse model of human tuberculosis that reflects the genetic diversity of the human population. Further, *M. tuberculosis*-infected mice present the same pathologic features as those in human patients. However, although AI analysis of cell clusters or *M. tuberculosis* bacilli clusters has been established ([4,6]), there is currently no study that has investigated the automatic detection of the individual cells within TB granuloma. As the complex host responses to TB infection involve many cells including antigen-independent and antigen-specific lymphocytes, Acid-Fast stained bacteria, and plasma cells, there is a need for additional digital pathology statistics of the number, location, and distribution of these cells within granuloma.

Methods

Aiforia is a cloud-based digital pathology image analysis service that will be used for development and training of AI algorithms to accurately detect, localize, and quantify individual cells within granuloma regions. We will use digital slide images of lung tissue from experimentally TB infected mice that are available in Dr.

Beamer's laboratory. Histopathological images from super-susceptible DO mice will be used to train, validate, and performance test an algorithm to allow the automatic detection of three different cell types within granuloma: lymphocytes, Acid-Fast stained bacteria, and plasma cells. The resulting new data will include quantification and spatial localization of these cells, which will be further analyzed for classification and statistical correlation with disease parameters.

Student Name:	Laura Coughlin V'23	Mentor:	Dr. Gillian Beamer and Dr. Felicia Nutter
Project Title:	Establishing Serum Biomarkers of Mycobacterium Tuberculosis Infection in African and Asian Elephants		
Funding Source:	Cummings School of Veterinary Medicine at Tufts University		

Summary:

Specific Aims

The primary aims of this study are as follows:

1. To determine which of the following biomarkers can be detected in the serum of African and Asian elephants: tumor necrosis factor alpha (TNF- α), interferon gamma (IFN- γ), interleukin-10 (IL-10), C-X-C Chemokine Ligand 1 (CXCL1), and matrix metalloproteinase-8 (MMP8);
2. To determine whether the serum concentrations of these five biomarkers are significantly elevated in elephants infected with *Mycobacterium tuberculosis* compared to healthy controls.

Based on previous studies of *M. tuberculosis* in elephants and translational research conducted in *M. tuberculosis*-infected mice and humans, we hypothesize that 1) the five biomarkers of interest will be detectable in African and Asian elephants and 2) the concentration of these five biomarkers will be significantly elevated in the serum of elephants infected with *M. tuberculosis* compared to healthy controls.

Experimental Designs/Methods

We will use commercial Enzyme Linked Immunosorbent Assay (ELISA) kits to determine the concentrations of the five biomarkers described above in serum samples from elephants positive for *M. tuberculosis* infection and elephants negative for *M. tuberculosis* infection (i.e., healthy controls). The concentrations of these biomarkers will be compared across tuberculosis (TB) positive and negative elephants using a variety of statistical methods to determine whether the concentrations of these biomarkers are significantly elevated in TB positive elephants compared to healthy controls.

Significance of the Proposed Research

Infection with *M. tuberculosis* is a substantial threat to elephant conservation and public health worldwide. As TB can be spread between elephants, humans, and a wide range of other susceptible mammals, TB infection in elephants not only jeopardizes the health of individual elephants but also that of other species living in close proximity and human veterinarians, zookeepers, and others that may have contact with infected elephants. The ability to quickly and accurately diagnose *M. tuberculosis* infection in elephants is of paramount importance, but diagnosis of these infections is often challenging due to the chronic and subclinical nature of TB infection in elephants and the lack of knowledge on the immune response that elephants mount following TB infection. Studies such as this one that seek to better characterize the immune responses of elephants infected with *M. tuberculosis* may offer new avenues for diagnosing these infections, allowing for more rapid treatment of infections in individual animals and better management of the risk of spread to humans and other species.

Student Name:	Vidharshi Dharmasena V'24	Mentor:	Dr. Ian Destefano
Project Title:	Likelihood of Infection in Veterinary Patients Treated for Sepsis in the Emergency Department		
Funding Source:	Elanco		

Summary:

In this retrospective study, we aim to evaluate the likelihood of sepsis and septic shock in dogs treated with a sepsis protocol in the emergency room at the Foster Hospital for Small Animals (FHSA) at the Cummings School of Veterinary Medicine at Tufts University. Within this study, we will characterize dogs treated with a septic kit from 2009 - 2017 as definitive sepsis, possible sepsis, or no sepsis. Sepsis is defined as dogs that meet the Systemic Inflammatory Response Syndrome (SIRS) and have an infection. Septic shock is defined as sepsis with hypotension (less than 70mmHg) and lactate > 2.5mmol. Descriptive statistics will be used to analyze the percentage of cases that received septic kits that did not have an infection requiring antibiotics. Our main objective is to bring awareness to inappropriate antimicrobial use in veterinary medicine as well as highlight the importance of antimicrobial stewardship. Antimicrobial resistance is one of the biggest threats facing the world – both in veterinary medicine and human medicine today. Being cognizant of the damage of overuse and inappropriate use of antimicrobials is imperative to being successful against the looming threat of antimicrobial resistance.

Student Name:	Samantha Dolan V'23	Mentor:	Dr. John Rush
Project Title:	Retrospective Evaluation of the Indication for and Outcome Following Sildenafil Administration in Cats with Heart Disease		
Funding Source:	Cummings School of Veterinary Medicine at Tufts University		

Summary:

Sildenafil is a phosphodiesterase type 5 (PDE5) inhibitor that has been used to manage certain cardiopulmonary disorders, including pulmonary hypertension and congestive heart failure. Pulmonary hypertension is characterized by an abnormally elevated pressure in the pulmonary artery. Affected humans and animals often have an adverse prognosis and a reduced quality of life without treatment. As pulmonary hypertension is more easily documented in humans and dogs, much of the current literature examines this condition in these populations, with improved clinical signs and survival times reported.

Congestive heart failure (CHF) results from a failure of the heart to move blood forward, leading to a buildup of fluid in the lungs and surrounding tissues for individuals with left-sided CHF. In humans, sildenafil administration is being evaluated for the treatment of heart failure with preserved ejection fraction (HFpEF) by reducing afterload. This type of heart failure can be caused by hypertrophic cardiomyopathy, the most common heart disease of cats. In dogs with myxomatous mitral valve disease and pulmonary hypertension secondary to left-sided CHF, sildenafil administration resulted in improved clinical status and a drop in estimated pulmonary artery pressure.

To date there have been no publications examining the safety or efficacy of sildenafil use in cats, and there are few reports of clinical indications for or clinical responses to sildenafil administration in cats. These reports mention sildenafil use in a very small number of cats. Evaluation of sildenafil in cats will provide clinicians with more information on clinical use and responses to sildenafil administration in cats.

The goal of this retrospective study is to examine the indications for the initiation of sildenafil in cats with heart disease and evaluate medical records for side effects that might be attributed to sildenafil. Based on similar studies in dogs, it is hypothesized that sildenafil will be well tolerated in cats with heart disease, may improve clinical signs as judged by the owner, and will reduce estimated pulmonary arterial pressure as measured by tricuspid regurgitation velocity.

Using the StringSoft and FIRST databases at the Foster Hospital for Small Animals, feline patients diagnosed with heart disease and treated with sildenafil will be identified and selected for the study. Medical histories will be examined for clinical indication for sildenafil administration, dose and frequency administered, concurrent treatments or therapies, and time until repeat hospitalization or initiation of additional medication. Clinical data will be recorded and examined for evidence of pulmonary hypertension and congestive heart failure. When serial cardiac and echocardiographic exams are available, the estimated pulmonary artery pressure will be evaluated before and after administration of sildenafil. The medical records will also be evaluated for evidence of possible adverse effects after the initiation of sildenafil treatment. All clients will be contacted to determine any missing data from the record, and to ask about change in clinical status of their pet after starting sildenafil. While a prospective clinical trial would ultimately be the most complete method to examine the efficacy of sildenafil use in cats for the treatment of cardiac disease, the data collected in this retrospective analysis will assist in the development of a future prospective study.

Student Name:	Stella Elwood V'23	Mentor:	Dr. Emily McCobb
Project Title:	Client Perceptions of an Educational Partnership to Provide Low-Cost Basic Veterinary Care to an Underserved Community		
Funding Source:	Pet Smart Charities		

Summary:

Multiple studies cite cost of service as the most prevalent barrier blocking pet owners from access to veterinary care. Tufts at Tech (T@T) has made great progress in lessening this widespread hardship. The organization accomplishes this goal by offering care at 25% the national average cost to individuals who rely upon governmental assistance, like food stamps. At the same time, the clinic allows fourth year veterinary students to complete a primary care rotation, which strengthens their communication skills and enhances their cultural humility. Furthermore, the program trains high school students, many of whom qualify for federal subsidies for breakfast and lunch, to become certified veterinary assistants. In this way, since its establishment in 2012, T@T has benefited the community in many different ways.

However, despite these achievements, those involved in the operations of T@T acknowledge the need for continued improvement in order to serve the clinic's patients, as well as their families, in the best way possible. As a result, this project aims to foster client-centered/driven progress by creating and employing an accessible client feedback system.

Through questions that ask clients about their experiences at T@T, we can identify areas in which further growth is necessary, which in turn, determine where future funding and research endeavors should be allocated. Consequently, client opinion will drive practice changes, as the clients themselves know and understand their own needs, and the ways in which we may best assist them and their pets, better than anyone else.

Moreover, this project will empower clients to explain their perceptions of how well student doctors conduct appointments. The feedback system, which will be monitored by project collaborators for misuse or abuse, will allow clients to convey the strengths and weaknesses of the student assigned to their case, so that the student may learn and grow from their clients. Prior research in human medical programs indicates that patient feedback has increased the experiential knowledge obtained and used by the rotating students, allowing them to become more effective doctors.

Student Name:	Jillian Hojsak V'24	Mentor:	Dr. Mark Pokras
Project Title:	Erosion of Paint-Coated Lead Fishing Gear in a Simulated Loon Gizzard		
Funding Source:	Private Donor Funds		

Summary:

The leading cause of death for common loons (*Gavia immer*) is lead poisoning from ingested lead fishing gear. Some fishing advocates contend that a coating of paint on fishing gear can prevent lead absorption and the subsequent negative health effects in wildlife. Many painted lead weights are sold commercially for fishing. The proposed study will simulate the function of the loon gizzard to document its effects in breaking down lead jigs with paint coatings. I will use rock tumblers to simulate the gizzard's digestive action in order to demonstrate that paint does not prevent the exposure and subsequent erosion of the lead. Small rocks and an acid solution placed in the tumblers will simulate the materials that are normally found in loon gizzards which aid in digestion. Additionally, through necropsy, gross measurements, and histological assessment I will characterize the anatomy of the common loon gizzard and proventriculus. The findings of this study will be important for shaping future environmental policies and regulations for fishing gear. We hope to publish these findings in an effort to support loon conservation legislation.

Student Name:	Callista Huang V'24	Mentor:	Dr. Jonathan Runstadler
Project Title:	Assessing the Prevalence of SARS-CoV-2 in Livestock Populations		
Funding Source:	USDA Formula Funds		

Summary:

The ongoing global pandemic has unearthed many questions about human-animal interdependence and brought forth its importance in the daily lives of the general public. In just over a year, the scientific community has discovered much about SARS-CoV-2. However, its reverse zoonotic transmission is still not well understood. Thus, it is imperative to investigate the prevalence of SARS-CoV-2 in a wide variety of species, including livestock, which have a unique and important role in society. We hypothesize that transmission of COVID-19 from humans to livestock species is possible. Specifically, this study aims to i) determine the presence or absence of antibodies to SARS-CoV-2 in the sera of various livestock species and ii) determine if samples positive for antibodies to SARS-CoV-2 are more prevalent in certain livestock species.

In the initial phase of the CoVERS study, blood samples and oral swabs were collected from mainly companion animals and wildlife and sent to the Runstadler lab for testing. While over 500 samples were screened and none were positive, the livestock domain was not examined in this first phase. Additionally, while some studies utilizing computer modeling to investigate species-specific ACE2 receptor binding to SARS-CoV-2 spike protein have been conducted, no broad screenings of livestock samples have been conducted to date.

To explore the potential transmission of SARS-CoV-2 in livestock, we propose to have blood samples from livestock collected by field service veterinarians and sent to the laboratory to be processed for extraction of serum. Testing of the serum from each sample will be conducted using screening ELISA tests utilizing SARS-CoV-2 antigen. Test plates will be analyzed using a spectrophotometer to quantify fluorescence.

Sample results will be grouped by species and analyzed to determine if prevalence is greater in a certain species or multiple species. If prevalent in that species or a number of species, inferential statistics will be used to assess how significant the difference is between species.

Student Name:	Vanessa Indeglia V'23	Mentor:	Dr. Alison Robbins and Dr. Meera Gatlin
Project Title:	Assessing Bat Diversity and Abundance in Massachusetts Using Acoustic Monitoring		
Funding Source:	Cummings School of Veterinary Medicine at Tufts University		

Summary:

The impact bats have on animal, human, and environmental health demonstrates the importance of taking a One Health approach to conserving bat populations and minimizing bat threats. Many bat species help our ecosystems flourish through their seed dispersal and pollination services. Insectivore bats are also extremely beneficial to farmers by providing a means of pest control, which in turn prevents crop destruction and economic losses. For viral research, bats serve as key models that can be used to gain knowledge on causes of disease spill over and future disease outbreaks. While bats provide numerous benefits, there are many threats to their populations. Urbanization in particular can be detrimental to bat populations through habitat loss, loss of food sources, and limited roosting sites. Natural threats, like White Nose Syndrome, has led to rapid declines in bat numbers across the U.S since 2006. At the moment, there is a lack of information on bat populations, distribution, and habitats of North American bat species in the North American continent. Acoustic monitoring, however, now provides researchers with an easily accessible and feasible means of assessing bat populations. A lack of bat population monitoring, at local and regional levels, can result in ecosystem changes and rising bat threats going undetected, reduced conservation efforts, and a potential loss of future research advancements. The goal of this study is to participate in collection and analysis of data regarding bat diversity and abundance using bioacoustics monitors. The specific aims of the study include establishing baseline acoustic data in four areas of Massachusetts (MA), survey each area for monitor placement, and identify bat activity to design bat walks at the Arnold Arboretum. Data collection will consist of acoustic monitoring using stationary detectors to record ultrasonic bat calls as they pass the detectors at four locations in MA: Boston (the Arnold Arboretum), Westford, Carlisle, and North Grafton (Tuft's Cummings Campus). Acoustic monitoring will follow protocols provided by the North American Bat Monitoring Program (NABat); an international program that collects acoustic surveying data and provides standardized surveying protocols. Prior to data collection, areas for best placement of the stationary monitors will be surveyed via walking or driving for at least 1.5 hours using mobile acoustic detectors (*Echo Meter Touch Pro for iOS*). Stationary acoustic surveying will take place over a span of two weeks at each of the four locations. Using two of the *Song Meter SM4 Acoustic Recorders*, detectors will record bat calls throughout the entirety of the night for a minimum of 4 nights per week. At the Arnold Arboretum, additional surveying will be conducted 3 to 5 nights during the two-week monitoring period using maps and mobile detectors to identify areas of bat activity at the site. Recordings from the stationary detectors will be analyzed using the *Wildlife Acoustics Kaleidoscope Pro* software to identify bat species. The number of calls collected will be aggregated and used to calculate diversity indices to assess bat abundance and diversity among the four locations. Through the use of GPS tracking from the mobile detector, geospatial analysis will be explored to better evaluate species distribution at the Arboretum. At the end of data collection, data will be submitted to the NABat program.

Student Name:	Shelby Karp V'23	Mentor:	Dr. Lisa Freeman and Dr. John Rush
Project Title:	Comparison of Echocardiography and Cardiac Biomarkers in Cats Eating Different Types of Cat Foods		
Funding Source:	The Barkley Fund		

Summary:

Dilated cardiomyopathy (DCM) is a severe heart disease seen in cats and dogs, which is typified by cardiac dilation and reduced contractility. As of September 2020, over 1100 cases of diet-associated DCM in dogs have been reported to the United States Food and Drug Administration and have been commonly associated with grain-free or high-pulse (e.g., peas, lentils) diets. While this form of DCM often improves with diet change, the specific cause remains unknown. Most reported cases have been dogs, but a small number of cats have also been reported.

Taurine deficiency is well known as a cause of diet-associated DCM in cats but, with the discovery of this association in 1987 and subsequent supplementation of commercial diets with higher taurine levels, taurine deficiency-induced DCM is now considered to be uncommon in cats. Nonetheless, some cardiologists have anecdotally noted a recent increase in the number of cats being diagnosed with DCM that have normal taurine levels.

Our recent retrospective, multicenter study of 37 cats with DCM showed that 41% were eating a high-pulse diet and that taurine deficiency was uncommon. More importantly, cats eating high-pulse diets that changed diet post-diagnosis lived significantly longer than cats eating high-pulse diets that did not change diet post-diagnosis or cats eating low-pulse diets. These findings support the possibility that some cats may have diet-associated DCM that can improve with diet change even in the absence of taurine deficiency. Investigating this issue is of critical importance since many cat foods now contain pulses, which could potentially put cats at risk for DCM or earlier, subclinical cardiac changes.

The purpose of this study is to evaluate a population of cats for subclinical cardiac damage by deficiency, comparing cardiac size and function, cardiac biomarkers, and taurine concentrations in healthy cats eating high- versus low-pulse diets. We hypothesize that cats eating high-pulse diets will have decreased cardiac contractility, larger hearts, and higher cardiac biomarker concentrations compared to cats eating low-pulse diets. Results of this study will help to determine the effect of high-pulse diets on cats and whether cats are susceptible to a diet-associated DCM unrelated to taurine deficiency.

Student Name:	Jingyi (Jenny) Li V'24	Mentor:	Dr. Mark Pokras
Project Title:	An Exploratory Research of the Common Loon Microbiome		
Funding Source:	Private Donor Funds		

Summary:

Common loons (*Gavia immer*) are aquatic birds which breed on freshwater lakes throughout Canada and northern US during summer and migrate as winter approaches. They are threatened in several states in the U.S. due to their susceptibility to several pathogens and sensitivity to climate change, water quality and other environment factors. The gastrointestinal microbiome plays a vital role in host health by helping normal metabolism and protection against pathogen infections. Composition of the microbiome varies with species, sex, diet, social interaction, and environmental factors. Despite the large amount of data available on the gut microbiome in vertebrates, information about common loon gut microbiome is unknown.

We will start 1) Characterize the host-associated bacterial diversity and composition of common loons (*Gavia immer*) sampled during the breeding season. 2) Determine to what extent microbiome changes postmortem and after cadavers have been frozen. 3) Compare the microbiome across different sections of the GI tract. Understanding the loon gut microbiome will provide us with novel insights with its relationship with environmental factors and host pathogen protection, will contribute to our interpretation of new aspects of loon microbiome structure and function. Also, having information about how the microbiome changes postmortem and after freezing will give us information on the usefulness of obtaining pathology samples for microbiome studies. We hope to be able to publish our finding.

Methods

The project includes both field work and lab work. Both live and dead bird samples will be collected at Loon Preservation Committee Center in New Hampshire. For postmortem samples, necropsies and sample collection from different part of the GI system will be completed with both field biologists and pathologists at LPC.

DNA extraction, 16S rRNA sequencing, and data analysis will be completed at Dr. Pauline Kamath's lab at University of Maine, Orono. Samples will be randomized and whole genomic DNA extracted using a DNeasy PowerLyzer PowerSoil kit(Qiagen Inc., Germantown, MD). To characterize the bacterial microbiome, the V4 region of the 16S rRNA gene will be amplified through PCR using primers 515F and 806R, and high throughput sequencing will be performed on an Illumina MiSeq. Sequences will be demultiplexed using Qiime2. All statistical data analyses will be conducted in R 3.6.3 (R Core Team 2015). The SILVA 132 taxonomy database will be used to assign taxonomy. A maximum likelihood phylogenetic analysis will be performed in RAxML. Alpha-diversity will be measured by the Chao1 index. Community-level diversity will be assessed using a multivariate analysis of dissimilarity, using both weighted and unweighted UniFrac measures.

Student Name:	Maria Maguire V'24	Mentor:	Dr. Heather Gardner
Project Title:	Interrogating the Role of β -dystroglycan in DMD-Mutant Osteosarcoma		
Funding Source:	Boehringer Ingelheim		

Summary:

Osteosarcoma is the most common primary bone tumor in dogs, with over 25,000 cases diagnosed annually¹. Even with aggressive treatment, including surgery and chemotherapy, over 90% of dogs develop metastasis and succumb to their disease within 10-12 months. Despite significant efforts, treatment of canine osteosarcoma has remained unchanged for three decades. As such, researchers are beginning to study unique features of the canine genome that may serve as novel therapeutic targets². For example, large somatic deletions and copy number loss of the gene *DMD* (which codes for dystrophin) were recently identified in about 50% of canine primary osteosarcoma tumors². Preliminary evidence has identified deletion and copy number loss of *DMD* in a subset of osteosarcoma cell lines, and CRISPRCas9 dystrophin deletion increases phosphorylation of β -dystroglycan, indicating activation of the dystrophin-associated dystroglycan complex (DGC). Additionally, preliminary data has shown that dasatinib, a Src inhibitor, reduces β -dystroglycan phosphorylation in canine OS cell lines, consistent with published evidence that Src facilitates phosphorylation (activation) of ezrin, a cytoskeletal reorganizing protein which binds β -dystroglycan. **It is therefore hypothesized that truncated dystrophin isoforms that do not associate with β -dystroglycan, thereby allowing Src to phosphorylate β -dystroglycan and ezrin. It is further hypothesized that this results in a more aggressive cancer phenotype.** I will investigate this hypothesis by studying the phosphorylation of *DMD* binding partners (β -dystroglycan and ezrin), and the localization of the DGC complex. Additionally, using small molecule inhibitors targeting Src in cell lines with and without *DMD* expression, I will evaluate the impact of dystrophin loss on cellular proliferation and invasive capacity.

Student Name:	Kylie McDaniel Pumphrey	Mentor:	Dr. Felicia Nutter and Dr. Stephanie
Project Title:	Identifying, Classifying, and Cataloging Retinal Lesions in Asiatic Black Bears (<i>Ursus thibetanus</i>)		
Funding Source:	Cummings School of Veterinary Medicine at Tufts University		

Summary:

Specific Aims

This retrospective medical record study aims to identify, classify, and report the prevalence of retinal lesions in a sample of 184 Asiatic black bears (*Ursus thibetanus*) housed at the Animals Asia Vietnamese Bear Rescue Center (VBRC). This is an exploratory study, as the retinal abnormalities are recognized in this captive population but have not been adequately described nor linked with etiologies. Therefore, I will develop a classification scheme to characterize different retinal lesions and will create a glossary for retinal lesions in Asiatic black bears to improve the diagnosis of different retinopathies and better identify their etiologies, with particular emphasis on systemic hypertension.

Background and Significance

Approximately 17,000 Asiatic black bears (*Ursus thibetanus*) live on bile farms across Asia. Animals Asia provides sanctuary for hundreds of these bears at their Chinese and Vietnamese Bear Rescue Centers (CBRC and VBRC). Cardiovascular disease has been identified as the third leading cause of mortality in previously bile-farmed bears, and systemic hypertension appears to be a common problem in the population of bears at the Animals Asia sanctuaries. Blood pressure, however, cannot be measured consistently in most rescued Asiatic black bears since direct measurement requires either extensive training or anesthesia. The primary criteria currently used by Animals Asia to diagnose systemic hypertension includes hypertensive retinopathy (i.e., retinal hemorrhage, retinal vessel caliber changes, and perivascular chorioretinal exudates), left ventricular hypertrophy, and aortic dilation. Target organ damage (TOD) is considered a sign of intermediate vascular disease and a strong predictor of cardiovascular risk in individuals with systemic hypertension. To date, however, no retinal lesion classification scheme exists in bears, and no published literature characterizes and catalogs retinopathies in bears. This makes it difficult to consistently and confidently diagnose retinal lesions and treat their underlying etiology.

Methods

We will analyze existing medical records from 184 bears housed at the Animals Asia Vietnamese Bear Rescue Center (VBRC). In 2018, Animals Asia began to incorporate fundic photographs into their bear's biennial health check and emergency health checks requiring anesthesia, using fundic videos recorded with a smartphone and 40D camera. Screenshots from the videos will be created and examined. I will review the photographs for each bear, describing the appearance, size, and location of the lesion. I will also record the date of onset and signs of visual impairment. Using ImageJ, I will calculate the ratio of the lesions' area to the area of the optic nerve head. For bears with multiple photograph sets, I will assess all images, but utilize the most recent set of images for any statistical analyses. Following this data collection, I will categorize the lesions using the classification scheme and calculate the percentage of each kind of lesion I identify. I will compare the different retinal classes with signs of visual impairment, mobility, and mortality to determine if the classification scheme can be used as a predictor for clinical outcomes. By running ANOVA and post-hoc Tukey analyses on R, I will determine if the presence of a specific class of retinal lesion correlates statistically with a specific clinical outcome. The variables used will be determined after developing the classification

scheme. Finally, grouping the images by class, I will create a glossary depicting examples of each lesion and report their possible diagnostic differentials.

Expected Outcomes

Creating a formal classification scheme and a glossary of lesions and their etiologies will help ensure that the retinal lesions are accurately diagnosed, improve treatment, and prolong the life of bears. Because a high proportion of the bears at the Animals Asia Sanctuaries have developed retinal lesions and bears are commercially farmed in some countries, this research will apply to thousands of bears held in captivity. Additionally, it may be useful for other captive wildlife species, for which no retinal lesion classification scheme exists.

Student Name: Cassandra Munroe V'24

Mentor: Dr. Claire Fellman

Project Title: Short Course Amoxicillin for Treatment of Sporadic Cystitis in Dogs

Funding Source: Blue Buffalo

Summary:

One of the most common reasons for prescribing antimicrobials in dogs is urinary tract infections. There are limited veterinary studies available examining the best antimicrobial treatment selection and duration and no studies showing the effectiveness of short course amoxicillin treatment for urinary tract infections in dogs. Despite this lack of evidence, the most recent guidelines recommend the use of amoxicillin or trimethoprim-sulfa for 3-5 days for treatment of sporadic urinary tract infections in dogs, less than the previously suggested 7-day duration. The goal of this research project is to determine if the current recommended short-term amoxicillin treatment for urinary tract infections in dogs is effective. We hypothesize that 3 days of amoxicillin is noninferior to 7 days of amoxicillin therapy for the treatment of sporadic bacterial cystitis in dogs.

A double-blinded, placebo-controlled trial will be performed in dogs with sporadic urinary tract infections until 100 dogs complete the trial. Female and castrated male dogs with urinary tract infections will randomly be assigned a treatment involving 3 days of amoxicillin followed by 4 days of a placebo or 7 days of amoxicillin. On day 1 a urinalysis, urine culture and serum chemistry will be performed. On day 21, a urinalysis and urine culture will be repeated.

Resolution of clinical signs will be evaluated by interviews with owners. The study will enroll patients at 3 study sites: a low-cost community clinic, an emergency clinic and local first opinion practice. The noninferiority of 3-day versus 7-day amoxicillin therapy will be established based on a margin of 20% for the primary outcome of microbiological cure defined as a negative urine culture at day 21 and for the secondary outcome of clinical cure defined as an absence of clinical signs noted by the owner. Clinical and microbiological cure rates will be compared for isolates identified as susceptible or resistant to amoxicillin on a sensitivity panel.

Determining the efficacy of short-term amoxicillin treatment for urinary tract infections in dogs will support or prompt revision of current guidelines used by general practitioners and specialists. Providing evidence-based support for these guidelines can help improve patient outcomes as amoxicillin has fewer adverse effects compared to trimethoprim-sulfa and will also aim to reduce the development of antimicrobial resistance. This study will inform the treatment recommendations for a common disease and help to improve antimicrobial stewardship in dogs.

Student Name: Jullian Nazzaro V'24 **Mentor:** Dr. Stephanie Borns-Weil

Project Title: The Effect of COVID-19 on Canine Separation Anxiety

Funding Source: Cummings School of Veterinary Medicine at Tufts University

Summary:

Separation anxiety (SA) is a common canine behavioral disorder addressed in many studies. Although well-represented in scientific literature, there are significant differences in findings and specific risk factors for SA are suspected, but unclear. Among the suspected risk factors for separation anxiety are insufficient socialization, lack of opportunity to be alone and shelter origin.

The COVID-19 pandemic presents a unique and shared experience resulting in widespread lifestyle changes characterized by increased human presence in the home and reduced alone time for dogs, limited social contact with nonfamily members and other dogs, changes in the delivery of veterinary care, and cancellation of socialization and training classes. Given the increase in dog acquisitions during the pandemic and the widespread nature of the environmental changes, we have a unique opportunity to investigate risk factors for this problem on a large scale. The data obtained will be novel, timely, and contribute significantly to the field of behavioral medicine. We believe that our findings have the potential to be directly relevant to professionals and pet owners alike.

Our proposed study aims to determine if rates and severity of separation anxiety in dogs adopted during the pandemic are different compared to pre-pandemic conditions. Additionally, we intend to identify risk factors that impact the above aims and determine if current suspected risk factors for separation anxiety align with our findings. We hypothesize that COVID-19 and its resulting social distancing measures led to an increase in the rate and severity of separation anxiety in dogs, and specific lifestyle changes engendered by the pandemic pose as risk factors for SA. We intend to carry out this study using an online survey administered to pet owners who adopted dogs between March 2020 and January 2021. This population and time frame will be used as COVID lockdown measures became widespread in the USA beginning around March 2020, and there is significant variability in adopted dog breeds/age/history/health status etc. It can be challenging to obtain sufficiently large sample sizes for behavioral studies and this range is both specific (COVID conditions, separation anxiety, adopted dogs), and general enough to attract a large participant pool.

A statistician will be hired to assist with project design and provide a statistical analysis of results. Although there are many shared experiences and restrictions in place, COVID spread and attitudes toward the pandemic vary significantly by region and personal beliefs, so we want to ensure our sample pool is an accurate reflection of pandemic-induced human and animal behavior while also accounting for these variants. The survey will identify the presence of separation anxiety/SA behaviors, severity of behaviors, and we aim to identify potential links or risk factors between these behaviors and lifestyle changes brought on by COVID.

Student Name:	Brianna O'Malley V'24	Mentor:	Dr. Sandra Ayres and Dr. Jennifer Graham
Project Title:	Non-Surgical Approach to Sterilizing Avian Species Using a Domestic Chicken Model		
Funding Source:	USDA Formula Funds		

Summary:

Overpopulation of certain avian species can have multiple detrimental effects to the natural and human environment. Zoonotic diseases, nitrogenous pollution, damage to human property and natural ecosystems are just a few concerns being raised by wildlife control agencies. [1,2] Current methods of population control include hunting, trapping, poisoning, and predator introduction, all of which have substantial impact on animal welfare and suffering of the target species. [16] In some species, introduction of oral contraception has proved to be a commercial failure due to political and social concerns. [11]

Companion bird species are subject to a myriad of different diseases related to reproductive dysfunction. [7] These diseases can run the gambit from behavior manifestations of aggression and feather picking to difficulties in egg production, neoplasia and cloacal prolapse. [8,9] Current therapies to treat reproductive disorders can be cost limiting, or in surgical cases, life threatening. [12,13] In both of these types of scenarios, there is a need for noninvasive sterilization in avian species.

The overall goal of this study is to develop a non-surgical solution to overpopulation and reproductive related diseases in avian species by using the domestic chicken as a research model. This proposal hopes to develop a pilot study for the summer of 2021 to investigate the effects of a high-dose single injection of lipid-nanocomplex in a small group of female domestic chickens. This study will provide crucial understanding on the lethality of the nanocomplex to determine effective and non-lethal dosing that can be used on larger testing groups.

The tested technique will use lipid-based nanocomplex carrying the cytotoxin saporin which has been shown to inhibit protein synthesis and induce apoptosis. [14] In order to introduce saporin to gonadal tissue, the nanocomplex will be guided by an antibody targeting specific receptors on gonadal cells. In females, granulosa cells and theca cells are support cells that produce hormones needed for the production of viable ova/eggs. Both the ca and granulosa cells have membrane bound anti-Mullerian hormone II (AMHII) receptors which are found exclusively on these cells.

This study will assess the ability of the AMHII receptor antibody guiding the nanocomplex to induce apoptosis in these gonadal support cells. This will be done by comparing egg laying and evaluating stained and processed gonadal tissue for apoptotic activity. The hypothesis is that the nanocomplex will destroy these support cells, causing lack of ova development and egg laying in treated hens.

Results from this study using the domestic chicken model can have implications beyond just poultry, including wildlife and companion avian species. The proposed project will provide an essential foundation for performing further research on non-surgical methods of permanent sex steroid suppression in domestic chickens. Developing a single-injection sterilization technique could potentially address overpopulation issues in various avian species, as well as serve as an alternative to other forms of reproductive control for reproductive-related diseases in companion birds.

Student Name:	Bill Orrico V'23	Mentor:	Dr. Marieke Rosenbaum and Dr. H. Frank
Project Title:	The Effect of Landscape Disruption on Herpesvirus Prevalence in Bat Species of Northwestern Ecuador		
Funding Source:	Cummings School of Veterinary Medicine at Tufts University		

Summary:

The goal of this project is to determine and compare the prevalence of herpesvirus in neotropical bat species across varying categories of habitat disruption. This project will occur in the Maché-Chindul Ecological Reserve in northwestern Ecuador, a region of rapid growth, development, and reforestation efforts resulting in a dynamic mosaic landscape of pristine primary tropical forest, mixed agriculture, and new pasture.

Anthropogenic disruption, specifically through changing land use practices, has been found to alter the distribution and behaviors of species within the habitat. These changes can alter the infection dynamics of endemic viral pathogens resulting in disease outbreaks leading to population declines in already stressed populations. Neotropical bats offer an excellent model to study this phenomenon. Bats are the most common neotropical mammal, occupy many important ecological niches, and are highly affected by habitat disruption. Bats are also known reservoirs for at least nine viral families, many of which have zoonotic potential. Understanding how habitat disruption is affecting viral pathogens in bat population is essential to future conservation efforts as well as effective zoonotic surveillance.

Specific Aim 1: Determine and compare the prevalence of herpesvirus in up to 10 common neotropical bat species in the Maché-Chindul Ecological Reserve in northwestern Ecuador.

Specific Aim 2: Determine associations between herpesvirus prevalence, landscape use (undisturbed forest, forest edge, mixed agriculture, pasture), and roosting behaviors (leaf, cave) within and between neotropical bat species.

Specific Aim 3: Determine the association between herpesvirus prevalence and total bat species richness in different landscape uses.

Over an 8-week period bats will be captured using ground level mist-nets in four distinct categories of habitat disruption: undisturbed forest, forest edge, mixed agriculture, and pasture. Saliva samples of each bat will be collected. Upon return from field collection directed PCR amplification to detect herpesvirus presence will be performed using primers for *pol* and glycoprotein B genes. Sanger sequencing of the products will be performed to assess relatedness. Statistical analysis will be performed to determine the relationship between herpesvirus prevalence and the variables important to the specific aims of the project.

Student Name:	Laura Richards V'23	Mentor:	Dr. Christopher Schonhoff
Project Title:	Nitric Oxide (NO) Signaling in the Female Developing Striatum: Investigating Oxycodone's Effects on NO on Development of Dendritic Complexity		
Funding Source:	Cummings School of Veterinary Medicine at Tufts University		

Summary:

As the opioid epidemic continues to impact the United States, one area of critical concern is children exposed to opioid drugs in utero. Work in the Schonhoff laboratory has previously shown that oxycodone negatively impacts the development of female, but not male, striatal neurons. However, the mechanism for this effect is not known. Because signaling plays a critical role in the development of female striatal dendritic patterning and nitric oxide is a signaling molecule known to play a necessary role in neuronal differentiation, we hypothesize in our first proposed experiment that oxycodone alters nitric oxide levels in female developing striatal neurons. To further investigate the role of nitric oxide on the developing striatum, we hypothesize in our second proposed experiment that adding exogenous nitric oxide to, or inhibiting endogenous nitric oxide in, the developing striatal neuron will alter development of non-primary dendrites.

In the first experiment (nitric oxide level analysis), male and female striatal neurons grown in vitro will be treated with either 0, 0.1, 1, or 10 μM of oxycodone. A sample of the cell media will be used to measure the production of NO at each dose. To measure levels of nitric oxide, a Griess assay will be used, in which we will treat samples with nitrate reductase and then measure the levels of the stable decomposition product, nitrite.

In the second experiment, effects of exogenous nitric oxide or nitric oxide synthase (NOS) inhibition on dendrite development will be investigated. Cells will be treated with varying concentrations of either the nitric oxide donor S-Nitrosocysteine or the NOS inhibitor N ω -Nitro-L-arginine methyl ester. After 10 days of treatment, cells will be fixed, blocked, incubated, treated with antibody, washed with PBS, and mounted onto glass slides. A microscope will then capture neuron images, and Sholl analysis will be performed on those neurons with clearly distinguishable dendritic tips. The Sholl analysis will be used to determine dendritic complexity.

We hope that these proposed experiments will illuminate whether there is a connection between nitric oxide signaling, female striatal neuron development, and opioid exposure.

Student Name:	Samantha Scott V'23	Mentor:	Dr. Vicky Yang
Project Title:	Role of Canine Hemangiosarcoma-Associated Extracellular Vesicles in Promoting Tumor Angiogenesis		
Funding Source:	Morris Animal Foundation		

Summary:

Specific Aims and Hypothesis

We hypothesize that extracellular vesicles produced by canine hemangiosarcoma cells (HSA-EVs) will activate endothelial cells and induce angiogenesis, aiding in the growth of the tumor and metastasis. To test this hypothesis, we will i). determine if HSA-EVs induce activation of normal canine endothelial cells in vitro; ii). determine if HSA-EV promotes angiogenesis in vitro; and iii). characterize the vascular endothelial growth factor (VEGF) content in HSA-EVs. Background and Significance: Hemangiosarcoma (HSA), a malignant tumor that is commonly seen in dogs, is derived from multipotent precursor cells and is vascular in nature (Gorden et al., 2014). It most commonly affects the spleen, liver, heart, and skin (Gorden et al., 2014). Treatments generally include doxorubicin-based chemotherapy and excision, but prognosis is poor due to high rates of metastasis (Smith, 2003). Because it has an endothelial phenotype, HSA may have strong angiogenic effects to aid in tumor survival. Furthermore, circulating endothelial cells have been found to be increased in cancer patients, demonstrating the importance of angiogenesis in tumor development (Feng et al., 2017) Extracellular vesicles (EVs) are membrane-bound nanoscale entities that transfer a variety of mediators from cell to cell, including mRNA, non-coding RNA, growth factors, lipids, and proteins. Their contents are highly regulated by the cell of origin and contribute to cell-to-cell signaling. 'Pathological signaling' through EVs has been documented in multiple disease entities from neurologic disorders to cancer. Our lab has already shown that HSA cell conditioned media containing HSA-EVs, proteins and growth factors promotes endothelial tube formation, a marker of angiogenesis. If proven that HSA-EVs are responsible for this increase in tube formation, interruption of the EV communication may represent a therapeutic strategy to slow tumor growth and metastasis.

Methods: Extracellular vesicles will be collected from the culture supernatant of 3 canine HSA cell lines and isolated using size exclusion chromatography. The isolated HSA-EVs will then be incubated with canine aortic endothelial cells, with untreated cells as negative control. RNA will then be isolated from the endothelial cells, and ten transcripts (i.e., CD34 and vWF) serving as markers for endothelial activation will be characterized using quantitative real-time PCR. EV-treated endothelial cell angiogenesis will be evaluated using tube formation assays, and endothelial cell proliferation and viability will be determined using MTT assay. To evaluate the role of VEGF in mediating cell activation and angiogenesis, we will determine the HSA-EV VEGF content using commercially available canine ELISA assays.

Student Name: Katie Shaffert V'23 **Mentor:** Dr. Heather Gardner

Project Title: Investigating the Role of SETD2 in Canine Osteosarcoma

Funding Source: Anivive

Summary:

Osteosarcoma is the most common neoplasia of bone in dogs, known for its highly invasive and metastatic behavior. However, there has been little successful application of novel treatment protocols over the last 30 years, and patient outcomes have remained stagnant with a long-term survival rate of only 10-15% beyond one year[1]. Therefore, there is opportunity for innovation in therapeutic approaches to this disease in order to improve patient outcomes. Recent research has identified recurrent mutations in *SETD2* in canine osteosarcoma samples[2, 3]. The *SETD2* gene encodes the sole histone methyltransferase responsible for the addition of a third methyl group to histone 3 lysine 36 (H3K36) to form H3K36me3. In human cancers, *SETD2* has been characterized as a tumor suppressor gene. Thus, *SETD2*-inactivating mutations that result in loss of H3K36me3 are associated with more aggressive human cancers. Due to the recent characterization of *SETD2* mutations in canine osteosarcoma, it is not yet understood how these mutations impact osteosarcoma tumor biology or may be a therapeutic vulnerability in this disease. The goal of this project is to use *in vitro* assays to characterize the cellular behavior of canine osteosarcoma cells with and without SETD2 expression and determine whether loss of SETD2 makes cells more vulnerable to certain therapeutic approaches.

I hypothesize that canine osteosarcoma cell lines with downregulated SETD2 expression will have decreased levels of H3K36me3, and that loss of H3K36 trimethylation mediates more aggressive biological behavior. Furthermore, I predict that treating *SETD2*-inactivated cell lines with a lysine demethylase inhibitor will restore levels of H3K36me3, thereby reducing the malignant osteosarcoma phenotype. I will test these hypotheses by first evaluating the biological behavior of canine osteosarcoma cell lines with and without SETD2 expression *in vitro*. Then, I will characterize how this process is mediated by trimethylation of H3K36 by evaluating the biological behavior of these canine osteosarcoma cell lines treated with a lysine demethylase inhibitor. This research will help to lay the foundation for further research focused on elucidating rational drug combinations in *SETD2*-mutant cancers for more targeted therapy.

Student Name	Rachel Sherinian V'23	Mentor: Dr. Marjorie Bercier
Project Title:	Bat Activity and Diversity Across Land Types on the Cummings School of Veterinary Medicine Campus, North Grafton, MA, from June to August 2021	
Funding Source:	Cummings School of Veterinary Medicine at Tufts University	

Summary:

Bats (*Order Chiroptera*) represent a disproportionate facet of mammalian diversity.⁸ Bats are crucial contributors to local ecosystems and even impact human economies through pest control and pollination services.^{1,2} They are also believed to impact human health through the mitigation of vector-borne diseases, while serving as a potential wildlife reservoir for emerging infectious disease themselves. Because of their exceptional characteristics, bats have become of increasing interest to scientists working within the One Health concept.⁷

Unfortunately, approximately 24% of all bat species are now listed as endangered or vulnerable.¹⁰ In recent decades, global bat populations have seen significant decline, due mainly to anthropogenic factors.⁸ White Nose Syndrome, caused by the fungus *Pseudogymnoascus destructans*, has also had a severe impact on cave-dwelling, hibernating bats in North America. Species on the East Coast of North America have been most severely impacted.^{9,13}

In order to protect vulnerable chiropterans, it is crucial that we form an understanding around the mutual relationships between humans, bats, and the environment. However, the very features that make bats so unique have also historically made them difficult to study.⁸ Recent advances in acoustic monitoring techniques have made wildlife population research more accessible than ever. Acoustic monitoring can be used to detect the ultrasonic echolocating calls of insectivorous bats. When recorded, the distinctive sonographic waveforms of these calls can be used to estimate species diversity and local activity levels.⁴ Our intention is to use acoustic monitoring equipment to survey bat activity and diversity on the Tufts Cummings School of Veterinary Medicine (CSVM) Grafton campus. We have selected three microhabitats within the campus that are of interest for bat conservation, where we intend to conduct the surveys: forested land, cropland and institutional areas centered around school buildings. Furthermore, we plan to compare two different acoustic surveillance methods: active, mobile transect surveys and passive, stationary surveillance.³ Data on species diversity and activity will be compared across location/land type, survey method, as well as temporal variables.

As part of the new course MCM1010 "A One Health Approach to Chiropteran Health", this project will offer opportunities for DVM and graduate students to participate in wildlife field work, from sampling to data analysis. As project manager, the Summer Research Student will assume a position of communicator and team leader. This research will also provide a baseline understanding of bat diversity and activity on the Tufts CSVM Grafton campus and serve as a starting point for future student-driven research on chiropteran health. We hope that our work will contribute to local and national bat conservation efforts through publications in the peer-reviewed literature. This project aligns with the CSVM mission by promoting the health of people, animals, and the environment under a One Health framework.

Student Name:	Lorena Sistig V'23	Mentor:	Dr. Abhineet Sheoran
Project Title:	Development of Anti-TNF α VHHs for Immunotherapy of Chronic Inflammatory Diseases in Humans and Canines		
Funding Source:	Cummings School of Veterinary Medicine at Tufts University		

Summary:

Tumor necrosis alpha factor TNF α plays a major role in inflammatory autoimmune diseases such as Rheumatoid Arthritis (RA), Crohn's Disease (CD) and ulcerative colitis. Treatment with monoclonal antibodies (MAbs) to block TNF α actively exist for humans, while animals, such as dogs, rely exclusively on the pain management and control. In human medicine, MAbs have shown great results within individuals that suffer from chronic autoimmune inflammatory diseases, but these molecules have some drawbacks. The production of MAbs is very time consuming and costly. Also a single MAbs targets only one epitode. This has led to the search for a smaller and more effective antigen-binding agent. Variable regions of heavy-chain-only antibodies (VHH) of camelids are smaller (~14kDa) and more effective antigen-binding agents then MAbs. Unlike MAbs, VHH can be functionally expressed in microbes and are cheaper to manufacture. The Shoemaker lab has identified and expressed 9 unique VHHs against human TNF α . They recognize human TNF α in enzyme linked immunosorbent assays, but their TNF α neutralizing ability is unknown. The goal of this research is to use ELISA, cell cytotoxicity assay, and immunoblotting to determine the ability of the 9 VHHs to neutralize human TNF α and cross neutralize canine TNF α . We will also include 12 VHHs against mouse TNF α to analyze their ability to cross neutralize human and/or canine TNF α . This research will provide new information on neutralization of both canine and human TNF α via these VHHs. These findings would be extremely important in helping develop less expensive immunotherapy for canine and human patients with chronic inflammatory diseases. It will also provide more information on the ability of using immunotherapeutic agents to help treat inflammatory autoimmune disease in animals such as dogs.

Student Name:	Aubrey Specht V'23	Mentor:	Dr. Marieke Rosenbaum
Project Title:	A Metagenomic Analysis of Antimicrobial Resistance Genes in the Boston Urban Rat Population		
Funding Source:	AVMA/AVMF		

Summary:

The WHO has declared antimicrobial resistance (AMR) to be one of the top 10 global public health threats facing humanity. As recent studies have shown, this multifaceted threat extends beyond human and veterinary medicine, and beyond agricultural and livestock management, into the natural ecosystem with the potential for propagation and spread among wild animals. With the continual encroachment of urban environments on natural ecosystems and wildlife, there is an urgent need to understand the determinants and spatial distribution of antimicrobial resistance and gene transfer. For centuries, rats have been vectors of infectious diseases that impact humans and animals; urban rats could also harbor and shed microbial genes encoding resistance to the very drugs we use to treat bacterial infections. Due to their proximity to humans, as well as potential environmental sources of AMR such as untreated wastewater, rats are of primary focus for their role as vectors of antimicrobial resistance genes (ARG). This study will take a metagenomic approach to characterizing antimicrobial resistance genes in the gastrointestinal tract of 100 Boston-dwelling rats using Nanopore long-read sequencing. Fecal samples will be taken from rats trapped throughout Boston and in the vicinity of South End hospitals and homeless shelters. Purified genomic DNA will be sequenced using the portable MinION sequencer. Long reads will be analyzed against databases of ARG, plasmids, transposons and integrons, and bacterial DNA to characterize the resistome and microbiome found in the gastrointestinal tracts of Boston city rats. The ARGs and associated microbial drug class, detected in each rat, will be mapped using ArcGIS Pro by rat trap location. We hypothesize rats dwelling in vicinity to the South End's hospitals and homeless shelters will possess genes encoding antimicrobial resistance and evidence for horizontal gene transfer. The results of this research may further imbue urgency to responsible antimicrobial stewardship from a One Health perspective and inform local and national pest control strategies and public health policy.

Student Name:	Jamie Wells V'24	Mentor:	Dr. Elizabeth Rozanski
Project Title:	A Novel Surgical Approach to Laryngeal Collapse in Brachycephalic Canines		
Funding Source:	Cummings School of Veterinary Medicine at Tufts University		

Summary:

The goal of this project is to test a new surgical technique for dogs experiencing laryngeal collapse as a complication of brachycephalic obstructive airway syndrome (BOAS). Brachycephalic dogs develop laryngeal collapse as an end-stage event in the BOAS complex. Laryngeal collapse manifests in extreme inspiratory respiratory distress. There is currently no effective surgical technique to correct laryngeal collapse; severely affected dogs are either palliated with a permanent tracheostomy or euthanized due to poor quality of life.

The model for this technique comes from pediatric medicine. Children with subglottic stenosis face similar challenges to dogs with laryngeal collapse, mainly, increased respiratory effort and decreased laryngeal lumen width, with collapse on inspiration. While children may be safely managed with tracheostomy tubes, surgical correction of laryngeal dysfunction is preferred. Laryngotracheal reconstruction, either with intercostal cartilage or plastic grafts, is effective in treating pediatric cases of subglottic stenosis and eliminating the need for permanent tracheostomy. This study will modify the technique for dogs and test its efficacy in expanding laryngeal lumen and decreasing airway resistance in a cadaver model. If successful, this surgical technique could permanently correct laryngeal collapse in dogs and revolutionize treatment of animals with brachycephalic obstructive airway syndrome.

Student Name:	Emma Wunderlich V'24	Mentor:	Dr. Allen Rutberg
Project Title:	An Evaluation of Behavioral Changes (Maternal Aggression & Social Group Size, Composition, and Affiliation) of White-Tailed Deer (<i>Odocoileus virginianus</i>) Treated with Immunocontraceptive PZP		
Funding Source:	Internal Funds from Center of Animals		

Summary:

Conflicts between high density deer populations and humans in suburban areas—particularly deer-automobile collisions—persist, causing researchers to search for solutions such as the immunocontraceptive porcine zona pellucida (PZP). The aim of this study is to determine how PZP immunocontraceptives affect white-tailed deer (*Odocoileus virginianus*) behavior, particularly maternal aggression/territoriality during fawning season as well as group size and composition. Female white-tailed deer are highly philopatric and travel in matrilineal groups. However, during fawning season does tend to isolate themselves to a core home range and exhibit aggressive behaviors toward conspecifics. We hypothesize that PZP-treated does will exhibit changes in social group size and composition compared to non-treated does. This could manifest as an increase in doe groups that are not genetically related or as a higher number of does traveling alone. We also predict that there will be a difference between the size of core home ranges of the two groups. Four major goals of this project include tracking adult females in order to evaluate core range size/territoriality between the two groups, determining family groups, matching fawns to does to evaluate mother-fawn relationships and determine which vaccinated individuals still reproduced, and observing sociality between female deer.

The study will be conducted in Head-of-the-Harbor Village, NY, with an emphasis on Avalon Nature Preserve. Approximately 50 does vaccinated with PZP in previous years are present on the site, and about 20 more females are expected to be captured, ear-tagged, and vaccinated in March 2021. Many of the deer vaccinated in previous years are expected to have yearlings while those vaccinated in March 2021 will most likely have fawns. Each treated doe will have a PIT tag as well as two unique numbered ear tags. Each captured deer will also receive solar powered GPS ear tags. Data collection will be divided into two parts. In the first half of the summer, GPS ear tag data and direct observation (three times daily in one-hour increments) will be combined to determine the size and overlap of home ranges and to record any aggressive behaviors. In the second half of the summer, social groups are expected to reunite, so this part of the project will combine trail cameras with direct observation to compare social group size and composition as well as affiliative behaviors between deer. Data analysis will be conducted using a Mann-Whitney U test for frequency of aggressive and affiliative behaviors as well as multiple t-tests to analyze average distances from fawns and other females in a group, percentage of time spent in a group with other females, group sizes, and the 95% home range polygon that overlaps with other females.

There is currently very little information on how immunocontraceptives like PZP may alter white-tailed doe behavior, which is critical to understand before considering the possibility of administering PZP to control deer populations on a wider scale.

Student Name:	Gianna Zavota V'23	Mentor:	Seana Dowling-Guyer
Project Title:	An Exploratory Study of Stress Behavior Patterns in Dogs		
Funding Source:	Cummings School of Veterinary Medicine at Tufts University		

Summary:

The stressors that dogs are exposed to can impact their behavior and welfare. Results from studies examining dogs' reactions to stressful stimuli tend to show that stress adversely affects welfare (Beerda et al., 1998; Tuber et al., 1999). Because of this, it is important for owners and animal caretakers to be able to identify signs of stress in dogs in an easy and non-invasive way. Since many methods for stress assessment rely on physiological measures such as cortisol collection, which is itself stressful, a non-invasive method to assess stress would be useful to help monitor dogs in a variety of situations. Behavioral measurements show promise to be utilized as a convenient stress assessment tool (Kerswell et al., 2009; Rooney et al., 2009), however, additional investigation is needed to fully understand which behaviors across varying contexts are useful indicators of stress in different dogs.

With the help of NIH funding last year, I began an exploratory study of stress-related behavior in dogs. I planned to examine 100 existing videos of kenneled and isolated dogs to look for stress-related behavior using Observer XT behavioral software by Noldus. In the first half of the summer, I developed a robust and reliable coding scheme to code the behaviors, uploaded all videos to the software, trained on the software with multiple practice videos to acceptable inter-rater reliability, and completed 40 out of the intended 100 videos to code. This summer, I aim to leverage that extensive work to continue the study, finish coding the remaining 60 videos, and analyze the complete data set to identify trends in stress-related behavior in dogs across a variety of settings. These data will contribute toward the development of a behavioral tool that can be used in a variety of settings, including the animal care industry, to improve the welfare of dogs in veterinary, laboratory, and shelter environments.