24TH ANNUAL RESEARCH DAY

SCIENTIFIC PROCEEDINGS

JAN. 28, 2023 | LORY STUDENT CENTER

COLLEGE OF VETERINARY MEDICINE
AND BIOMEDICAL SCIENCES
COLORADO STATE UNIVERSITY

Our 24th annual Research Day showcases the work of more than 100 aspiring scientists in Colorado State University's College of Veterinary Medicine and Biomedical Sciences. The event gives our rising stars vital experience presenting their research findings to a scientific audience through poster displays and talks. The day also provides young researchers with an avenue for feedback to help them develop ideas that, in many cases, will become lifelong scientific pursuits.

The research projects on display are sponsored by companies, foundations, and institutions concerned with improving human, animal, and environmental well-being. Thank you for supporting and engaging with our presenters – undergraduate students, graduate students, veterinary residents, and post-doctoral fellows – as they pursue research that will improve the health of animals, people, and the planet!

CONTENTS

RESEARCH DAY 2023

SCHEDULE OF EVENTS

10:00-10:45 a.m.	Poster Set up Oral Presentation	LSC Grand Ballroom AB Set up in assigned rooms
11:00 a.m.	Opening Remarks	LSC Theater
11:10-11:50 a.m.	Zoetis Research Excellence Award Winner – Dr. Dan Regan	LSC Theater
11:50 a.m.-12:15 p.m.	Break	
12:15-4:15 p.m.	Oral Presentations: Basic Science	Rm 1: LSC 308-310
	Oral Presentations: Basic Science	Rm 2: LSC 312
	Oral Presentations: Clinical Science	Rm 3: LSC 322
	Oral Presentations: Clinical/Translational Science	Rm 4: LSC Longs Peak
12:15-2:00 p.m.	Poster Session I Judging: Odd-Numbered Posters	LSC Grand Ballroom A/B
2:00-2:15 p.m.	Break	
2:15-4:00 p.m.	Poster Session II Judging: Even-Numbered Posters	LSC Grand Ballroom A/B
4:15-6:30 p.m.	Social Hour and Awards	LSC Grand Ballroom C/D

DEPARTMENTAL ABBREVIATIONS

BMS: Biomedical Sciences
CS: Clinical Sciences
ERHS: Environmental and Radiological Health Sciences
MIP: Microbiology, Immunology, and Pathology

CONGRATULATIONS TO THE 2022 CVMBS RESEARCH DAY WINNERS!

POSTER PRESENTATIONS

First Early Basic	Julietta Sheng, Graduate Student, BMS, "Maternal stress alters hypothalamic development." Mentor: Stuart Tobet
Second Early Basic	Mikaela Linch, DVM Student, MIP, "Development of a novel vaccine for Feline Enteric Coronavirus using recombinant Lactobacillus acidophilus." Mentor: Gregg Dean
First Advanced Basic	Reed Woyda, Graduate Student, MIP, "Campylobacter prevalence differs across and within broiler houses with re-used poultry litter." Mentor: Zaid Abdo
Second Advanced Basic	Samantha Labb, PhD Student, ERHS, "Closing the Nuclear Fuel Cycle: Minor Actinide Separations." Mentor: Ralf Sudowe
First Early Clinical	Ashley Parker, Resident, CS, "Early Failure of CHOP Protocol Indicates Poor Response to Rescue Protocol in Dogs with Lymphoma." Mentor: Doug Thamm
Second Early Clinical	Hannah Patterson, DVM Student, CA, "Use of computed tomography and nuclear scintigraphy for diagnosis and staging of primary anterior uveal osteosarcoma in a client-owned rabbit." Mentor: Miranda Sadar
First Advanced Clinical	Patricia Mara Lopes Sicupira Franco, Staff, CS, "Molecular Characterization of Giardia intestinalis isolates in Dogs from a Rescue Shelter in Northern Colorado." Mentor: Michael Lappin
Second Advanced Clinical	Sera Lee, DVM Student, CS, "Ocular penetration of oral acetaminophen in the horse" Mentor: Rachel Hector
First Early Translational	Hayley Templeton, Graduate Student, BMS, "Intestinal Model for Parkinson's Disease Development." Mentor: Stuart Tobet
Second Early Translational	Laurel Haines, DVM PhD Student, MIP, "Osteosarcoma exosomes selectively home to the lung and elicit pro-metastatic changes in resident alveolar macrophages." Mentor: Dan Regan
First Advanced Translational	Ariel Timkovich, Graduate Student, MIP, "Differential transcript expression in compensatory limbs following DMM or sham surgery in male and female mice." Mentor: Kelly Santangelo

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ORAL PRESENTATIONS

First Early Basic
Luke Whitcomb, Gradute Student, BMS, "Glucocorticoid receptor signaling is required for acclimation of skeletal muscle to hypobaric hypoxia." Mentor: Adam Chicco

Second Early Basic
Brandon Lowry, Undergraduate Student, BMS, "Exploring the effects of standard two-dimensional anatomical models and virtual reality models on Visio spatial skills." Mentor: Tod Clapp

First Advanced Basic
Jessica Kincade, Graduate Student, BMS, "Transient and Persistent BVDV Infections and a Unique Post-natal Phenotype." Mentor: Thomas Hansen

Second Advanced Basic
Taylor Locklear, Resident, CS, "Retrospective review of causes of mortality for zoo giraffe and okapi (1991-2020)." Mentor: Miranda Sadar

First Early Clinical
Rachel Conway, DVM Student, CS, "Behavioral and physiologic effects of a single dose of oral gabapentin in rabbits." Mentor: Miranda Sadar

Second Early Clinical
Jordan Marsh, DVM Student, BMS, "Phenotypic assessments of metabolic status and potential for dietary supplements to mitigate insulin resistance in the obese mare." Mentor: Elaine Carnevale

First Advanced Clinical
Rachel Maison, Graduate Student, BMS, "Serosurveillance for anthrax exposure in Texas feral swine: A potential biosurveillance tool for mapping risk." Mentor: Angela Bosco-Lauth

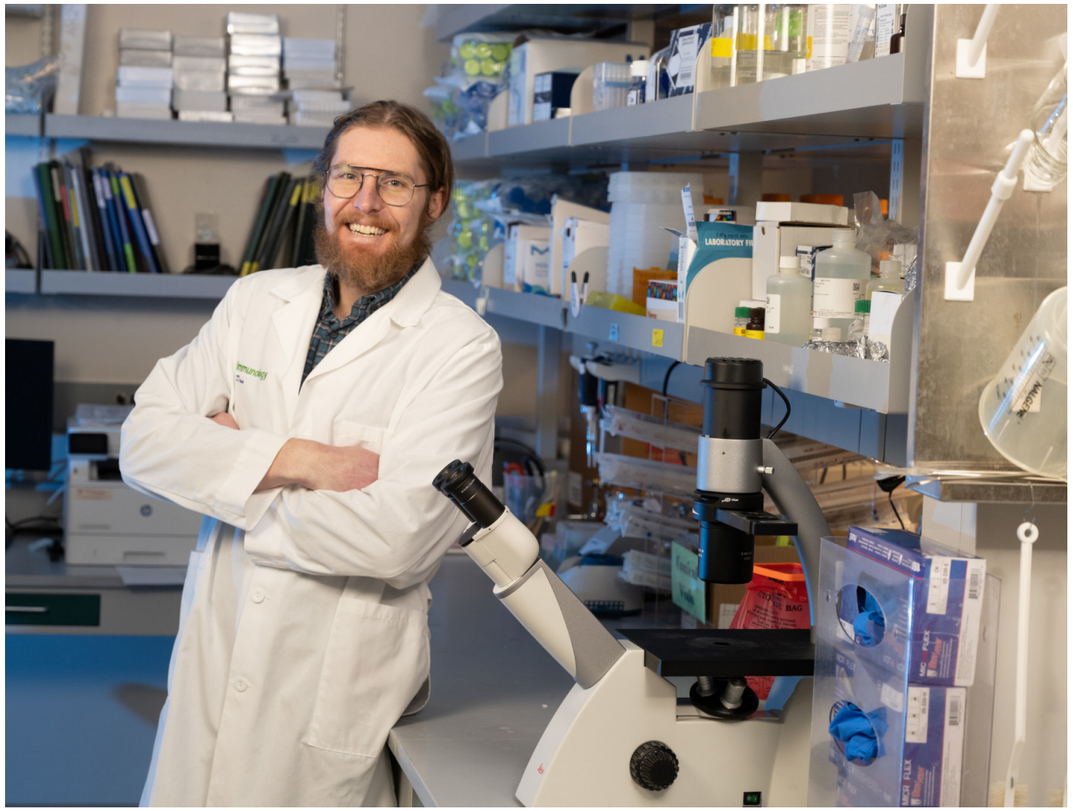
Second Advanced Clinical
Jennifer Kelley, Resident Post-Doc Fellow, CS, "Prognostic indicators for feline craniofacial trauma: a retrospective study of 130 cases." Mentor: Jennifer Rawlinson

First Translational
Katherine Bisazza, Graduate Student, CS, "Comparison of Advanced Imaging Modalities to Assess Bone Mineral Density in the Sheep Model." Mentor: Jeremiah Easley

Golden Pipette Award
Biomedical Sciences

CANINES KEY TO CANCER RESEARCH FOR ZOETIS AWARDEE

By Madelein Lopez



Dr. Dan Regan was awarded the 2022 Zoetis Award for Veterinary Research Excellence. (John Eisele/CSU Photography)

DRIVEN BY CURIOSITY AND CARE, DR. DAN REGAN'S translational work and collaboration within the College of Veterinary Medicine and Biomedical Sciences and broader field of comparative oncology research earned him the 2022 Zoetis Award for Veterinary Research Excellence.

A pathologist and assistant professor in the Department of Microbiology, Immunology, and Pathology, his initial osteosarcoma research progressed a therapeutic combination from novel canine models to human clinical trials at the Children's Hospital Colorado and Children's Healthcare Atlanta. Regan is also director of the Investigational Pathology Laboratory at the Flint Animal Cancer Center, a role that allows him to contribute his pathology expertise through a team science approach to a variety of translational animal models of disease.

As the Zoetis awardee, Regan will deliver the keynote talk at the upcoming College of Veterinary Medicine and Biomedical

Sciences Research Day on Jan. 28, titled "Everything I know I learned from dogs: A comparative metastasis biology approach to accelerate development of tumor microenvironment targeted immunotherapies for osteosarcoma."

DAN, THE ALMOST WEATHERMAN

Collecting turtles, snakes, and "anything [he] could find" as a child on Saint Simons Island off the southern coast of Georgia served as a formative start to Regan's journey to becoming a veterinarian.

After moving to Ohio, the anticipation of his first snow and a certain skiing weatherman, Herb Stevens, inspired an additional interest in weather and winter activities.

"By the time I hit high school, I was like, 'yeah, I'm just not smart enough to be a veterinarian,' but I'm going to get a Ph.D. and be a winter weather specialist," Regan said.

Drawn to the University of Georgia by family and a reputable meteorology program, Regan instead rediscovered his desire to become a veterinarian during his first year, prompting him to change his major from meteorology to biology.

"THE ACADEMIC COMMUNITY TO ME WAS WHERE, FOR THE FIRST TIME IN MY LIFE, I FELT ACCEPTED AND COULD BE MYSELF"

Regan developed a passion for the mechanisms of disease in veterinary school and a unique opportunity in an ophthalmology course funded his first-ever research. Initially drawn to research immunology by a family history of autoimmune diseases, his father's cancer diagnosis cemented Regan's research interests. One Nature paper "blew his mind" at the time, demonstrating a tumor could signal immune cells to visit and prepare future sites of metastasis in the body prior to the tumor's arrival, like preparing the soil for the seeds of cancer.

Regan was intent on further pursuing this paradigm, known as the "pre-metastatic niche," when he began the Anatomic Pathology Residency and Ph.D. program at Colorado State University in 2011.

COMMITTING TO CANCER RESEARCH

"I just thought osteosarcoma was the perfect model," Regan said. "It has this extreme propensity to metastasize to the lungs."

Luckily, his primary advisor, Dr. Steve Dow, agreed. Both veterinarians and pathologists, they were uniquely equipped to investigate this type of bone cancer more prevalent in dogs.

In humans, osteosarcoma is a rare yet primarily pediatric disease, with little improvement in therapeutic outcomes in recent decades. Over a third of patients still develop recurrent disease, almost exclusively in the lungs, within an average 1.6 years of initial treatment.

Using a canine model, Dow and Regan's initial research focused on targeting monocytes, which tumor cells convert to macrophages and progress metastasis. A resulting clinical trial for dogs with a drug combination aimed at these monocytes successfully advanced to a human clinical trial, underscoring the value of the canine model to rapidly deploy potential therapeutics.

Now, as a CSU faculty member with his own lab, Regan takes a highly collaborative and multi-pronged approach to understanding different cell types and mechanisms involved in the tumor microenvironment and metastatic seeding. With his lab members, Regan is investigating exosomes released by tumors that arrive at the lung first and possibly signal for additional arrivals, such as tumor macrophages that influence changes to residential macrophages in the lung. The presence of these macrophages could provide a potential diagnostic method for patient monitoring, using a bronchoalveolar lavage to wash fluid through the lungs and analyze the present cell types.

Additionally, responding to the signals, fibroblasts in the lung are thought to trigger the overall transformation of the soil of the lungs and recruit additional tumor-promoting cells.

"Understanding these mechanisms of priming this soil might lead to something that you can monitor to predict who would relapse and why," Regan said.

The early success and further potential of his translational osteosarcoma research was recognized and supported with several honors, including a Boettcher Foundation Webb-Warring Biomedical Research Award in 2020. Regan was also recently awarded an Outsmarting Osteosarcoma grant by the Making It Better Agents, a nonprofit organization that brings together the small community of patients, caregivers, doctors, and researchers to prompt awareness and developments for pediatric osteosarcoma.

As one of the few veterinarians involved, Regan had the chance to meet and hear the stories of "osteo-warriors" and their families. He carries those personal connections with him to the lab bench.

"I would love to make a difference for some of these patients. They have their efforts to fight, but they've been through a lot of stuff; it's tough therapy," Regan said. "Once this tumor recurs, it would be great to make a difference there."

GIVING BACK WITH GRATITUDE

Many people along the way have been instrumental in Regan's education and career.

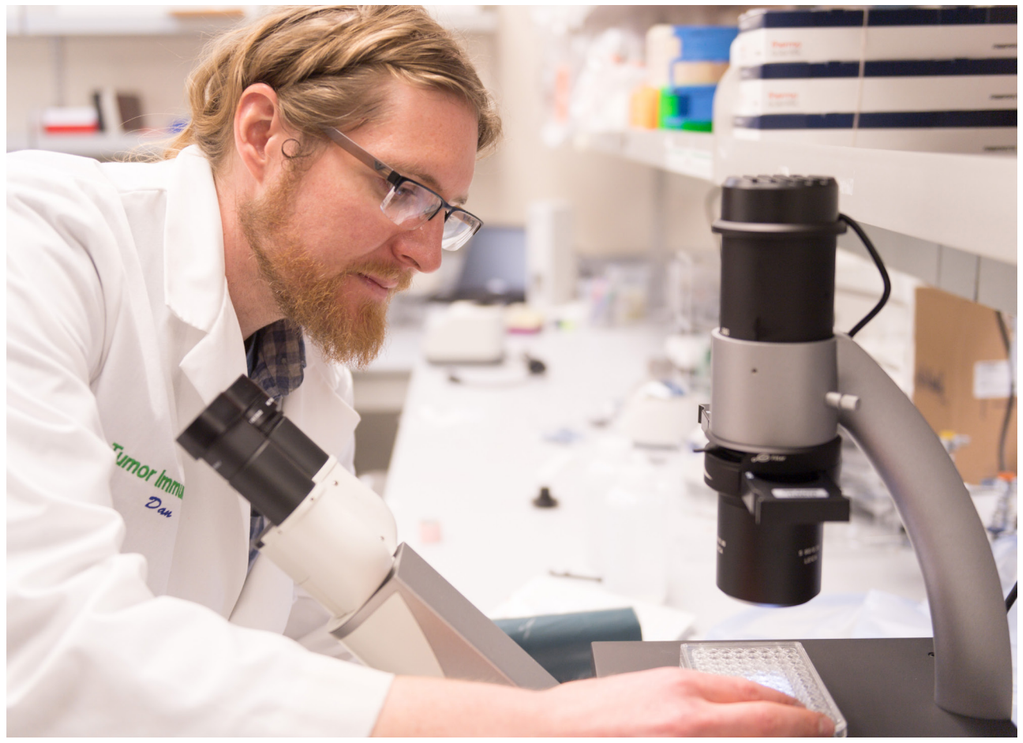
"The academic community to me was where, for the first time in my life, I felt accepted and could be myself," Regan said.

Early on, mentors helped him navigate the process of college, train in research, and apply for a residency program, setting him on his current path. He values the opportunity to give back and ignite a similar spark in students, through teaching, offering research experience in his lab, and his leadership of the D.V.M./Ph.D. Combined Degree Program.

Regan can continue to move ideas from the lab, thanks to his proximity to other translational scientists, clinical staff, dedicated pet owners, and growing connections to fellow osteosarcoma researchers and oncologists beyond the university.

Receiving the news of his selection for the 2022 Zoetis Award for Veterinary Research Excellence is motivating for his efforts to make a difference for people and pets, through ups and downs.

"Honestly, I'm just grateful for the opportunity to do this as a career and maybe help somebody," Regan said.



SESSION 1: Basic Science

12:15–3:45 p.m. | LSC 308-310

Time	Presenter	Topic	Dept.
12:15	Anderson, Isla	Peripheral *Mycobacterium tuberculosis* Infection Causes Neurodegeneration and Memory Loss in Guinea Pigs | J Moreno	ERHS
12:30	Barnes, Summer	Use of Etorphine for Immobilization of Giraffe for Snare Removal: A Retrospective Study | K Mama and M Sadar	CS
12:45	Brady, Rachel	CD206 activation results in growth of canine histiocytic sarcoma cell lines | D Thamm	CS
1:00	Braun, Hana	Interferon-tau protects the corpus luteum from lysis by prostaglandin F2-alpha | Thomas Hansen	BMS
1:15	Fletcher, McKenzie	Localization of a probiotic *Lactobacillus acidophilus* rotavirus vaccine within the host | G Dean	MIP
1:30	Hatch, Nizhoni	The cultivation and characterization of soil bacterium in Navajo Nation to identify the natural soil biome | P Charley	MIP
1:45	Kerley, Anne	Evaluation of ketamine-midazolam in combination with either medetomidine or dexmedetomidine in Bennett's wallabies (*Notamacropus rufogriseus*) under human care | M Sadar	CS
2:00	BREAK		
2:15	Kim, Elizabeth	Integron-encoded antimicrobial resistance and virulence determinants among *Salmonella enterica* serovar Typhimurium isolated from poultry | S Rao	CS
2:30	Love, Joy	Exploring the Fecal Microbiome of Mice Vaccinated Against the Rotavirus Using Python | Z Abdo	MIP
2:45	McKee, Hannah	Transcutaneous identification of implanted microchips as a method of tracking equine large colon migration | D Hassel	CS
3:00	Patterson, Hannah	The effect of various perineural analgesia techniques of interleukin-1βinduced synovitis of the equine metacarpophalangeal joint | E Contino	CS
3:15	Ratnayake, Oshani	Key players of membrane remodeling: Role of Phospholipase A2 in flavivirus infection of the human host | R Perera	MIP
3:30	Ring, Molly	The Role of Niemann Pick Type C2 Genes During Ivermectin Blood Meal Response in Mosquito Plasmodium Vectors | B Foy	MIP

SESSION 2: Basic Science

12:15–3:30 p.m. | LSC 312

Time	Presenter	Topic	Dept.
12:15	Risen, Sydney	Non-toxic Nanoligomers™ targeting key neuroinflammatory pathways are neuroprotective in prion disease | J Moreno	ERHS
12:30	Rockow, Megan	Toll-Like Receptor Activation of Mesenchymal Stromal Cells for Improved Cellular Treatment of Osteoarthritis | L Pezzanite	CS
12:45	Souza, Luca	Investigating the levels of LHCGR in extracellular vesicles and its relationship with ovarian follicle development in bovine | D Tesfaye	BMS
1:00	Stratton, Hayley	Prognostic value of rectal temperature, packed cell volume, and blood glucose at hospital admission in client-owned ferrets |M Sadar	CS
1:15	Zaiger, Megan	Evaluation of Nanodiamonds for the Adsorption of Radioactivity from Ocean Waters | R Sudowe	ERHS
1:30	Carpenter, Molly	Characterizing the effect of coinfection ratios on bluetongue virus reassortment in *Culicoides sonorensis* | C Mayo	MIP
1:45	Charley, Phillida	MERS-CoV passaged on Jamaican fruit bat cells leads to mutations in Orf5 gene | T Schountz	MIP
2:00	BREAK		
2:15	DeFranco, Joseph	Diverse prion strains result from selective propagation of distinct quasispecies conformations in different host compartments | G Telling	MIP
2:30	Kincade, Jessica	Fetal BVDV infections and postnatal epigenetic dysregulation | T Hansen	BMS
2:45	Labb, Samantha	Development of a sodium bismuthate-coated polyacrylonitrile resin for the separation of oxidized actinides from used nuclear fuel | R Sudowe	ERHS
3:00	Latham, Amanda	Characterizing Infectious Disease-Induced Neurodegeneration in a Guinea Pig Model of *Mycobacterium tuberculosis* Infection | J Moreno	ERHS
3:15	Thompson, Riley	Bovine oviductal organoids as a biomimetic system to evaluate extracellular vesicles | F Hollinshead	CS

SESSION 3: Clinical Science

12:15–4:00 P.M. | LSC 322

Time	Presenter	Topic	Dept.
12:15	Baughman, Jennifer	Epidemiology of Traumatic brain injury (TBI) and head injury in cats: an ACVECC-VetCOT registry study (April 2017 - December 2021) | K Hall	CS
12:30	English, Zach	Functional anatomy of the mitral valve in canine degenerative mitral valve disease | B Potter	CS
12:45	Jones, Katrina	The effects of two different air quality categories ('good' versus 'moderate') on ophthalmic parameters in normal dogs | M de Linde Henriksen	CS
1:00	Larson, Blaine	Synovial transcriptomic response in osteoarthritis progression determined by single cell sequencing in an equine model | L Pezzanite	CS
1:15	Lee, Ber-In	Local immune response to radiation therapy and combined myeloid cell targeted therapy in a dog model of sinonasal carcinoma | K Boss	ERHS
1:30	Marchell, Nina	Radiation dose to patients and staff during feline esophagrams | E Randall	ERHS
1:45	Patton, Elizabeth	Development of a porcine oocyte collection protocol for intracytoplasmic sperm injection (ICSI) with stallion spermatozoa | J Hatzel	CS
2:00	BREAK		
2:15	Rice, Hannah	Increasing enclosure complexity to improve geriatric African elephant welfare at the Cheyenne Mountain Zoo | M Johnston	CS
2:30	Riedemann, Macy	Outcome of hyperthyroid cats treated with radioactive iodide (I-131) therapy | M Lappin	CS
2:45	Spitznagel, Katya	Detection of pro-inflammatory cytokines in healthy canine tear film using mass spectrometry via multiple reaction monitoring (MRM-MS) | M de Linde Henriksen	CS
3:00	Talbot, Charles	Evaluation of tissue oxygenation saturation as a marker of shock and resuscitation in a canine hemorrhagic shock model | K Zersen	CS
3:15	Diaz, Devon	Impact of lyophilized platelet infusions on point-of-care viscoelastic test VCM-Vet™ in canine cardiopulmonary bypass | J Guillaumin	CS
3:30	Kaliq, Muhammad	Seroprevalence and potential risk factors for brucellosis among small ruminant farmers in Punjab, Pakistan, 2019-20 | S Rao	CS
3:45	Maison, Rachel	Feral swine as indicators of anthrax contamination and potential vectors of infectious spores: an experimental infection study | A Bosco-Lauth	BMS

SESSION 4: Clinical/Translational Science

12:15–3:45 p.m. | LSC LONGS PEAK

Time	Presenter	Topic	Dept.
12:15	Tucker, Claire	Epidemiology of Traumatic brain injury (TBI) and head injury in dogs: an ACVECC-VetCOT registry study (April 2017 - December 2021) | K Hall	CS
12:30	Weber, Annika	Rice bran in ready-to-use therapeutic foods (RUTFs) for microbiota-targeted treatment of childhood malnutrition | E Ryan	ERHS
12:45	Alsulami, Abdullatif	*C. elegans* Nrf Homolog, Skn-1, May Play a Role in Cannabidiol Neuroprotection | J Moreno	ERHS
1:00	Cagle, Shelby	Stop the Spread: a community-based science communication approach to address misinformation | N Kelp	MIP
1:15	Hines, Amelia	Antemortem diagnostic for canine cognitive dysfunction using plasma biomarkers | J Moreno	ERHS
1:30	Klosowski, Marika	Breast cancer cell co-culture with primary lung fibroblasts is associated with modulation of cytokine secretion and extrinsic stromal-mediated drug resistance | D Regan	MIP
1:45	Michalko, Bridget	Biomechanics of the LDE and ACL in ovine stifle stability for a novel model of posttraumatic osteoarthritis | B Nelson	CS
2:00	BREAK		
2:15	Murphy, Katlyn	Undergraduate STEM students' science communication skills, science identity, and science self-efficacy influence their motivations and behaviors in STEM community engagement | N Kelp	MIP
2:30	VanZeeland, Emily	Sex differences in the response to anterior cruciate ligament injury following mechanical rupture in a mouse model | K Sikes	CS
2:45	Berezin, Casey-Tyler	Opioids in the retina modulate sleep/wake behavior | J Vigh	BMS
3:00	Menjivar, Nico	Granulosa cell-derived extracellular vesicles alter oocyte function and promote survival under heat stress conditions | D Tesfaye	BMS
3:15	Pfluger, Brigitte	The role of dietary intake and diversity of mother-infant pairs for malnutrition prevention in Guatemala | E Ryan	ERHS
3:30	Sullivan, Elizabeth	SolaVAX: a whole virion vaccine platform produced via a novel inactivation method against coronaviruses | I Ragan	BMS

POSTER PRESENTATIONS

SESSION 1 | ODD-NUMBERED POSTERS | 12:15–2:00 p.m.

SESSION 2 | EVEN-NUMBERED POSTERS | 2:25–4:00 p.m.

NOTE: The presenters listed below may be found according to their assigned poster numbers.

No.	Presenter	Title | Mentor	Dept.
1	Alshammari, Ibrahim	A physiologically based toxicokinetic model to predict the disposition and fate of N, N-Dimethylformamide in animals and humans | B Reisfeld	ERHS
2	Anderson, Sarah	Evaluation of the validity of joint stress tests in dogs with and without musculoskeletal disease | F Duerr	CS
3	Aragon, Juan	Dietary rice bran and navy beans are digestible during chemotherapy and beneficially-modulate fecal and plasma metabolomes in dogs undergoing chemotherapy | E Ryan	ERHS
4	Bashor, Laura	Notable characteristics of the oral microbiome of domestic cats infected with Feline Immunodeficiency Virus in the presence and absence of antiretroviral therapy | S VandeWoude	MIP
5	Bennett, Lauren and Janapati, Ria	Are wild armadillos a reservoir for *leprosy bacilli* in Ecuador? | M Jackson	MIP
6	Bergum, Nikolas	A retinal contribution to opioid-induced sleep/wake disturbances | J Vigh	BMS
7	Bollegar, Andrew	Mutation to the *Synaptotagmin* Calcium Binding Pocket Produces Congenital Myasthenic Syndrome-like Symptoms in Drosophila | N Reist	BMS
8	Breuning, Anneliese	Chinese water dragons and goldfish as hosts for maintaining *Burkholderia pseudomallei* | R Bowen	BMS
9	Burnett, Thomas	Quantitative assessment of genes involved in early lymphopoiesis using droplet digital PCR (ddPCR) to distinguish immature lymphoid neoplasms in the dog | A Avery	MIP
10	Cao, Jenna	Canine osteosarcoma as a translational model for advancing solid tumor CAR T cell therapy | S Dow	CS
11	Cardillo, Jenna	Examining the Vital Vascular Network: Evaluation of the Microvasculature and Endothelial Glycocalyx in a Canine Hemorrhagic Shock/Resuscitation Model | J Guillaumin	CS
12	Castellanos, Emily	Got Stim? Check the Brainstem | S Tobet	BMS
13	Cherry, Christian	Investigating T cell responses to an orally delivered probiotic vaccine platform | G Dean	MIP
14	Coffin, Kathryn	The Detection of *Plasmodium* Species in Anopheles Vectors in Burkina Faso, Africa to Determine the Role of Plasmodium Species in Malaria Epidemiology | B Foy	MIP
15	Cole, Jesse	Blood serum-derived extracellular vesicles as a potential peripheralization mechanism for Chronic Wasting Disease in White-tailed deer | C Mathiason	MIP
16	Davis, Kaleb	Mosquito tracking through larval barcoding | R Kading	MIP
17	Droeger, Alice	Glial inflammation in aging canines | J Moreno	ERHS
18	Erickson, Caitlin	Role of the CD40/Th40 inflammatory pathway in canine autoimmune diseases | T Webb	CS
19	Farrell, Kristen	Omacetaxine reduces c-MYC expression and demonstrates antitumor effects in osteosarcoma | D Thamm	CS
20	Gallagher, Brooke	The use of cannabidiol as adjunct therapy for refractory idiopathic epilepsy in 51 dogs: a double-blinded crossover design | S McGrath	CS
21	Garrick, Amanda	Comparison of C-reactive protein measurement on three assays and following storage | M Lappin	CS
22	Gasparini, Molly	Retrospective investigation of the correlation between clinical cause of death and necropsy cause of death due to malignant neoplasia in a tertiary academic hospital population | K Vickery	CS
23	Geer, Charlize	Peripheral high-dose *Mycobacterium tuberculosis* infection induces neuroinflammation and reduces the integrity of the blood-brain barrier | J Moreno	ERHS
24	Hafey, Alexandra	The Effects of Prostatic Extracellular Vesicles on Canine Frozen-Thawed Semen Function and Viability | F Hollinshead	BMS
25	Haines, Laurel	Osteosarcoma exosome priming of alveolar macrophages promotes formation of a pre-metastatic niche | D Regan	MIP
26	Hall, Jack	Leishmania major inactivation using riboflavin and ultraviolet light | I Ragan	BMS
27	Hart, Cullen	Local immune responses to radiation therapy and myeloid cell-targeted therapy in naturally occurring canine sinonasal carcinoma | K Boss	ERHS
28	Hess, Hannah	Effects of long-term cannabidiol administration in dogs | S McGrath	CS
29	Hessock, Emma	Modulation of embryonic oxidative stress response to mitigate oxidative damage during the preimplantation period and beyond | D Tesfaye	BMS
30	Hogan, Parker	Effects of short and long term frozen storage of recombinant hamster prion protein in real time quaking induced conversion assay | C Mathiason	MIP
31	Hunt, Dakota	A New Way to Think about Recovery | S DiCecco	Other

No.	Presenter	Title | Mentor	Dept.
32	Isdale, Rachael	Incidence of suspected blood transfusion reactions in the Veterinary Teaching Hospital | M Lappin	CS
33	Jauch, Linzy	Nonspecific stimulation of mucosal immunity and protection against severe COVID-19 disease in hamsters | R Bowen	BMS
34	Jones, Katie	Investigation of four methods to identify infectious ulcerative keratitis in horses; standard microbiology culture, real-time polymerase chain reaction (RT-PCR), next-generation DNA sequencing (NGDS), and corneal cytology | M de Linde Henriksen	CS
35	Kern-Allely, Caroline	Waste not want not: Piloting a clinical waste audit at a university veterinary teaching hospital | C Duncan	MIP
36	Kloser, Heidi	Connection: Osteoarthritis and Tuberculosis | K Santangelo	MIP
37	Laurence, Hannah	Sphingolipid biosynthesis pathways are altered during flavivirus infection | R Perera	MIP
38	Lian, Elena	The functional impact of ubiA mutations associated with chronic *Mycobacterium abscessus* infections – implications for host adaptation | M Jackson	MIP
39	Lott, Linda	Implantation and early placentation in the mare: the role of kisspeptins in trophoblast invasion | C Magee	BMS
40	Lowe, Diana	Emergent prion disease in Swedish moose causes remarkably rapid disease in mice | G Telling	MIP
41	Lugo, Emily	Improving the reproductive success in the Black-Footed Ferret using new minimally invasive Assisted Reproductive Techniques | B Wolfe	CS
42	Martens, Elise	A Retrospective Review of Chemotherapy-Related Extravasation Events in Dogs and Cats | K Vickery	CS
43	Martin, Brittany	Characterization of Retroviral Elements in Jamaican Fruit Bats | T Schountz	MIP
44	Mayer, Treana	Don't cry wolf on Echinococcosis: retrospective analysis of a neglected zoonosis in US patients with regional canine spillover risk | S VandeWoude	MIP
45	Melgoza, Cynthia	Evaluation of the nutritional content in senior vs adult dog food diets | C Torres-Henderson	Other
46	Mulligan, Lisa	The potential role of behavioral testing in the diagnosis of canine cognitive dysfunction | S McGrath	CS
47	Natter, Nicole	Longitudinal SARS CoV-2 identification in human stool and relationships with systemic immune activation markers | E Ryan	ERHS
48	Nelson, Alysa	Triple Drug Immunotherapy for Treatment of Metastatic Osteosarcoma: Understanding Immune Mechanisms of Action | S Dow	MIP

No.	Presenter	Title | Mentor	Dept.
49	Neuheardt, Margaret	CBD protects UV stress in a skn-1 dependent pathway in C. elegans | J Moreno	ERHS
50	Orozco, Paloma	Examination of *in vitro* Proliferation Properties of Bone Marrow Derived Mesenchymal Stem Cells Harvested from Osteoporotic Sheep | J Easley	BMS
51	Padayachee, Brittany	Comparison of mercury concentrations in grey seals (*Halichoerus grypus*) from two United Kingdom colonies | L Rea	Other
52	Parker, Ashley	Retrospective evaluation of large granular lymphocyte (LGL) lymphoma in dogs | J Burton	CS
53	Patlin, Brielle	Nanocrystals as a potential drug delivery mechanism for intranasal vaccination | S Tobet	BMS
54	Pinto, Samantha	Role of Triacylglycerol during dengue virus replication in *Aedes aegypti* | R Perera	MIP
55	Read, Margaret	Biofeedback Application to a Military Training Task | B Clegg	Other
56	Rubio, Hennio	Evaluating Zika viral proteins capacity for CXCL10 induction in human placental cells | C Mathiason	MIP
57	Scherner, Samantha	RT-QuIC detection of amyloid seeds in brain tissues harvested from aged canines | C Mathiason	MIP
58	Schumaker, Mason	Colostrum Depletion in the Post-partum Mare | P McCue	BMS
59	Sheng, Julietta	Not All Models of Maternal Stress Are Created Equal | S Tobet	BMS
60	Shirley, Payton	Targeting neuroinflammatory pathways using Nanoligomers™ reduces glial inflammation and protects the brain from spongiosis and neuronal loss in prion disease | J Moreno	ERHS
61	Silvey, Justin	Investigation of NCAM1 as a potential biomarker of feline infectious peritonitis | G Dean	MIP
62	Slaughter, Megan	Proprietary probiotic for lessening pruritus in atopic dogs | M Lappin	CS
63	Snow, Aimee	Investigating Transtyretin's presents within immortalized ovine trophoblast cell lines | Q Winger	BMS
64	Snyder, Taylor	Alterations in the ocular surface immunome and microbiome in horses with corneolimbal squamous cell carcinoma | K Wotman	CS
65	Sparrer, McKenzie	Surveillance of companion and exotic animals for SARS-CoV-2 and evaluating transmission potential within veterinary medicine | C Mathiason	MIP

No.	Presenter	Title | Mentor	Dept.
66	Sun, Julianna	A single residue of the prion protein controls disease pathogenesis in deer and elk | G Telling	MIP
67	Swiderski, Maya	Effect of alpha-casozepine supplementation on stress-associated findings in cats | M Lappin	CS
68	Templeton, Hayley	Intestinal Neuroimmune Involvement in Parkinson's Disease Development | S Tobet	BMS
69	Thomas, Brodie	The Effect of Water Bowl Material and Volume on Downstream Biofilm Formation in Canines | T Kinkel	MIP
70	Thometz, Mary Jo	Epidemiology of canine B cell leukemia and lymphoma | A Avery	MIP
71	Threatt, Alissa	Omega-3 fatty acid metabolite Resolvin D1 regulates the pulmonary immune response to agricultural dust | T Nordgren	ERHS
72	Thumarat Phabpim	The prevalence of increased serum cobalamin in cats with disease | C Torres-Henderson	CS
73	Tipton, Madison	Serum metabolome and proteome reveals small molecules and metabolic pathways associated with COVID-19 disease severity and risk of development of post-acute sequalae of COVID-19 | E Ryan	ERHS
74	Trujillo, Courtney	Educational Healing Gardens: Intersection of Human, Animal, and Environmental Health at Veterinary Medical Facilities | C Duncan	MIP
75	Tyer, Leo	Exploratory use of RT-QuIC to quantify and compare kinetics of amyloid formation in North American and Scandinavian CWD prions | G Telling	MIP
76	Uzan, Olivia	Placement characteristics and flow rates of a novel manual intraosseous device versus an automatic device in canine and feline cadavers | L Guieu	MIP
77	Venator, Camden	Detection of gastrointestinal parasites in fecal samples from dogs attending dog-parks in New York State | M Lappin	CS
78	Vise, Alina	Using a novel high-volume throughput process to establish optimal energy dose for Zika virus inactivation | I Ragan	BMS
79	Wilford, Megan	Effects of dietary omega-3 and -6 polyunsaturated fatty acids on muscle mitochondrial function in old mares | E Carnevale	BMS
80	Lonergan, Jacob	Correlations between DTI and NODDI parameters in the healthy aging white matter | A Burzynskag	Other

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O-1. Peripheral *Mycobacterium tuberculosis* Infection Causes Neurodegeneration and Memory Loss in Guinea Pigs.

Isla Anderson, Amanda Latham, Charlize Geer, David F Ackart, Amelia Day Hines, Randall J Basaraba, Julie A Moreno

Tuberculosis (TB), a bacterial infection caused by *Mycobacterium tuberculosis* (Mtb), infects approximately ten million people each year. Primarily a disease of the lungs, TB induces a robust peripheral immune response of cytokine producing macrophages and T cells. Cross-sectional studies have shown associations between TB and an increased risk for neurodegenerative diseases, including Parkinson's Disease (PD) and dementia. TB patients co-infected with HIV also experience accelerated cognitive decline compared to infection with HIV alone. These correlations exist without diagnoses of central nervous system (CNS) Mtb infection or tuberculosis meningitis (TBM), where infection progresses to the brain causing inflamed meninges.To better understand the CNS effects of peripheral TB disease, we used guinea pigs, a pathologically relevant model to human infection. Guinea pigs were then infected with Mtb by aerosol exposure. Through use of behavior testing and immunohistochemical staining, animals with systemic Mtb infection show impaired cognitive functioning and markers of neurodegeneration compared to uninfected controls. Guinea pigs 60- and 90-days post infection demonstrate non-spatial memory loss and anxiety-like behaviors indicative of declined cognition. Neuropathological analysis, including quantification of neurons and H&E staining, also shows death and degradation of neurons in the hippocampus. Interestingly, these results occur without any detectable bacteria in the brains of these animals. This helps us establish a correlation between peripheral Mtb infection and damage to the CNS. These findings will enhance our ability to prevent human patients with TB from experiencing permanent deficiencies, as well as deepen our understanding of how the peripheral immune response affects the brain and contributes to neurological disorders.

Undergraduate Student/ Environmental and Radiological Health Sciences

O-2. Use of Etorphine for Immobilization of Giraffe for Snare Removal: A Retrospective Study.

Summer Barnes, Joshua Lubega, Khursheed Mama, Sangeeta Rao, Sara Ferguson, Miranda J Sadar

The Nubian giraffe (*Giraffa camelopardalis camelopardalis*) found in focal areas across central and western Africa is classified as critically endangered. A critical element for their long-term survival is the use of immobilization to facilitate translocation and care. However, a historically high incidence of reported complications, including mortality, suggests a need for refinement of immobilization techniques. This retrospective study utilized immobilization data acquired during snare removals from 80 giraffes in Murchison Falls National Park. Animals were immobilized with etorphine hydrochloride, with adults on average receiving a dose of 12.4mg and subadults receiving a dose of 11.6mg and were subsequently reversed with either naltrexone or diprenorphine. Naltrexone doses ranged from 50-200mg and diprenorphine from 14-26mg. Data included age (adult, subadult), sex, estimated weight, body condition score, induction drug dose, reversal drug dose, induction time, procedure time, time to reversal administration, quality of induction, and snare wound characteristics. Through chi-square, Fisher's exact, and Mann-Whitney tests it was determined that there were no statistically significant differences between males and females, for induction quality ($p>0.99$) and time ($p=0.72$) and procedure time ($p=0.18$). No significant differences were noted between adults and subadults for induction quality ($p=0.16$) and procedure time ($p=0.35$). However, there was a significant difference in induction time for adults and subadults ($p<0.0001$). Similarly, wound severity did not have a significant impact on induction quality. These results support that at the dosages reported, a purely etorphine-based protocol may be suitable for short term immobilization for minimally invasive procedures in giraffe. This may assist organizations working towards the conservation of these animals by minimizing complications such as mortality when immobilization is necessary.

DVM Student/ Clinical Sciences

O-3. CD206 activation results in growth of canine histiocytic sarcoma cell lines.

Rachel V Brady, Kristen B Farrell, Douglas H Thamm

Small molecule NCGC00413972 (referred to as 972) is a CD206 agonist that was recently developed to deplete tumor-associated macrophages. The purpose of this project was to assess the effects of 972 on canine histiocytic sarcoma (HS) cell lines, a cancer derived largely from dendritic cells, which share a common precursor with macrophages. It was hypothesized that 972 would inhibit growth on HS cell lines similar to its known effect on primary macrophages. Previous experiments demonstrated a half maximal inhibitory concentration (IC50) of 200 nmol. A range of 972 concentrations encompassing the IC50 (0 – 2000 µmol) were incubated under a variety of conditions with three HS cell lines (DH82, MH588 and Nike) as well as one negative control osteosarcoma cell line (D17) that did not express CD206 based on RNA-sequencing data. Using complete media or tumor-conditioned media, 72 hours of incubation stimulated growth with increasing concentrations of 972 in all HS cell lines but not in D17 as assessed by measuring cell viability via resazurin fluorescence. The average growth stimulation was roughly 160% of a DSMO-control for all HS cell lines incubated with the highest concentration of 972. Under stressed growth conditions, including anchorage-independent and serum-starved growth, cell viability was largely stable in all cell lines. Under no conditions did 972 inhibit growth. In conclusion, activation of CD206 stimulates growth of HS cell lines under normal growth conditions. Future experiments will explore the potential mechanisms behind this phenomenon, specifically investigating NF-κB activation.

Graduate Student/ Clinical Science

O-4. Interferon-tau protects the corpus luteum from lysis by prostaglandin F2-alpha.

Hana E Braun, Jessica R Wittenstein, Aydin Guzeloglu, Jeanette V Bishop, Thomas R Hansen

Purpose: It was hypothesized that subcutaneous delivery of recombinant ovine interferon-tau (roIFNT) would protect the corpus luteum (CL) from exogenous challenge with prostaglandin F2-alpha (PGF). **Materials/Methods:** On Day 10 of the estrous cycle, osmotic pumps loaded with bovine serum albumin (BSA, Control) or roIFNτ to deliver 20 µg/day were inserted subcutaneously into the neck near the jugular vein (0h, n=4-5 ewes/group) for 72 hours. After 24 hours of exposure to either BSA or roIFNτ, a single dose of PGF (4mg/58 kg bw) was injected. Blood serum was collected for determination of progesterone (P4) concentrations twice daily for 72 hours. CL were collected from ewes at 24, 48, and 72h after the pump insertion to test the ability of roIFNT to block lysis of the CL by exogenous PGF. **Results:** In BSA-infused controls, serum progesterone concentrations declined ($P<0.05$) within 48 h after PGF2 injection. In roIFNT-treated ewes, serum progesterone concentrations remained high ($P<0.05$) and were not affected by PGF. Interferon stimulated gene (i.e., *ISG15*) mRNA was not detected in BSA-infused controls. However, ISG15 was detected in CL by 48 h and remained at high ($P<0.05$) concentrations through 72 h following the start of roIFNT infusions. *LHCGR*, *TNFaIP6*, *TGFβ2* mRNA levels were greater ($P<0.05$) in the CL from roIFNT- compared to the BSA-infused ewes, indicating maintenance of these luteotropic mechanisms**.** Doses of roIFNT in osmotic pumps with catheters directly into the center of the CL have been identified that stimulate ISGs in the CL, without action on the endometrium. These ongoing studies will test direct action of IFNT on the CL to see if it is protected from PGF-induced luteolysis, without also acting on the endometrium. **Conclusions:** Systemic delivery of 20 µg/day of roIFNτ subcutaneously protects the CL from exogenous PGF challenge through induction of ISGs and maintaining luteotrophic gene expression.

Graduate Student/ Biomedical Sciences

O-5. Localization of a probiotic *Lactobacillus acidophilus* rotavirus vaccine within the host.

McKenzie L Fletcher, Sophie M Kiehl, Allison C Vilander, Gregg A Dean

Rotavirus causes a severe diarrheal illness responsible for the deaths of over 215,000 children worldwide every year. Due to safety concerns and limited effectiveness of existing live attenuated rotavirus vaccines in lower income countries, a new type of vaccine is needed. Our lab engineered the probiotic bacterium, *Lactobacillus acidophilus*, as a next generation rotavirus vaccine. This recombinant *Lactobacillus acidophilus* (rLA) expresses the VP8 rotavirus capsid protein and two proven adjuvants—the *Salmonella typhimurium* flagellin protein (FliC) and the *Escherichia coli* fimbrial protein (FimH). FliC acts as a TLR5 agonist while FimH serves as an M-cell targeting protein and TLR4 agonist. To better understand the interactions between this live vaccine and the host, we sought to 1) temporally characterize rLA passage through the gastrointestinal (GI) tract and localization within immune induction sites and 2) determine the extent adjuvant/antigen expression over time and location. We tracked the migration of rLA 6 hours post oral administration in mice. The GI tract, Peyer's patches, mesenteric lymph nodes, and spleen were collected. Immunofluorescence staining and spectral microscopy were employed to visualize rLA and proteins expressed by rLA. The images generated from microscopy showed rLA throughout the extent of the GI tract (stomach to colon) but no penetration of the epithelium into immune inductive sites. Next, tissues will be collected 6, 12, 18, 24, and 48 hours as well as 1 and 2 weeks post oral administration of rLA to capture when rLA reaches immune inductive sites, interacts with immune cells to trigger a response, and is ultimately excreted from the host. The results of this project will contribute to a larger understanding of appropriate immunization schedules with this vaccine and the use of probiotic vaccine platforms in general.

Graduate Student/ Microbiology, Immunology and Pathology

O-6. The cultivation and characterization of soil bacterium in Navajo Nation to identify the natural soil biome.

Nizhoni A Hatch, Phillida A Charley

From the 1940s to the 1980s, the demand for nuclear weapons during World War II caused the extraction of nearly 30 million tons of uranium ore from Navajo Nation lands. The Navajo Nation is still dealing with the lasting effects of uranium mining and remediation. Currently, the mines are closed, but over 500 abandoned uranium mines are in different stages of remediation or reclamation. Since the 1990s, the EPA has been closing mine portals and replacing contaminated soil with non-radioactive soil. Little research has been done on the impact of remediation on the soil biome, which is crucial for plant, wildlife, environmental, and human health. To understand how the remediation process affects the natural soil biome, we are analyzing the bacteria in samples from disturbed areas such as uranium mines in the Sweetwater area of northeastern Arizona in the Navajo Nation and comparing them to an undisturbed control area. Presently, we are characterizing the control bacteria by identifying anti-bacterial/anti-fungal/quorum quenching/sensing capabilities in preparation for comparison to bacteria from disturbed areas. The control group had nine bacteria isolates with anti-bacteria activity, four bacteria isolates with anti-fungal activity, four bacteria isolates with quorum sensing, and 15 bacteria isolates with quorum quenching activity. This research is essential to understanding the impact of uranium contamination on Navajo Nation soil and provides crucial information for the remediation process.

Undergraduate Student/ Microbiology, Immunology and Pathology

O-7. Evaluation of ketamine-midazolam in combination with either medetomidine or dexmedetomidine in Bennett's wallabies (*Notamacropus rufogriseus*) under human care.

Anne Marie Kerley, Miranda J Sadar, Jon Romano, Sangeeta Rao, Khursheed Mama

Many non-traditional species under human care require sedation or anesthesia for medical treatments for their own wellbeing, as well as that of their human caretakers. In numerous states it is legal to own Bennett's wallabies (*Notamacropus rufogriseus*) as pets, which adds to the necessity of finding safe anesthetic protocols in this species, as manual restraint can be stressful and dangerous to both the animal and the handler. It will also help veterinarians to better care for wallabies, both as pets and for individuals in zoological collections. This retrospective study aims to draw conclusions regarding the current protocols of the drug combinations of ketamine-medetomidine-midazolam and ketamine-dexmedetomidine-midazolam and their dosages, as well as their corresponding adverse effects. 113 anesthetic records between June 1, 2015 and April 15, 2022 from 15 Bennett's wallabies housed in a zoological facility were evaluated. Reasons for anesthetic events included, but are not limited to, dental procedures, emergencies, and pre-shipment exams. Of the 113 records, during 63 events, the animal was administered ketamine-medetomidine-midazolam and during 25 events, the animal was administered ketamine-dexmedetomidine-midazolam. Quality of induction/recovery is subjectively described as good, fair, and poor. In preliminary assessment of the data, in anesthetic events where animals given medetomidine, 6% of events were subjectively described as fair, with doses ranging from 0.03mg/kg to 0.45mg/kg. In anesthetic events where animals were given dexmedetomidine, 4% of events were subjectively described as fair, with the dose administered being 0.01mg/kg. Based on preliminary assessment of the data, the author determines that while true anesthetic plane is not reached, these protocols may sedate Bennett's wallabies enough to approach them safely to intubate or mask for isoflurane gas anesthesia. Forthcoming statistical analysis will further analyze these findings..

DVM Student/ Clinical Sciences

O-8. Integron-encoded antimicrobial resistance and virulence determinants among *Salmonella enterica* serovar Typhimurium isolated from poultry.

Elizabeth Kim, Nora J Nealon, Cydney Jardine, Roberta Magnuson, Joy Scaria, Sangeeta Rao

Antimicrobial resistance (AMR) is an escalating global public health concern in *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*), which is an enteric pathogen known for its broad range of hosts, including food-producing animals and poultry. In tackling issues regarding AMR in *S. Typhimurium*, previous studies indicated that integron-encoded *S. Typhimurium* contains increased numbers and diversity of AMR genes; however, little is known about the association of virulence factor genes with integron-encoded *S. enterica*. Thus, the aim of this study is to understand the interrelationship between the presence of integrons, AMR genes, and virulence factor genes among *S. Typhimurium* utilizing whole genome sequencing (WGS) methods. For this study, poultry isolates (n=26) were sourced from veterinary diagnostic laboratories at University of Pennsylvania and Colorado State University. Three of the 26 isolates were initially found to contain integrons, each with a length of 1000 basepairs. Genetic data extracted via WGS were then used to construct heat maps and graphs to conduct descriptive analysis. The results show that the percentage of isolates with integrons have resistance to certain drugs such as beta-lactams, phenicols, and tetracyclines (100%, 66.7%, and 100% respectively) more than those with no integrons (8.7%, 0%, and 21.7% respectively). On the other hand, virulence factor profile was similar among most isolates. *Bacteriocin* was the only notable gene class with more virulence factor genes present in isolates with integrons than those with no integrons. Furthermore, both *AMR* and *virulence factor* genes were found to be more localized on chromosomes than on plasmids regardless of integron presence. Overall, these results can be utilized to help better predict the pathogenicity of *S. Typhimurium* and potentially develop antimicrobial therapies targeted against these virulence mechanisms, which would result in preventing the further spread of disease caused by this AMR pathogen.

DVM Student/ Clinical Sciences

O-9. Exploring the Fecal Microbiome of Mice Vaccinated Against the Rotavirus Using Python.

Joy Love, Zaid Abdo, Reed Woyda

Rotavirus is the most common cause for dysentery in young children and the leading cause of mortality due to diarrhea and dehydration. Unfortunately, the vaccine for Rotavirus is less effective in developing countries, although these countries are more heavily affected. Over 85% of deaths from Rotavirus dysentery occur in developing countries but the vaccines reduce severe disease by 30-60%. This is compared to a 90% decrease in other settings. To create a more effective vaccine, it is important to understand the biological response for potential vaccines. Specifically, the vaccines impact on the host microbiome. This study used a vaccine developed by the Dean lab at Colorado State University, which used a recombinant *Lactobacillus acidophilus* as a rotavirus oral vaccine vector to assess immune and microbiome responses in mice. The fecal contents of two male mice were taken before and after they received the vaccine. DNA was extracted from the fecal samples, quantified, and then sequenced. From there, Metagenomic analysis was performed using a computation pipeline to determine if the difference was significant. We hypothesize that there will be no significant difference between each male at the two timepoints, but there will be a significant difference in the microbiome before and after the mice receive the vaccine. A secondary goal to this study was to increase the robustness of the computational pipeline used in this experiment.

Undergraduate Student/ Microbiology, Immunology, and Pathology

O-10. Transcutaneous identification of implanted microchips as a method of tracking equine large colon migration.

Hannah McKee, Sara KT Steward, Allison Watson, Mo Salman, Diana M Hassel

Colic due to large colon displacement is one of the leading causes for equine hospitalization and surgery, yet there is not an adequate model to study the pathophysiology of this condition. The objective of this proof-of-concept study was to determine if subserosal implantation of bioinert microchips in the large intestine would be detectable by a RFID (radio-frequency identification) receiver when the implanted microchips were adjacent to the body wall, thus identifying the location of the colon within the abdomen. The goal is to develop a model that can be used in future studies to monitor colonic movement in relation to various stimuli. A horse with no history of gastrointestinal disease underwent a ventral midline celiotomy to implant twelve bioinert microchips into the subserosa at predetermined locations within the large colon and cecum. A RFID scanner was used to monitor the location of the colon via transcutaneous identification 1-3 times daily for a one-month period. Following humane euthanasia, postmortem examination of the horse was performed to assess microchip implantation sites for migration and histologic assessment. Eleven out of the twelve implanted microchips were successfully identified transcutaneously at occurrences as high as 100%. Odds ratios were calculated for the likelihood of identifying each chip in a location different from its most common location. Microchips with both high identification frequency and high odds ratios were associated with locations that are thought to be more mobile within the abdomen, whereas microchips that had high identification frequency but low odds ratios were associated with locations that are expected to be less mobile within the abdomen. Microchips implanted into the subserosa of the equine large colon can be used as a means of identifying the approximate location of the equine large colon via transcutaneous identification by a RFID scanner.

DVM Student/ Clinical Sciences

O-11. The effect of various perineural analgesia techniques of interleukin-1β-induced synovitis of the equine metacarpophalangeal joint.

Hannah Patterson, Alicia Yocom, Katie Seabaugh, Christopher Kawcak, Erin Contino

Perineural analgesia is an important tool in diagnosing equine lameness. This study compared the ability of four distal limb diagnostic nerve blocks to resolve synovitis-induced lameness of the equine metacarpophalangeal joint (MCPJ). Eight horses had one randomly-selected MCPJ injected with recombinant equine interleukin-1β (reIL-1β) to induce lameness. Half of the horses received palmar digital (PD) followed by abaxial sesamoidean nerve blocks, while the other half received palmar metacarpal followed by palmar nerve blocks. Lameness was evaluated subjectively (AAEP Grade 0-5) and objectively at five and ten minutes following each block. After a one-week washout, the horses switched groups and lameness was induced in the opposite MCPJ. Statistical analysis was performed using a mixed model analysis. On average, following reIL-1β injection, horses were grade 3.2 lame. Mean lameness was not significantly decreased following PD or abaxial sesamoidean nerve blocks. Lameness significantly decreased five and ten minutes following palmar metacarpal (mean= grade 2.7 and grade 2.2; $p \leq 0.02$) and palmar (mean= grade 1.5 and grade 1.2; $p < 0.0001$) nerve blocks. Three horses had clinically-relevant levels of mepivacaine detectable in the MCPJ; all three were associated with the low 4-point nerve block. In horses with induced fetlock synovitis, blocking only the palmar metacarpal nerves decreased mean lameness by one full grade. As expected, lameness further decreased with addition of the palmar nerve blocks. On average, lameness grades did not decrease following PD or abaxial nerve blocks. However, some individual horses had notable reduction in lameness following these blocks, which could have important clinical implications.

DVM Student/ Clinical Sciences

O-12. Key players of membrane remodeling: Role of Phospholipase A2 in flavivirus infection of the human host.

Oshani C Ratnayake, Elena Lian, Camryn S Guenther, Paul S. Soma, Laura St Clair, Rebekah Gullberg, Angel Balmaseda, Jason M Mackenzie, Eva Harris, Rushika Perera

Dengue viruses (DENVs) are transmitted by *Aedes aegypti* mosquitoes causing over 400 million infections annually. Upon infection, these viruses hijack host lipid metabolic pathways to support their replication. Specifically, we and others have shown that *de novo* lipid biosynthesis is actively upregulated via viral protein NS3 to support the formation of replication factories on modified membranes. Interestingly, we have shown that metabolites arising from the opposing pathway, lipolysis, are also altered during infection of both human and mosquito hosts. In the human host, inhibition of lipolysis disrupted viral replication. Analysis of serum isolated from dengue patients also show an enrichment of lipolytic metabolites specifically in patients with severe disease. In the mosquito host, lipolytic metabolites were elevated during infection, prominently differentiating between infections with DENVs as well as Chikungunya, and Zika viruses. Cytosolic and secreted phospholipase A2 enzymes (PLA2s), releases arachidonic acid (AA) and lysophospholipids from cell membrane phospholipids. AA is a well-known precursor of anti-viral inflammatory mediators. The current study shows how reduced AA and lysophospholipids, through a knockdown of AA producing PLA2 isoforms (2A, 4A, and 4C) decreased DENV2 infectious viral titer. Further, the effect of exogenous AA addition on viral infection and rescue of infectious virus production in PLA2 knockdown cells were investigated. We hypothesize that PLA2 and AA may have dual roles during virus infection, both in supporting viral replication and creating the anti-viral inflammatory precursor molecules.

Graduate Student / Microbiology, Immunology, and Pathology

O-13. The Role of Niemann Pick Type C2 Genes During Ivermectin Blood Meal Response in Mosquito Plasmodium Vectors.

Molly Ring, Paula Lado, Brian Foy

The endectocide ivermectin (IVM) is being used as a novel vector-borne disease control strategy given its ability to kill mosquitoes and interfere with egg production in surviving mosquitoes. We are studying the administration of IVM-dosed bird feed as a method to curb the spread of West Nile Virus and mass drug administration of IVM to humans in West Africa to disrupt the spread of malaria. These strategies depend on drug pharmacokinetics in the treated host, and so mosquitoes invariably will ingest sublethal concentrations depending on when they bite a host relative to the last time they were treated. When mosquitoes ingest a blood meal containing sublethal IVM concentrations, more genes are upregulated than downregulated, and certain classes of upregulated genes predominate, but the roles of many of these IVM-responsive genes are not well understood. We have found that members of a specific gene family (Niemann Pick Type C2 family; NPC2) are the most highly upregulated following ingestion of an IVM-containing blood meal. NPC2 traffics cholesterol in vertebrates but may also serve as carriers for other semiochemicals and hydrophobic compounds in arthropods. We used qPCR to measure the transcript levels of two IVM-responsive NPC2 genes AGAP002848 and AGAP002847, and the bloodmeal-responsive NPC2 gene AGAP002851. Transcript levels will be detected in female *Anopheles gambiae* mosquitoes that have been given varying concentrations of IVM-dosed bloodmeals. In future experiments, we will clone these three transcripts to make dsRNA that can be used for RNAi knockdown assays via intrathoracic injections. We hypothesize that transcript knockdown of AGAP002848 and AGAP002847 will lead to increased mortality following digestion of an ivermectin spiked blood meal while knockdown of AGAP002851 transcript will lead to sterol transport disruption and ultimately egg production interference..

Undergraduate Student/ Microbiology, Immunology and Pathology

O-14. Non-toxic Nanoligomers™ targeting key neuroinflammatory pathways are neuroprotective in prion disease.

Sydney J Risen, Sean Boland, Sadhana Sharma, Grace Weisman, Amielia D Hines, Arielle JD Hay, Vincenzo Gilberto, Stephanie McGrath, Anushree Chatterjee, Prashant Nagpal, Julie A Moreno

Neuroinflammation is a key factor in the development of neurodegenerative diseases, including prion disease. There are currently no effective treatments to halt pathogenesis and progression, which includes accumulation of misfolded proteins, neuroinflammation, and cognitive/behavioral deficits followed by irreversible neuronal death. We hypothesized that downregulation of two key neuroinflammatory targets, NF-κB and NLRP3, would be neuroprotective. To test this, we utilized transcriptional inhibiting Nanoligomers™ in a prion-diseased mouse to assess the impact on glial inflammation, behavioral/cognition deficits, and loss of neurons. Established by PK/PD studies, Nanoligomers™ are systemic but nontoxic to mice, at doses up to 5% of body weight. Nanoligomer™ treated mice displayed decreased numbers of glia, and improved behavior and cognitive tests. Critically, the Nanoligomer™ protected the brain from prion-induced spongiotic change, neuronal loss, and significantly increased life span, indicating that Nanoligomer™ inhibition of inflammatory pathways can prevent neuronal death and slow the progression of neurodegenerative diseases.

Graduate Student/ Environmental and Radiological Health Sciences

O-15. Toll-Like Receptor Activation of Mesenchymal Stromal Cells for Improved Cellular Treatment of Osteoarthritis.

Meagan Rockow, Steven Dow, Lyndah Chow, Renata Impastato, Meghan Webster, Ariel Timkovich, Kelly Santangelo, Dean Hendrickson, Lynn Pezzanite

Osteoarthritis (OA) represents one of the most common disorders in humans and animals. Despite high prevalence of the disease, there remains a lack of effective treatment options to reduce pain and improve quality of life without inducing adverse effects. Cellular therapies such as mesenchymal stromal cells (MSCs) are increasingly popular to treat OA but remain controversial due to lack of rigor in study design and consistency in product composition. In the presence of inflammation, as in OA, MSCs upregulate toll-like receptors (TLR) on their cell surface. Therefore, we hypothesize that preactivation of MSCs with TLR ligands will improve their effectiveness to treat OA. Osteoarthritis was induced in mice via surgical destabilization of the medial meniscus. Mice were injected in the affected joint at weeks 3 and 5 postoperatively with one of three treatments (needle insertion alone, MSCs, or TLR-activated MSCs) and monitored until week 8. Outcome parameters assessed were mouse mobility evaluated by motion activated camera (AnyMaze®), histopathology of joint tissues graded via established osteoarthritis scoring systems, and gene sequencing of formalin fixed joint tissues using Nanostring technology. To further assess mechanistically the effect of TLR activation on MSC, RNA sequencing of MSC and TLR-activated MSC from mice will be performed. Preliminary analysis of gait data indicates mice treated with TLR-MSCs traveled further total distances, had greater total time mobile, and traveled at greater mean speeds compared to those receiving needle insertion alone at week 8 postoperatively. Collectively, these findings support further evaluation of TLR-activated cellular therapies for treatment of OA in both humans and animals.

DVM Student/ Clinical Sciences

O-16. Investigating the levels of LHCGR in extracellular vesicles and its relationship with ovarian follicle development in bovine.

Luca A Souza, Nico G Menjivar, Ahmed Gad, Juliano C da Silveira, Dawit Tesfaye

Ovarian dysfunction is a major cause of reproductive inefficiencies in dairy herds leading to delayed ovulation and lowered conception rates. The prevalence of ovarian dysfunction may range from 10% to 50% of cows across herds. It has been reported that dominant follicles failing to ovulate have decreased production of estradiol with diminishing numbers of LH receptors (LHr). Therefore, it's important to understand the multifactorial process by which granulosa cells (GCs) acquire the LHr, ultimately leading to follicular dominance needed to induce ovulation. Here we aimed to identify the role of extracellular vesicles (EVs) derived from small and large follicles in propagating the LHr acquisition of GCs. For this, we evaluated the expression levels of *LHCGR*, involved with LHr acquisition, in EVs from follicular fluid (FF) and in GCs from small (3-6 mm in diameter) and large (8-14 mm) bovine follicles. Initially, FF samples were submitted to a series of centrifugations and ultracentrifugation to isolate the FF-EVs. Statistical analyses were performed using an unpaired t-test (significance level of 5%) with each experimental group having 4 biological replicates (>200 follicles each). Using *GAPDH* and *RPL15* as housekeeping genes, RTq-PCR indicated *LHCGR* was higher ($p = 0.0174$) in GCs from large follicles than from small follicles. Inversely, *LHCGR* was found in higher amounts ($p = 0.0321$) in the EVs from small follicles than from large follicles. In conclusion, these results indicate that during the initial stages of follicular development, EVs are secreted with copious amounts of *LHCGR* in order to induce the LHr acquisition in GCs to promote follicular dominance aiding to the potential successful ovulation.

DVM Student/ Biomedical Sciences

O-17. Prognostic value of rectal temperature, packed cell volume, and blood glucose at hospital admission in client-owned ferrets.

Hayley S Stratton, Sangeeta Rao, Miranda Sadar

The objective of this study was to determine whether rectal temperature, packed cell volume (PCV), or blood glucose at presentation were associated with all-cause mortality in client-owned ferrets. A medical record database was searched for ferrets from 8/1/2019 through 9/1/2022. Records from 414 individual examinations were evaluated. Inclusion criteria were rectal temperature, PCV, and/or blood glucose measured at presentation and data on survival status 7 days post-presentation. Data were included from 144 ferrets with 247 individual examinations. Rectal temperature in 130 ferrets from 192 examinations, PCV in 73 ferrets from 109 examinations, and blood glucose in 110 ferrets from 168 examinations were available. The percentage of ferrets that died within 7 days of presentation was higher with temperature derangements compared to normothermic animals, with mortalities of 51.5% (35/68) with hypothermia (<100°F) and 40.0% (2/5) with hyperthermia (>104°F) compared to 16.0% (19/119) of normothermic (100-104°F) individuals. For PCV, mortality was higher for anemic ferrets compared to those with a normal PCV, with mortalities of 60.0% (3/5) for anemia (<33%) compared to 20.5% (15/73) of individuals with a normal PCV (33-57%). For blood glucose, mortality was higher for hyperglycemic ferrets compared to normoglycemic, with mortalities of 52.6% (10/19) with hyperglycemia (>152mg/dL) compared to 22.1% (21/95) of normoglycemic (75-152mg/dL) individuals. In conclusion, derangements in rectal temperature, anemia, and hyperglycemia may be predictors of mortality in client-owned ferrets. Due to its potential association with mortality and ease of measurement, a rectal temperature should be measured during physical examinations of ferrets.

Resident/ Clinical Science

O-18. Evaluation of Nanodiamonds for the Adsorption of Radioactivity from Ocean Waters.

Megan Zaiger, Ralf Sudowe

The determination of low levels of radioactivity in the environment is challenging, because the sample amount required to meet low minimum detection limits can be difficult to manage and can give rise to self-shielding which may lead to inaccurate measurements. In this work, the use of a hexacyanoferrate(II) nanodiamond adsorbent is investigated to preconcentrate dissolved radiocesium in contaminated waters in order to improve the counting efficiency and accuracy for low activity samples. The adsorption of various radioisotopes on nanocarbon materials from aqueous solutions has been studied previously; however, low adsorption rates together with a strong dependence on pH and temperature have limited their use. Detonation Nanodiamonds (ND) are a new technological approach to the use of carbon-based nanomaterials for adsorption-based separations. They have a much higher adsorption rate of 98% and are not strongly affected by changes in pH or temperature. NDs were originally created by detonating explosives in a closed chamber under reduced conditions; however, can now be synthesized in a lab. They have a high biocompatibility, high surface area, radiation resistance, low weight, and are less toxic than other nanocarbon based materials; therefore, have attracted great interest for radiochemical research. Past studies saw a smaller absorption rate for very low levels of radiocesium, such as 0.01 Bq/L. However, this may be caused by the radioactive cesium adhering to the container. To address this issue, future research will be conducted by adding stable cesium to the solution as well as radioactive cesium. The non-radioactive cesium should adhere to the container and allow the radioactive cesium to bind to the nanodiamonds and increase the absorption rate. Finally, the uptake of radiocesium on coated nanodiamonds from a variety of water samples, including synthetic and real ocean waters will be studied to determine absorption rates in the presence of other competing ions.

Graduate Student/ Envirounmental and Radiological Health Sciences

O-19. Characterizing the effect of coinfection ratios on bluetongue virus reassortment in *Culicoides sonorensis*.

Molly J Carpenter, Jennifer H Kopanke, Case Rogers, Justin S Lee, Barbara Graham, Mark Stenglein, Christie E Mayo

Bluetongue virus (BTV) is a segmented, double-stranded RNA virus transmitted by *Culicoides* biting midges that can result in devastating disease in susceptible ruminants. Reassortment between BTV strains may enhance its ability to spread to new regions. In prior in vitro BTV coinfection studies, progeny genotypes were dominated by the parental strain with the higher initial dose. Our study evaluated *in vivo* reassortment of progeny virus in *Culicoides sonorensis* midges coinfected with different ratios of BTV-10 and BTV-17. Midges were fed blood containing BTV-10, BTV-17, or a combination of BTV-10: BTV-17 at 90:10, 75:25, 50:50, 25:75, or 10:90 ratios. Midges were collected every other day for pan BTV and COX (housekeeping gene) qRT-PCR. A curve was fit to the deltaCt values (pan BTV Ct - COX Ct) for each ratio group and linear portions evaluated by pairwise comparisons with *P* values adjusted with Tukey's method. On day 10, midges were processed for BTV plaque-isolation. Genotypes of plaques were determined by next generation sequencing. Comparison of linear portions of deltaCt curves demonstrated no differences between ratio treatment groups. Plaque genotyping indicated that most plaques fully aligned with one of the parental strains. However, there was reassortment evident in a pool of midges coinfected with BTV-10: BTV-17 ratio of 75:25. Thus, reassortment within the midge may be an infrequent event, but reassortants may have an advantage over the parental strains and overtake parental strain populations. Bluetongue virus reassortment patterns and resulting biological consequences will add an important dimension to the understanding of viral emergence and evolution.

Graduate Student/ Microbiology, Immunology and Pathology

O-20. MERS-CoV passaged on Jamaican fruit bat cells leads to mutations in Orf5 gene.

Phillida A Charley, Kira W Douglas, Tony Schountz

Middle East respiratory syndrome coronavirus (MERS-CoV) is a merbecovirus (genus *Betacoronavirus*, subgenus *Merbecovirus*) that causes Middle East respiratory syndrome in humans. MERS first emerged in 2012 in Saudi Arabia. Recently, dromedary camels have been shown to be a secondary reservoir host, but its ancestral virus likely originated in bats. Bats are believed to be another primary reservoir because they host many merbecoviruses. Furthermore, thousands of coronavirus sequences have been identified in bat species spanning Asia, Africa, the Americas, and Europe. However, there are no data on the interactions between reservoir bats and MERS-CoV, although experimental infection of Jamaican fruit bats (*Artibeus jamaicensis)* found limited viral infection without visible signs of disease. Previous studies have used human cells or mouse models to study this virus but do not give insight into a natural reservoir interaction. This study aims to determine if MERS-CoV can be adapted for more efficient infection in the Jamaican fruit bat model. We have determined Jamaican fruit bat kidney (AJK) cell lines are susceptible to MERS-CoV. MERS-CoV causes more cytopathic effects after ten viral passages on our AJK cells. Additionally, in the MERS-CoV passage ten virus had the most mutations occur in the Orf5 gene and it had eight nonsynonymous substitutions. Overall, we show MERS-CoV ability to adapt to a New World bat species.

Post-doctoral Fellow/ Microbiology, Immunology and Pathology

O-21. Diverse prion strains result from selective propagation of distinct quasispecies conformations in different host compartments.

Joseph DeFranco, Sehun Kim, Jifeng Bian, Jenna Crowell, Julie Sun, Glenn Telling

Chronic wasting disease (CWD) is a rapidly spreading, uncontrolled prion disease in wild and captive cervids in North America, Europe, and Asia. This pathogen transmits through bodily fluids, shedding skin during infection, is invariably fatal, and has incomparable, robust environmental persistence. Although it is assumed that the primary mode of natural CWD is likely horizontal transmission, either through direct contraction between cervids or indirect infection via contaminated fomites in the environment, there is still limited understanding of how these different transmission routes affect disease pathogenesis. Despite PrP^{Sc} (i.e., the pathogenic form of host-encoded PrP^{C}) lacking informational nucleic acids, prions share strain diversity analogous to conventional pathogens. These strain properties affect prion infectivity and pathogenesis, PrP^{Sc} biochemical properties, and host-range dynamics. It is well established that different routes of prion exposure engender varying disease incubation times; however, this was attributed to the duration of time needed for the pathogen to enter the central nervous system to cause disease. Using newly developed gene-targeted models, we have found that the route of inoculation has a profound effect on prion pathogenesis. Specifically, we have discovered that, at the terminal stage of disease, there are distinct biochemical conformations of the protein, varied titers of the infectious pathogen, and different depositions of prions in the brain. This data suggests that unique prion strains are propagated as a result of the route of prion exposure. This research is critical to understanding the natural transmission and zoonotic potential of CWD, as well as fostering the study of prion biology.

Graduate Student/ Microbiology, Immunology, and Pathology

O-22. Fetal BVDV infections and postnatal epigenetic dysregulation.

Jessica N Kincade, Jeanette V Bishop, Hana Van Campen, Terry E Engle, Hanah M Georges, Carolina L Gonzalez-Berrios, Thomas R Hansen

Bovine Viral Diarrhea Virus (BVDV), a vertically transmissible virus, induces profit loss through spontaneous abortion, immunosuppression, and decreased performance. The impact of BVDV upon the fetus is dependent upon gestational age: fetal infection occurring prior to ~125 of gestation generates a persistently infected (PI) calf that develops immunotolerance to the infecting strain, while fetal infection occurring after ~150 days of gestation generates a transiently infected (TI) calf capable of clearing the virus via immune response. Postnatal TI and PI animals experience a lower body weight, higher incidence of illness requiring treatment, as well as altered complete blood count and immune cell profiles observed in peripheral mononuclear blood cells (PBMCs). It was hypothesized that postnatal defects observed in animals experiencing a fetal BVDV infection occur due to dysregulation of the epigenome during gestation, a critical period of development that can impair postnatal health and growth. To test this hypothesis, pregnant heifers were inoculated with non-cytopathic BVDV 2 or phosphate buffered saline (PBS) on day 175 of gestation to generate TI and control calves, respectively. Age-matched PI calves were identified at a cooperating ranch. DNA isolated from PBMCs collected at 4 months of age was subjected to reduced representation bisulfite sequencing (RRBS) via Zymo Research. When TIs were compared to controls, 3876 differentially methylated regions (DMRs) were identified, 1799 hypomethylated regions (associated with increased activity) and 2077 hypermethylated regions (associated with decreased activity). When PIs were compared to controls, 4816 DMRs were identified, with 2723 hypomethylated regions and 2093 hypermethylated regions (meth.diff>25; p-adj.<0.01). Gene ontology characterization indicates affected pathways to include immune function, metabolism, anatomical development, and reproduction. Fetal BVDV infections induce postnatal epigenomic dysregulation contributing to the associated abnormalities in physiological function in both TI and PI animals. USDA NIFA Grants: 2019-67015-29866 and 2021-38420-34040.

Graduate Student/ Biomedical Sciences

O-23. Development of a sodium bismuthate-coated polyacrylonitrile resin for the separation of oxidized actinides from used nuclear fuel.

Samantha A Labb, Ralf Sudowe

Currently there are no long-term storage options for the radioactive nuclear waste that is generated through energy production. However, the need for clean energy through nuclear power will result in an increase in the nuclear waste inventory making the development of waste management solutions a high priority. The long-lived radioactive elements present in nuclear waste (uranium, neptunium, plutonium, americium, and curium) are the main contributors to the radiotoxicity and long shelf-life of this waste, but the ability to generate energy from these elements make them good candidates for new nuclear fuels. The process of retrieving these elements from waste and using them as fuel in nuclear reactors is called Partitioning and Transmutation (P&T) which aims to generate additional clean energy from waste while simultaneously decreasing the radiotoxicity and time required to store the waste. A major scientific challenge for the implementation of P&T is due to the fact that the chemistry of these elements is nearly identical, making separating them from waste difficult. However, the exploitation of the redox chemistry of these elements provides a pathway to achieve these separations. This research, in collaboration with the French separation materials company, TrisKem Int., aims to develop a resin that incorporates a solid oxidizing agent, sodium bismuthate, that utilizes redox chemistry to perform these separations. The behavior of these elements on this material has been characterized through batch contact studies and the potential for separation has been explored through chromatographic separation studies. Based on this data, further characterization of sodium bismuthate and tailoring of the separation material is necessary to apply this method to large-scale waste reprocessing operations. This project is funded by the Mountains and Plains Education and Research Center training grant and the U.S. Nuclear Regulatory Commission.

Graduate Student/ Environmental and Radiological Health Science

O-24. Characterizing Infectious Disease-Induced Neurodegeneration in a Guinea Pig Model of *Mycobacterium tuberculosis* Infection.

Amanda S Latham, Charlize Geer, Isla Anderson, David F Ackart, Brendan Podell, Jessica Elf, Randall J Basaraba, Julie A Moreno

Approximately one billion people suffer from a neurological disorder worldwide; this encompasses the 50 million people diagnosed with a neurodegenerative disease, including Alzheimer's Disease (AD), Parkinson's Disease (PD), and dementia. Neurodegenerative disease cases are estimated to double over the next twenty years, further establishing the importance of research in this field. This estimated escalation in cases will occur due to the rising aging population as well as the detrimental effects of environmental and infectious agents. Although the mechanism is not fully understood, increasing evidence demonstrates a role of viral and bacterial infections as contributors to disease susceptibility, progression, and pathology. Notably, epidemiological studies show that tuberculosis (TB), caused by infection with *Mycobacterium tuberculosis* (Mtb), predisposes individuals for neurodegenerative diseases and is correlated with neurological deficits. Patients with TB disease, but no diagnosis of central nervous system (CNS) infection, show decreased neuropsychological functioning, cognitive impairments, and loss of interest in social activities. They are also predisposed for the development of dementia and PD. Through studies examining the effects of low-dose Mtb exposure by aerosol, we identify pathological markers of inflammation and toxicity in the central nervous system (CNS) of outbred, Dunkin Hartley guinea pigs. This includes migration and proliferation of microglia followed by the activation of astrocytes in multiple brain regions. The misfolded proteins phosphorylated tau and amyloid beta, which are pathological biomarkers found in diseases including AD and dementia, are also detected. Importantly, these effects occur despite the absence of detectable bacteria in the brains of infected animals. Through this data, we establish that the characteristic hallmarks of neurodegenerative disease, neuroinflammation and misfolded proteins, are found in our guinea pig TB model. These studies will allow us to obtain a better understanding of the role peripheral infections play on the progression of disease in the central nervous system.

Graduate Student/ Environmental and Radiological Health Sciences

O-25. Bovine oviductal organoids as a biomimetic system to evaluate extracellular vesicles.

Riley E Thompson, Mindy A Meyers, Nico G Menjivar, Ahmed Gad, Dawit Tesfaye, Fiona K Hollinshead

Novel *in vitro* models are needed to recapitulate the *in vivo* environment. Organoids are three-dimensional *in vitro* cell clusters that regenerate and organize with similar functions to their tissue of origin. Extracellular vesicles (EVs) are nanoparticles containing bioactive molecules that are secreted by cells for intercellular communication. Our objectives were to i) characterize bovine oviductal organoids and ii) evaluate their utility as a biomimetic model. Bovine oviductal organoids (n=13 cows) were cultured for up to 77 days (passaged every 7-14 days), and spent medium was collected for isolation of EVs via ultracentrifugation and characterization by nanoparticle tracking analysis. Organoids were assessed via histology and immunohistochemistry and were co-cultured with fluorescently-labelled (PKH26) EVs to evaluate EV uptake. Histology demonstrated round cellular clusters with a central lumen, which is consistent with other reports. Immunohistochemistry showed positive immunostaining for progesterone receptor, oviduct-specific glycoprotein (OVGP1), FOXJ1 (cell ciliation), and periodic acid-Schiff (secretory function). EV production by the organoids was confirmed after isolation from the spent medium, and EVs were 50-150 nm in diameter. EV uptake was confirmed by fluorescence signal in recipient organoid cells. In conclusion, bovine oviductal organoids display *in vivo*-like morphology, protein expression, and function, indicating they are a biomimetic model of the bovine oviduct to assess EV interaction with oviductal cells. Additionally, bovine oviductal organoids may be used to generate EVs that are similar to those produced *in vivo* to improve *in vitro* production of embryos and to utilize EVs for delivery of novel therapeutics. Funding provided by ARBL TRB Program.

Post-doctoral Fellow/ Clinical Sciences

O-26. Epidemiology of Traumatic brain injury (TBI) and head injury in cats: an ACVECC-VetCOT registry study (April 2017 - December 2021).

Jennifer E Baughman, Claire D Tucker, Kelly E Hall

Traumatic brain injury (TBI) is a leading cause of traumatic death in companion animals, and yet the epidemiology is still poorly understood, especially in cats. This retrospective study of 5742 cats utilizes data from the American College of Veterinary Emergency and Critical Care (ACVECC) Veterinary Committee on Trauma (VetCOT) registry to further characterize cats with TBI and head injury. TBI was defined as a modified Glasgow Coma Score (mGCS) of less than 18, a novel definition in clinical veterinary research. Feline trauma patients with TBI had a significantly lower chance of surviving to discharge compared to those without TBI (47.3%, 93.7%, $p<0.00001$). Cats were also more likely to have TBI when compared to altered cats (32.7%, 21.0%, p value <0.00001). Age and size significantly correlated with the presence of TBI, with younger and smaller cats more likely to demonstrate TBI ($p<0.0001$ and $p<0.0001$, respectively). TBI was most commonly caused in animals with both blunt and penetrating trauma (36.9%) with a lower percentage caused by independent blunt (28.2%) or penetrating (10.5%) injury. When evaluated based on severity of initial injury (mild, moderate, severe) as determined by the Animal Trauma Triage (ATT) score, TBI injuries occurred most frequently in severely injured patients (ATT>5/18 41.7%, ATT <5/18 3.7%, $p<0.00001$). The presence of hyperglycemia, a prognosticator in human TBI, was significantly correlated with the presence of TBI ($p<0.0001$). This study enhances the description and understanding of TBI in cats, paving the way for additional studies aimed to improved diagnostics, interventions, and outcomes of trauma in veterinary patients overall. Additionally, this study helps to further develop a translational model of TBI, leading to informed and impactful knowledge-sharing across human and veterinary medicine.

DVM Student/ Clinical Sciences

O-27. Functional anatomy of the mitral valve in canine degenerative mitral valve disease.

Zack English, Chris Orton, Brianna Potter

Degenerative mitral valve disease (DMVD) is the most common acquired heart disease in small breed dogs as well as the number one cause of cardiac-related death in this population. With recent advancements in procedural options for DMVD, it has become increasingly important to understand the functional anatomy of the mitral valve to determine candidacy for these procedures. The hypotheses of this study are: 1. that excessive leaflet motion originates at the A2 segment of the anterior leaflet of the mitral valve, with additional segment involvement in later stages of disease and 2. there will be an additional component of functional mitral regurgitation (MR) secondary to cardiac remodeling with increasing disease severity. Excessive leaflet motion of the A2 and P2 segments of the mitral valve was assessed on the short-axis and multiple long-axis inflow-outflow views from previous prospectively acquired echocardiograms from clinical patients at varying stages of DMVD. Functional MR was evaluated by measurement of vena contracta width compared to anatomic diameter on the commissural view. When evaluating MR jet direction and leaflet morphology throughout the stages of DMVD, the results suggest a heterogeneous population within stage B1 with various locations of leaflet prolapse and resultant jet morphologies. These findings are in contrast to the more homogeneous findings within stage B2, which consisted of isolated A2 prolapse and resultant posterior wall-hugging jets. When comparing stage B2 to C, the data suggests progressive segment prolapse to include the P2 segment with posteriorly directed jets. Estimates of secondary functional MR increased almost linearly with DMVD progression. This data seems to support the hypotheses that excessive leaflet motion originates in the A2 segment in earlier stages of disease with progressive segment involvement in later stages. Further investigation of stage B1 populations is warranted due to the heterogeneous findings within this group in this study.

DVM Student/ Clinical Sciences

O-28. The effects of two different air quality categories (‘good’ versus ‘moderate’) on ophthalmic parameters in normal dogs.

Katrina EV Jones, Joshua B Daniels, Michael R Lappin, Michala de Linde Henriksen

The purpose of this study was to identify changes in ocular surface parameters and evaluate conjunctival microbial flora in healthy dogs when exposed to different air quality index (AQI) categories. This was a prospective cohort study involving 15 client-owned dogs. The AQI was monitored using the EPA’s AirNow database during the expected 2022 wildfire season in Colorado. An ophthalmic examination was performed when AQI was reported to be 0-50 ‘good’, and 51-100 ‘moderate’. Clinical and diagnostic parameters were evaluated during both categories; conjunctival chemosis and hyperemia (scored 0-4), tear production (mm/min), tear film break-up time (seconds), fluorescein stain (positive or negative), Rose Bengal stain (positive or negative), and conjunctival microbial culture. The median ± SD for AQI three days prior to examination was 69.8±16.9 for ‘moderate’ and 45.1±6.9 for ‘good’. No differences were found between hyperemia and chemosis scores for the two AQI categories (P=0.3040, P=0.1713, respectively). Tear production (STT) for ‘good’ and ‘moderate’ were 27.5±3.96 mm/min and 29.5±4.22 (respectively). No differences between STT for ‘good’ versus ‘moderate’ were found (P=0.4008). TFBUT for ‘good’ and ‘moderate’ were 12.33±3.54 sec and 14.83±5.02 sec (respectively). A difference was found between TFBUT for ‘good’ versus ‘moderate’ (P=0.0075). No differences were found between conjunctival culture growth with ten dogs in the ‘good’ category culturing one or more bacteria on their conjunctival culture compared to seven dogs in the ‘moderate’ category group (P=0.7104). Coagulase-negative Staphylococcus spp., various gram-negative nonfermentative coccobacilli, and Bacillus spp. were the most common bacteria in the ‘good’ category, whereas coagulase-negative Staphylococcus spp. and various Coryneform bacteria (diphtheroids) were the most common in the ‘moderate’ category. In conclusion, the present study did not reveal significant differences in ocular surface parameters between the two AQI categories. Future studies should expand on this work and evaluate these parameters at higher AQI categories.

DVM Student/ Clinical Sciences

O-29. Synovial transcriptomic response in osteoarthritis progression determined by single cell sequencing in an equine model.

Blaine Larson, Dylan Ammons, Lyndah Chow, Laurie Goodrich, Steve Dow, Lynn Pezzanite

Osteoarthritis (OA) impacts more than 10% of the US population, resulting in pain, disability, and economic burden. Understanding of the pathogenesis is evolving, but OA is increasingly thought to be a multifactorial disease in which the innate immune system, particularly myeloid cells, play a role in regulating and perpetuating low-grade inflammation. The goal of this study was to improve understanding of OA immunopathogenesis, with emphasis on the transcriptomic response of cells in the joint following OA induction and throughout progression, which will facilitate development of targeted therapies tailored to disease stage. The immune response of equine synovial fluid and synovium was established using single cell RNA sequencing techniques and compared to that of normal joints from the same horses. We hypothesized that information regarding novel therapeutic targets may be gained by more in-depth analysis of the functional shift in transcriptome in early OA, and that single cell RNA sequencing (scRNA-seq) would allow identification of cellular heterogeneity in diseased tissue and subpopulations of immune and synovial cells within the osteoarthritic joint as OA progresses that would not be detected using other currently available techniques. Synovial fluid was obtained from three horses with OA and healthy joints. Transcriptome wide scRNA-seq and computational analysis were performed on cells isolated from synovial fluid. Preliminary findings showed differentially expressed genes with upregulation of CCL2 (monocyte recruitment marker) and FABP5 in OA and upregulation of MARCO (anti-inflammatory marker) in healthy synovial fluid. These findings support further horse enrollment and evaluation of OA progression over time to classify the temporal immune response and its role in OA pathophysiology. This study highlighted the role of immune infiltration contributing to disease biology in OA progression towards the overall objective of developing treatments tailored to disease stage.

DVM Student/ Clinical Sciences

O-30. Local immune response to radiation therapy and combined myeloid cell targeted therapy in a dog model of sinonasal carcinoma.

Ber-In Lee, Braden Burdekin, Dylan Ammons, Lyndah Chow, Patricia Gualtieri, Erin Trageser, Sana Karam, Steven Dow, Keara Boss

Sinonasal carcinoma (SC) is an aggressive cancer needing better local tumor control despite treatment with surgery and/or radiotherapy. Consistent with human SC, we've found the canine SC tumor immune microenvironment (TiME) consists of immunosuppressive myeloid cells and regulatory T cells (T-reg). Propranolol, a β2-adrenergic receptor antagonist, and losartan, an angiotensin II receptor antagonist, may reverse TiME by reducing the density and influence of tumor-promoting immunosuppressive myeloid cells. We hypothesize that combining Stereotactic Body Radiation Therapy (SBRT) and TiME-modulation (propranolol + losartan) will reduce immunosuppressive myeloid cells, restore T-cell immunity, and lead to a more durable local response for dogs with SC. Dogs with spontaneous SC were randomized into 1) SBRT alone and 2) SBRT + propranolol & losartan (SBRT-PL). This trial is ongoing; 6 dogs have been enrolled, 3 in each group. Local immune responses were assessed via serial nasal lavage. For the lavage samples, cytokines were quantified by Milliplex cytokine assay, and cells were analyzed by flow cytometry (FC) and single-cell RNA sequencing (scRNAseq). Cytokines evaluated at 2-week post-radiation, SBRT-PL v.s. SBRT dogs, had decreased levels of the fold-change in various pro-tumor cytokines including IL-6 (46% v.s. 497%), IL-8 (55% v.s. 363%), KC-like (82% v.s. 687%), and MCP-1 (193% v.s. 364%). Cells quantified at 3-month post-radiation, SBRT-PL v.s. SBRT dogs had higher fold-change in cytotoxic T cells (2933% v.s. 15%), helper T cells (1114% v.s. 38%), co-stimulatory MHCII+ cells (159% v.s. 80%), and less fold-change in immunosuppressive T-regs (80% v.s. 420%) and M2 macrophages (146% v.s. 619%). Preliminary scRNAseq data collected from one dog was consistent with corresponding FC results. Concurrent administration of SBRT and propranolol/losartan may suppress immunosuppressive myeloid cells and encourage anti-tumor T cell infiltration. Next, we will evaluate gene expression of serially collected circulating cells, nasal lavage cells, and tumor biopsy samples by NanoString sequencing.

Post-doctoral Fellow/ Environmental and Radiological Health Sciences

O-31. Radiation dose to patients and staff during feline esophagrams.

Nina Marchell, Justin Bell, Kate Goodman, Elissa Randall

There currently is limited published information on radiation dose to veterinary staff and patients during procedures requiring ionizing radiation. Radiation worker doses are calculated monthly at CSU, but data for individual studies is generally unavailable. The purpose of the study was to document the radiation dose to staff and patients during feline fluoroscopic esophagrams. Electronic Personal Dosimeters (EPD) were used to estimate the radiation dose to patients and staff members involved in the procedure. Fluoroscopic images were captured using the Philips Veradius C-arm. Patients were free-fed to maximize patient compliance and radiation safety. A total of 17 studies had complete radiation dose monitoring data. The average dose recorded by the dosimeter badges was 0.003 mrem to the feeder, 0.002 mrem to the table mover and fluoroscopy operator, and 0.035 mrem to the patient. However, the average dose to the patient recorded by the fluoroscopy machine was 454.8 mrem. The EPD used to monitor patient dose was likely outside of the direct fluoroscopy beam, which may indicate there is minimal radiation exposure in this area. This study shows that the hands-free method of performing esophagrams in cats is advantageous in limiting dose to staff. Maximum allowable dose is 100 mrem/yr for members of the public and 5,000 mrem/yr for radiation workers; maximum allowable dose for veterinary patients is unregulated. The EPDs used have a reported energy response of ≤ ±20% from 60 keV to 1.5 MeV, ^{137}Cs, applicable to the energy of the fluoroscopy beam. However, dose contributions to staff from low energy, scattered photons, may require further investigation. Furthermore, the machine reported patient dose may be more specific to human patients. Large anatomical variances between humans and animals warrant the development of an accessible methodology to rapidly verify the radiation dose to veterinary patients during diagnostic procedures.

DVM Student/ Environmental and Radiological Health Sciences

O-32. Development of a porcine oocyte collection protocol for intracytoplasmic sperm injection (ICSI) with stallion spermatozoa.

Elizabeth A Patton, JoAnne E Stokes, Jennifer N Hatzel

Assisted reproductive technologies (ART), such as intracytoplasmic sperm injection (ICSI), are commonly utilized in clinical equine practice for mares and stallions suffering from infertility. For ICSI, a mare's oocyte is injected with a singular sperm from the desired stallion. Embryonic development to the stage of transfer remains low and research advancements are stifled due to lack of equine oocyte availability. We propose using porcine oocytes as a model to then evaluate stallion fertility and semen processing techniques. Collection of oocytes from abattoir porcine ovaries, establishment of appropriate maturation timing, and optimization of maturation media (MM) were the three main variables evaluated. Formation of a polar body (PB) indicates maturation of an oocyte to undergo ICSI and served as an end point. Follicular aspiration techniques were developed through modification of a bovine protocol and provided 760 total oocytes. There was a statistical difference in PB formation when oocytes were placed directly into control MM (cMM) post-aspiration compared to holding for 24 hours (24.4% vs. 3.3%, $p = 0.004$) and when 50% porcine follicular fluid (PFF) was added to MM compared to cMM (26.1% vs. 11.3%, $p = 0.019$). There was not a statistical difference in PB formation when 33% PFF was added to MM compared to cMM (23.7% vs. 24.2%, $p = 0.938$), or when MM contained 20% fetal calf serum vs. 10% in cMM (19.1% vs. 17.1%, $p = 0.753$). Therefore, porcine oocytes can be collected and successfully matured in an equine based MM in preparation for ICSI. Further development and implementation of this protocol provides a foundation for research opportunities devoted to improvement of a multitude of ART procedures across species.

DVM Student/ Clinical Sciences

O-33. Increasing enclosure complexity to improve geriatric African elephant welfare at the Cheyenne Mountain Zoo.

Hannah Rice, Rick Hester, Jason Bredahl, Liza Dadone, Matthew Johnston

Stereotypic behaviors commonly seen in elephants include weaving, head-nodding, pacing, and repetitive trunk swaying. These behaviors have been linked exclusively to animals in human care and are manifested in response to a variety of welfare issues, including an environment with limited opportunities to perform highly motivated behavior patterns. Our study aimed to test methods for improving African elephant welfare for any institution wanting to reduce stereotypic behavior and enhance quality of life. Our hypothesis was that stereotypic behavior would be reduced in response to providing more opportunity for species specific behavior. Our sampling method was partial interval recording with one minute intervals. We measured the behaviors of five female African elephants ranging in age from 39 to 53. A behavior was recorded at its onset and only marked once for each interval period. The behaviors recorded were foraging, log interaction, substrate interaction, wallowing, stereotypy and waiting. Baseline behavior frequencies were measured prior to changing their environment. Our materials were the SIT application for interval timing, multiple large mud wallows, 6 to 10′ tall sand mounds and logs ranging in height from 10′ to 18′. Our preliminary results were that when this environmental enrichment was added stereotypy reduced significantly and interaction with enrichment increased, resulting in increased behavioral diversity. This study is a continuation of one performed the previous Summer which had failed to show stereotypy reduction when enclosure complexity was increased. We believe the more recent study's success was attributed to combining the enrichments, maintaining fresh mud wallows and providing new logs for foraging when they were stripped of bark. From our preliminary results, we concluded that stereotypy can be reduced in zoo African elephants when opportunities to perform highly motivated species typical behaviors are made naturally available.

DVM Student/ Clinical Sciences

O-34. Outcome of hyperthyroid cats treated with radioactive iodide (I-131) therapy.

Macy A Riedemann, Petra Černá, Michael R Lappin

Hyperthyroidism is a common endocrine disease in older cats caused by excess release of thyroid hormones resulting in an increased metabolic state. Radioactive iodine therapy (I-131) is one of the treatments available for hyperthyroidism; however, this is an irreversible treatment that can result in hypothyroidism as well as some cats failing the treatment and remaining hyperthyroid. The main objective of this study was to evaluate the success rate and survival of cats post radioactive iodine treatment. A total of 96 hyperthyroid cats treated with I-131 therapy at Colorado State University Veterinary Teaching Hospital between August 2019 and May 2022 were retrospectively analyzed and data including age, sex, breed, clinical signs at time of diagnosis, prior methimazole therapy, thyroid hormone concentrations, kidney function, and overall survival was assessed by Kaplan Meier analysis. A total of 96 records were reviewed and 57 cats with a minimum of 6 month follow up were included in the survival analysis. The main clinical signs on presentation were weight loss (62/96 cats), vomiting (33/96) and polyphagia (19/96). A total of 80.7% of cats achieved euthyroidism, 10.5% of cats remained hyperthyroid, and 8.8% of cats became hypothyroid post I-131 treatment. The survival rates varied amongst cats that became euthyroid (945 days), remained hyperthyroid (601 cats), or became hypothyroid (317 days). In conclusion, the overall success of I-131 therapy was high; however, monitoring cats post I-131 therapy is important to start supplementation for cats that develop hypothyroidism as hypothyroid cats have shorter survival time post I-131 therapy.

Staff/ Clinical Sciences

O-35. Detection of pro-inflammatory cytokines in healthy canine tear film using mass spectrometry via multiple reaction monitoring (MRM-MS).

Katya Spitznagel, Railey Mikeska, Haley Jost, Isabella Corsato Alvarenga, Stephanie McGrath, Carolina Mehaffy, Michala de Linde Henriksen

Pro-inflammatory cytokine analysis in ocular fluid is a useful screening tool in the diagnosis of ocular inflammatory diseases. Several methods of cytokine analysis exist, including PCR, ELISA, multiplexed bead assay, and mass spectrometry. Multiplexed bead assay analysis is often chosen due to its simplicity and speed, but important pro-inflammatory cytokines such as interleukin 1-beta (IL-1ß) are not measured by this assay in the commercially available canine assay, and there are associated quality control limitations. In contrast, targeted mass spectrometry with multiple reaction method (MRM-MS) is highly specific, highly sensitive, requires low volume samples to screen for multiple cytokines even in low abundance. This method has been used to evaluate canine aqueous humor, but not canine tears. The purpose of this study was to evaluate the pro-inflammatory cytokine level in canine tears from healthy research beagles using Canine Cytokine SpikeMix™ MRM-MS. Tears were collected from both eyes of 16 healthy research beagles by placing a Weck-Cel® cellulose spear in the ventral conjunctival fornix for one minute. The Weck-Cel® spear was placed in a 2.0 mL Eppendorf tube and was spun down in a centrifuge. Tears were analyzed using Canine Cytokine SpikeMix™ MRM-MS, a method capable of detecting 144 different cytokines. Previous experiments with canine aqueous humor have demonstrated that 0.1-0.2 mL samples of fluid were sufficient to target and identify 15 key pro-inflammatory cytokines in dogs with primary glaucoma as well as post-operative intraocular hypertension and uveitis post-phacoemulsification. In our current experiment, we hypothesize that when analyzed by Canine Cytokine SpikeMix™ MRM-MS, we will detect more different pro-inflammatory cytokines in canine tear film than when the samples are analyzed by multiplexed bead assay. Canine tear film analysis by MRM-MS may offer insight into the roles of pro-inflammatory cytokines in surface ocular pathology in a minimally invasive yet highly specific manner.

DVM Student/ Clinical Sciences

O-36. Evaluation of tissue oxygenation saturation as a marker of shock and resuscitation in a canine hemorrhagic shock model.

Charles T Talbot, Taylor N Baird, Kelly E Hall, Ann M Hess, Kristin M Zersenn

The purpose of this study was to determine if non-invasive tissue oxygen saturation is correlated with oxygen delivery and cardiac output in a canine hemorrhagic shock model, and therefore able to be applied clinically as a marker of shock. The second purpose was to determine if tissue oxygen saturation can be used as a marker of resuscitation. Eight healthy dogs were anesthetized and instrumented with pulmonary arterial, peripheral venous, and peripheral arterial catheters (cardiac output by thermodilution, sampling/medication administration and direct blood pressure, respectively). Tissue oxygen saturation (StO_2) was measured using a portable near-infrared spectroscope (NIRS). After a 10-minute period of blood pressure stabilization (T_1), dogs were hemorrhaged to a fixed mean arterial blood pressure of 40 mmHg (+/- 5 mmHg) for 10 minutes (T_2). Dogs were then resuscitated with shed blood in 2 equal aliquots [50% and 100% of volume removed (T_3 and T_4, respectively)]. Tissue oxygen saturation, systolic blood pressure, heart rate, cardiac output, and volume of blood removed (replaced) were documented at T_1, T_2, T_3 and T_4. Oxygen delivery (DO_2) was calculated at each time point (DO2 = CO x [(1.34 x Hb x SpO2) + (0.03 x PaO2)]). Statistical tests included Shapiro-Wilk for normality, a mixed model to investigate changes over time, a Dunnett's method to compare downstream time points verses baseline, and residual diagnostic plots to evaluate model assumptions of normality and equal variance. Strong statistical correlations were identified between tissue oxygen saturation and cardiac output (R=0.8), and tissue oxygen saturation and oxygen delivery (R=0.75). These results suggest that non-invasive tissue oxygen saturation may be used in conjunction with clinical signs and other diagnostic parameters as a marker of shock and resuscitation.

Resident/ Clinical Sciences

O-37. Impact of lyophilized platelet infusions on point-of-care viscoelastic test VCM-Vet™ in canine cardiopulmonary bypass.

Devon Diaz, Christopher Orton, Julien Guillaumin

To assess the hemostatic impact of canine lyophilized platelets (LP) on cardiopulmonary bypass (CPB) recovery, our study included dogs undergoing CPB for surgical correction of a naturally-occurring cardiac disease. Variables collected were demographics, cardiac disease, and VCM-VET(VET) variables: Clot time (CT), Clot formation time (CFT), Angle (Angle), Maximum Clot Formation (MCF), Amplitude at 10 (A10) and 20 minutes (A20), Clot Lysis at 30 (LY30) and 45 minutes (LY45). LP were administered at a dose of 1 mL/kg (Single) or 2 mL/kg (Double) after weaning off CPB and protamine reversal (T1). VET variables were compared at 4 timepoints: baseline (T0), (T1), 5-minutes-post-LP infusion (T2), and 1-hour-post-LP infusion (T3). Differences between timepoints, and groups, were compared using an ANOVA for repeated measures, and a T-test or Mann-Whitney's test, respectively. Twelve dogs were included (six in each group). Median age was 1.8 years (0.7-6.9). Median body weight was 28.5 kg (12.4-38.2). Underlying cardiac diseases were tricuspid (n=6) or mitral (n=3) valve dysplasia, atrioventricular septal defect (n=2), and tetralogy of Fallot (n=1). For the Single group, CFT was prolonged from T0 to T1. Angle was decreased from T0 to T2. A10 decreased from T0 to T1 and from T1 to T2. For the Double group, CFT was prolonged from T0 to T1. Angle was decreased from T0 to T1 and from T1 to T2. Angle was increased from T2 to T3. A10 decreased from T1 to T2 and increased from T2 to T3. A20 decreased from T1 to T2 and increased from T2 to T3. MCF was decreased from T0 to T2 and from T1 to T3. No other differences between timepoints were found. No differences between groups were found for each of the timepoints. This pilot study showed changes in VET variables overtime during CPB recovery after LP infusion.

Resident/ Clinical Sciences

O-38. Seroprevalence and potential risk factors for brucellosis among small ruminant farmers in Punjab, Pakistan, 2019-20.

Muhammad S Khaliq, Muhammad H Mushtaq, Mo Salman, Sangeeta Rao

This study was performed to estimate the seroprevalence and associated risk factors for brucellosis among sheep and goat farmers in Punjab, Pakistan during 2019-20. A cross-sectional study was conducted in three different districts of Punjab, Pakistan. The relevant data on potential risk factors were collected and statistically analyzed using the serological test results as the outcome. The Rose Bengal Brucella Test (RBT) and Indirect ELISA tests were used for serological diagnosis of human brucellosis. There were 122 farmers enrolled from 70 different villages of districts Chakwal, DG Khan and Jhang in the study. The results revealed overall seroprevalence of 4.9% with no significant difference between three districts of Punjab. The individuals with age group 30-50 years have higher prevalence of 7.3% (n=55) as compared to the age group <30 years and above 50 years i.e. 2% (n=50) and 5.9% (n=17) respectively. The risk factor analysis showed that the odds of seropositivity was higher in people who consume raw unheated milk (OR: 6.83, 95% CI: 0.7-60.3). Co-housing of small ruminants with large animals and assistance in parturition of animals without wearing PPEs had higher likelihood of brucella antibodies among tested livestock farmers (OR: 2.73, 95% CI: 0.5-15.5 and OR: 2.83, 95% CI: 0.5-16 respectively). Although the results are not statistically significant, the study lays a platform for further investigation on this zoonotic disease. Moreover, the study found substantial level of agreement between RBT and indirect ELISA tests for diagnosis of human brucellosis (kappa value: 0.65, CI: 95% 0.29-1.00). This study provides an insight on acquiring brucellosis among livestock farmers and its associated risk factors especially related to livestock management, farmers attitudes and practices.

DVM/PhD Student/ Clinical Sciences

O-39. Feral swine as indicators of anthrax contamination and potential vectors of infectious spores: an experimental infection study.

Rachel M Maison, Maggie Priore-Bush, Michael J Bodenchuk, Vienna R Brown, Richard A Bowen, and Angela M Bosco-Lauth

Anthrax is a disease that affects livestock, wildlife, and humans worldwide; however, its relative impact on these populations remains underappreciated, particularly for wildlife, as individuals are not always easily observed. Despite being a reportable disease in the United States, surveillance is largely passive. Active management, where present, primarily consists of livestock vaccination, but this practice is not standard across regions. Feral swine (*Sus scrofa*) are less susceptible to anthrax than ruminants, and past serological surveys have alluded to their potential utility as sentinels for surveillance, yet empirical data to support this is lacking. Moreover, whether feral swine may assist in the spread of anthrax by transporting infectious spores after natural exposure has not been investigated. To address these, we exposed 15 juvenile feral swine to *Bacillus anthracis* strain Sterne 34F2 spores and measured seroconversion and bacterial shedding over time. Animals were divided into subgroups, and intranasally exposed to 10^4 or 10^7 colony forming units of *B. anthracis* Sterne. Individuals also were exposed either one or three times based on group assignment. Sera were evaluated by enzyme-linked immunosorbent assay for the presence of antibodies to *B. anthracis*, and nasal swabs were cultured to detect viable *B. anthracis*. We report that feral swine exhibit measurable antibody responses to *B. anthracis* after intranasal exposure, and that the strength of response correlates positively with both inoculum dose and number of exposure events experienced. We additionally report that feral swine may assist in the spread of infectious spores on the landscape, as viable bacteria were isolated from the nasal passages of most study animals throughout the study period. These findings have implications for the identification of landscapes contaminated with *B. anthracis*, and as well exposure risk to other species. This research was supported by the U.S Department of Agriculture, Wildlife Services.

Graduate Student/ Biomedical Sciences

O-40. Epidemiology of Traumatic brain injury (TBI) and head injury in dogs: an ACVECC-VetCOT registry study (April 2017 - December 2021).

Claire D Tucker, Jennifer E Baughman, Kelly E Hall

Traumatic brain injury (TBI) is an understudied topic in veterinary medicine. Despite TBI being a leading cause of traumatic death in companion animals, the epidemiology is poorly understood. This retrospective study of 9669 dogs from April 2017 to December 2021 used data from the American College of Veterinary Emergency and Critical Care (ACVECC) Veterinary Committee on Trauma (VetCOT) registry to characterize the canine TBI population. TBI was defined as a modified Glasgow Coma Score (mGCS) of less than 18, a novel definition in clinical veterinary research. Canine trauma patients with TBI had a significantly lower chance of surviving compared to those without TBI (68.3%, 97.7%, p value <0.001). Age and size significantly correlated with the presence of TBI, with younger and smaller dogs more likely to demonstrate TBI ($p<0.0001$ and $p<0.001$). Intact canines were also more likely to have TBI when compared to altered canines (41%, 30.8%, p value <0.001). TBI was most commonly caused by blunt trauma (69.1% blunt, 23.9% penetrating, 7.0% both blunt and penetrating). Dogs without TBI had a head injury incidence of 12% and dogs with TBI had a head injury incidence of 42.2%. When evaluated based on severity of initial injury (mild, moderate, severe) as determined by the Animal Trauma Triage (ATT) score, TBI injuries occurred most frequently in severely injured patients (ATT>6/18) ($p<0.001$). The presence of hyperglycemia, a prognosticator in human TBI, was significantly correlated with the presence of TBI in dogs ($p<0.001$). Canine TBI demonstrates clinically significant injury patterns that can be used to prognosticate and treat dogs presenting with trauma. This study enhances understanding of TBI in dogs, paving the way for improved diagnostics, interventions, and outcomes of trauma in veterinary patients. Additionally, this study helps to develop a translational model of TBI, leading to impactful knowledge-sharing across human and veterinary medicine.

DVM/PhD Student/ Clinical Sciences

O-41. Rice bran in ready-to-use therapeutic foods (RUTFs) for microbiota-targeted treatment of childhood malnutrition.

Annika Weber, Frank Wieringa, Damayanti Soekarjo, Silvia Barbazza, Elizabeth P Ryan

Severe acute malnutrition (SAM) affects more than 29 million children around the world and causes nearly half of all child deaths under the age of five. SAM is associated with high mortality due to the vicious cycle of food insecurity, recurring and co-existing infections, and impaired gut barrier functions, including immunity. In Indonesia, SAM affects more than 2 million children under 5 years of age, though only ~1% receive adequate treatment. SAM treatment in involves provisions of ready-to-use therapeutic foods (RUTF) which provide vital nutrients and calories for immediate benefits to the malnourished child and has greatly reduced mortality. However, recovery is often short-term as children remain highly susceptible to infections and malnutrition relapse. Emerging attention is placed on the infant and child gut microbiota in the protection against enteric infections and for enhancing immune strength. This study seeks to characterize the gut microbial changes related to a gut targeted RUTF (RUTF + rice bran) in the improvement of SAM treatment and cessation of the vicious cycle of malnutrition. To explore this objective, we will use 16S rRNA and Shotgun metagenomics to analyze changes in the recovering child's gut microbiome during and after RUTF treatment. This study will yield new information on the improved treatment outcomes in SAM treatment as well as use of affordable local products.

Graduate Student/ Environmental and Radiological Health Sciences

O-42. *C. elegans* Nrf Homolog, Skn-1, May Play a Role in Cannabidiol Neuroprotection.

Abdullatif Alsulami, Vincenzo Gilberto, Margaret Neuheardt, Stephanie McGrath, Julie A Moreno

Alzheimer's disease (AD) is a neurodegenerative disease that is affecting an increasing number of the aged population worldwide. AD is characterized by the accumulation of amyloid-b and tau hyperphosphorylation along with a failure in redox homeostasis. The hallmarks of neurodegenerative diseases include the increased generation of reactive oxygen species (ROS) which is tightly controlled by an antioxidant defense mechanism under physiological conditions. The nuclear factor erythroid 2-related factor (Nrf-2) is a transcription factor that is responsible for the regulation of redox balance and antioxidant detoxifying responses. Since oxidative stress is believed to contribute to age-dependent neurodegenerative diseases, it could be predicted that Nrf-2 system may function in its prevention. Furthermore, Nrf-1 regulate proteosome activity to maintain protein homeostasis. The proteosome is a branch in the protein quality control system that degrades misfolded proteins in neurodegenerative diseases, thus; upregulation of proteosome activity would ameliorate proteotoxicity. This research aims to utilize various strains of the model organism *C. elegans* to understand the mechanism of cannabidiol at the cellular level in stressed models. In *C. elegans*, skn-1a plays a role in proteotoxic stress through upregulation proteosome subunits and is negatively regulated by the abundance of proteosome complex protein. Cannabidiol (CBD) is a non-psychoactive phyto-cannabinoid that has multiple beneficial effects including neuroprotection. We hypothesize that CBD-mediated survival extension is through the activation skn-1 isoforms. UV crosslinker is used to mimic the ROS generated from misfolded protein and to test if CBD can scavenge them independently of Skn-1b/c. Bortezomib, a proteosome stressor, is used to test if CBD's upregulation of skn-1a could increase proteosome subunit expression. Our preliminary data show that CBD activates skn-1a and c. Also, CBD acts independently of the repressor (wdr-23). Knowledge gained will allow for a better understanding of how CBD is helping neurons survive due to increased misfolded proteins.

Graduate Student/ Environmental and Radiological Health Sciences

O-43. Stop the Spread: a community-based science communication approach to address misinformation.

Shelby Cagle, Sera Choi, Joy Enyinnaya, Marilee Long, Colleen Duncan, Ashley Anderson, Nicole Kelp

The Covid-19 pandemic ushered in a new interest for science, but also enabled the dissemination of misinformation. To better understand and mitigate such misinformation, we established a three-part study: first, to survey scientists and gauge their current perceptions of misinformation; second, to engage stakeholders through focus groups and interviews to understand patterns of misinformation within their communities; third, to develop a course that will train graduate students to address misinformation through interdisciplinary teams and mixed methods research with community partners. The cornerstone of our approach is a Community-Engaged Model, which recognizes that stakeholders have crucial expertise and experience which strengthen the overall study. From the survey of scientists, our results indicate that science communication experience does in fact enable scientists to feel able to address misinformation. Specifically, science communication that avoids a deficit view of non-scientists is better at addressing misinformation. From our focus group data, we gained evidence to support that a distrust in science drives misinformation within communities. Therefore, our course development is aimed at training scientists in science communication in a way that builds trust. Several key course competencies we hope to accomplish and build upon include: interpersonal skills, self-efficacy, deficit- versus asset-based views of communities, and knowledge about theory, methods, and practice. We are currently gathering pre-course and post-course data on students through interviews and course surveys aimed at validating the efficacy of the course on training students on science communication that addresses misinformation. Overall, this multi-pronged project provides insight on how academic-community partnerships can be instrumental in co-creating solutions to address misinformation. This research was supported by Colorado State University's Office of the Vice President for Research's "Accelerating Innovations in Pandemic Disease" initiative, through support from The Anschutz Foundation.

Graduate Student/ Microbiology, Immunology and Pathology

O-44. Antemortem diagnostic for canine cognitive dysfunction using plasma biomarkers.

Amelia D Hines, Stephanie McGrath, Nhu Linh Trinh, Nikole Z Madrid, Abdullatif Alsulami, Amanda Latham, Lisa Mulligan, Breonna Kusick, Julie A Moreno

Canine Cognitive Dysfunction (CCD) is a well-recognized neurodegenerative disease, affecting up to 35% over the age of 8. As canines age, they may experience a build-up of misfolded proteins, causing decline of cognition with similarities to Alzheimer's disease (AD). Canines provide an excellent translational model for human dementias due to their natural development of clinical signs. Currently, antemortem diagnostic testing for CCD is limited to owner questionnaires and magnetic resonance imaging to rule out other causes of cognitive decline. The objective of this project is to design non-invasive antemortem diagnostic methods able to detect biomarkers of cognitive decline in plasma. Immunohistochemical (IHC) staining of canine brain tissue from both young and aged canines has shown presence of Aβ and hyperphosphorylation of tau, as well as increased glial cell inflammation, marked by GFAP, Iba1 and S100β. The biomarkers of CCD found by IHC staining are also present within extracellular vesicles, specifically exosomes, in both plasma and cerebrospinal fluid (CSF). Concentration of extracted exosomes can then be run on a western blot and probed for the same biomarkers of CCD as found in IHC. To date, we have observed neurofibrillary light chain (NfL) to be present in both the plasma and in the CSF at significantly higher levels in canines with CCD compared to young or aged canines without clinical signs of CCD. Detection of NfL in plasma and CSF supports our hypothesis that other biomarkers of neurodegeneration will be present in the plasma and CSF of CCD patients. Development of an antemortem plasma and CSF based assay would be instrumental in helping veterinarians to diagnose CCD, and due to the similarities between CCD and AD, these diagnostics have the potential for strong translation to human medicine.

Staff/ Environmental and Radiological Health Sciences

O-45. Breast cancer cell co-culture with primary lung fibroblasts is associated with modulation of cytokine secretion and extrinsic stromal-mediated drug resistance.

Marika Klosowski, Kathryn Cronise, Gwyneth Knott, Daniel Regan

Breast cancer is the most frequently diagnosed cancer worldwide, with over 2 million new diagnoses yearly. While many breast cancer patients with localized tumors will experience lasting remissions, the 5-year relative survival rate for metastatic breast cancer is only about 30% due largely to a lack of effective anti-metastatic therapies. Cancer-associated fibroblasts (CAFs) are key extrinsic drivers of tumor progression and therapeutic resistance in the *primary* breast cancer tumor microenvironment, but little is known about how tissue-resident fibroblasts in metastatic organ sites support outgrowth and chemoresistance of disseminating breast cancer cells. Since the lung is a top metastatic site in breast cancer, we developed a high-throughput breast cancer cell (BCC) - primary human donor-derived lung fibroblast (LF) co-culture model to identify therapeutic vulnerabilities and mechanisms of fibroblast-mediated extrinsic chemoresistance in lung-metastatic breast cancer. We characterized growth and chemoresistance phenotypes of BCCs co-cultured with primary LFs via bioluminescence and fluorescence imaging, RNAseq, and multiplex cytokine analysis. Using this model, we also performed a high-throughput screen evaluating a library of 900 FDA approved and experimental kinase inhibitor compounds to identify therapeutic vulnerabilities specific for metastatic breast cancer. Human BCC co-culture with primary LFs resulted in significant subtype-dependent differences in tumor cell growth and drug resistance compared with conventional monoculture. Moreover, we observed significant increases in tumor-promoting cytokines including IL-6, CXCL8, and CCL2 in co-culture vs. conventional monoculture. High-throughput kinase inhibitor library screening defined a subset of compounds, namely inhibitors of the class III PI3K and autophagy regulator VPS34, with increased efficacy in lung fibroblast co-culture, suggesting autophagy induction as a mechanism underlying fibroblast-mediated drug resistance in breast cancer. Thus, breast cancer cell co-culture with primary lung fibroblasts represents a scalable *in vitro* model for therapeutic discovery and mechanistic evaluation of extrinsic modulation of chemotherapy drug response in metastatic breast cancer.

Resident/PhD Student/ Microbiology, Immunology and Pathology

O-46. Biomechanics of the LDE and ACL in ovine stifle stability for a novel model of posttraumatic osteoarthritis.

Bridget Michalko, Katie Sikes, Chloe Brekhus, James Johnson, Jeremiah Easley, Brad Nelson

Posttraumatic osteoarthritis (PTOA) is a degenerative disease that affects 630 million people worldwide. Surgical ovine models via anterior cruciate ligament (ACL) transection require 40 weeks to develop clinical signs associated with PTOA. This may be due to the long digital extensor (LDE) tendon on the craniolateral aspect of the femoral condyle. We hypothesized that the LDE tendon provides sufficient anterior support for the stifle following injury to the ACL, thus slowing the progression of PTOA in ovine models. The study aim was to identify the biomechanical influence of the LDE tendon on joint stability. For this, ovine cadaver stifles were assessed for joint mobility using a load frame to simulate a cranial drawer test. To delineate the role of the LDE tendon and/or ACL, specimens were divided into two groups which differed only in order of ACL/LDE transection. For each testing sequence, specimens were pulled at 0.1mm/sec until a standard load of 50N was achieved, with 5 minutes of rest between testing. Interestingly, transecting the ACL first resulted in a 6.1x increase in maximum displacement and a 4.2x reduction in stifle stiffness relative to an intact limb. Sequentially cutting the LDE resulted in an additional 1.3x increase in maximum displacement with no further decrease in stifle stiffness. Notably, when transecting the LDE first, a change in these parameters was not detected until the ACL was sequentially transected. These data suggest that the ACL provides the major stability in the ovine stifle, and that transecting the LDE in conjunction with the ACL increases joint instability. Future studies will elucidate the effects of PTOA development in the ovine stifle through a dual ACL/LDE transection model.

DVM Student/ Clinical Sciences

O-47. Undergraduate STEM students' science communication skills, science identity, and science self-efficacy influence their motivations and behaviors in STEM community engagement.

Katlyn M Murphy, Nicole C Kelp

While numerous studies have examined how scientists perceive doing public communication and engagement, there is limited research on undergraduate STEM student attitudes towards these meaningful activities. Undergraduate students are more diverse than STEM faculty and may serve as boundary spanners in communities, so exploring their motivations and behaviors in STEM engagement is valuable. For scientists, self-efficacy in communication skills is one driver of public engagement behavior. In this study, we utilize a survey to examine how undergraduate STEM students' science communication skills as well as their science identity and science self-efficacy may drive motivation and behaviors in STEM community engagement. Our findings reveal that STEM students are motivated to do community engagement but lack opportunities to actually do these behaviors. Regression analyses reveal that science communication skills, science identity, and science self-efficacy are all predictors of student motivation and behaviors in STEM community engagement. However, year in academic progression does not increase STEM students' attitudes and behaviors in community engagement. These findings suggest that universities should intentionally provide training in science communication, continue providing support for students developing science identity and self-efficacy, and develop opportunities for undergraduate STEM students to do science outreach and engagement activities.

Undergraduate Student/ Microbiology, Immunology and Pathology

O-48. Sex differences in the response to anterior cruciate ligament injury following mechanical rupture in a mouse model.

Emily M Van Zeeland, Brandon Kassel, Travis Montoya, Kelly S Santangelo, Jeremiah T Easley, Katie J Sikes

Following anterior cruciate ligament (ACL) injury, up to 87% of individuals develop post-traumatic osteoarthritis (PTOA) in their knee joint. Although it is well established that females are more likely to experience an ACL injury compared to males, it is poorly understood if sex differences contribute to the development of PTOA. The aim of this project was to examine the variable injury responses of males and females following ACL rupture using a refined mechanical (non-surgical) rupture model. Unilateral ACL rupture was achieved via mechanical compression in male and female mice. Longitudinal mobility analyses were conducted prior to ACL rupture and weekly out to eight weeks post-injury. Significance for all comparisons was set to $p<0.05$. Data at each timepoint was normalized to baseline. Individual parameters for males and females were compared using an un-paired Student's t-test. No significant differences were observed between male and female mice at baseline for all parameters. Relative to uninjured baseline values, male mice exhibited decreased voluntary distance traveled compared to female mice at five weeks following ACL injury ($p=0.0086$). Compared to female mice, males showed increased hindlimb stance width on a flat treadmill relative to baseline values ($p=0.0271$). These changes suggest male mice are minimizing voluntary movement after ACL rupture. Further, increased hindlimb stance width seen in male mice compared to females suggests male mice are modifying their gait due to a pain response and/or compensating for decreased stability. These observations could indicate differences in injury responses, pain perception, and/or PTOA development between males and females. Additional work is being conducted to examine histologic and proteomic changes in both sexes following ACL rupture. Understanding the early injury differences between males and females may elucidate novel therapeutic mechanisms and surgical reconstruction techniques for an overall goal of improving ligamentous healing following rupture to mitigate PTOA progression.

DVM/PhD Student/ Clinical Sciences

O-49. Opioids in the retina modulate sleep/wake behavior.

Casey-Tyler Berezin, Nikolas Bergum, Jozsef Vigh

Therapeutic interventions aimed at improving opioid-induced sleep/wake disturbances (OISD) are expected to ameliorate other negative outcomes associated with chronic opioid use (e.g. affective disorders, drug craving, hyperalgesia). While the mechanisms underlying OISD have been largely unknown, our recent work suggests that opioid bioavailability in the eye may modulate opioid signaling by retinal neurons to affect sleep/wake behavior. We use a variety of techniques - immunohistochemistry, qRT-PCR, mass spectrometry and telemetric sleep recording - to dissect the contributions of opioid signaling and transport in the retina to OISD. Using a mouse line with a cell-specific knockout of mu-opioid receptors (MORs), we show that endogenous activation of MORs expressed by cells in the retina modulates natural sleep/wake cycles. Specifically, activation of these receptors appears to be important for maintaining activity ($p<0.01$, ANOVA, Tukey adj.) and suppressing slow-wave sleep ($p<0.01$) during the mouse's active period. Since opioid accumulation in the eye can be reliably detected post-mortem in both mice and humans, we investigate transporters expressed at the blood-retina barrier that may contribute to this phenomenon. We find that low expression of permeability glycoprotein (P-gp) in the retina compared to the brain ($p<0.0001$) is associated with greater ocular morphine accumulation compared to brain ($p<0.0001$) in both male and female mice. We discuss how upregulation of P-gp with the non-steroidal anti-inflammatory drug diclofenac may be a novel therapeutic target for reducing opioid bioavailability in the eye. This work presents a previously underappreciated role of the opioid signaling in the retina in contributing to OISD.

Graduate Student/ Biomedical Sciences

O-50. Granulosa cell-derived extracellular vesicles alter oocyte function and promote survival under heat stress conditions.

Nico G Menjivar, Ahmed Gad, Dawit Tesfaye

Elevated temperatures as a result of a warming planet have direct implications on reproductive function. The follicular microenvironment provides an optimal cavity for oocyte growth and maturation, largely regulated by its crosstalk with granulosa and theca cells. Cell-secreted nanoparticles and their encapsulated bioactive components, broadly categorized as extracellular vesicles (EVs), are known to mediate cellular communication with the potential to promote protection against heat stress (HS). Previously, we have shown that intrafollicular-derived granulosa cells (GCs) release EVs, with the capacity to shuttle protective messages to induce thermotolerance in recipient GCs against recurrent HS *in vitro*. Therefore, here we aimed to investigate the ability of GC-derived EVs (GC-EVs) to modulate bovine oocytes tolerance to HS during *in vitro* maturation (IVM). For this, EVs were isolated from cultured granulosa cell-conditioned medium under normal (38.5°C) and thermally stressed (41°C) conditions using ultracentrifugation. At the time of maturation, cumulus-oocyte complexes (COCs) were arranged in a 2 x 4 factorial design for temperature (38.5°C or 41°C) versus supplemented treatments (non-treated controls [NTC], PBS Vehicle (VE), normal EVs [N-EVs], or stressed EVs [S-EVs]) at 20% of the total maturation medium. Statistical differences between mean values were compared using One-way ANOVA followed by Tukey's Multiple Comparisons Tests. Results indicate that S-EVs improve the survival of oocytes under thermal stress conditions by reducing reactive oxygen species (ROS) accumulation, improving mitochondrial function, and suppressing the expression of stress-associated genes thereby reducing the severity of HS on oocytes. Moreover, our findings indicate a carryover impact from the addition of GC-EVs during oocyte maturation in the development to the blastocyst stage with enhanced viability. Conclusively, EVs derived from GCs exposed to HS induce tolerance in oocytes against HS, thereby mitigating the negative impact of thermal stress on oocyte developmental competence. This research was supported by USDA-NIFA 2021-38420-34040.

Graduate Student/ Biomedical Sciences

O-51. The role of dietary intake and diversity of mother-infant pairs for malnutrition prevention in Guatemala.

Brigitte Pfluger, Alexis Giunta, Diva M Calvimontes, Molly Lamb, Roberto Delgado-Zapata, Elizabeth P Ryan

Malnutrition can have long-lasting consequences, and between 6 to 24 months of age is a period particularly sensitive to inadequate nutrition. Rice bran, a byproduct of rice milling, is a food ingredient rich in vitamins, amino acids, and prebiotics that promote healthy child growth. This 3-month study investigated the nutritional impacts of rice bran integration among 30 mother-infant pairs in Guatemala. Baseline and endline 24-hour dietary recalls were administered to mothers (≥ 18 years) and infants (6 to 24 months). Infant length and weight measurements were taken at baseline, midline, and endline and entered in Nutritionist Pro™. Heat stabilized rice bran was provided in manufacturer sealed packets (60g each) and distributed based on household size: <5 members received one packet (60g) per day and ≥5 members received two packets (120g) per day. To gauge household acceptability, minimal instructions were provided on rice bran dietary incorporation. At enrollment, most children (90%; n=27) were healthy and breastfed (80%, n=24). Females represented nearly half (47%) of infants. The average baseline infant length-for-age z-score was -0.6 (SD=1.1) and weight-for-age z-score was -0.7 (SD=1.0). Male infants had higher food consumption than females in 13 of 15 food groups, including animal-source foods (78-85% more). Rice bran was commonly added to atol (a traditional hot grain-based beverage), eggs, beans, and soups. Compared to baseline, 32% more mothers achieved minimum dietary diversity at endline, largely due to an increased bean (67% change) and meat (163% change) consumption. For 25 nutrients, maternal mean intake fell below dietary reference intakes for 17 (68%) at baseline and endline, Correlations will identify possible relationships between maternal and infant dietary intake and diversity with infant growth z-scores. Malnutrition prevention requires household-level participation. Research on dietary habits across the lifespan may inform household-level interventions and improve nutritional status.

Graduate Student/ Environmental and Radiological Health Sciences

O-52. SolaVAX: a whole virion vaccine platform produced via a novel inactivation method against coronaviruses.

Elizabeth Sullivan, Lindsay Hartson, Raymond Goodrich, and Izabela Ragan

During the last 20 years, three novel coronaviruses from the genus Betacoronavirus have emerged and caused a threat to public health, beginning with Severe Acute Respiratory Syndrome coronavirus (SARS-CoV-1) in 2002 and followed by Middle East Respiratory Syndrome (MERS) coronavirus in 2012 as well as the current pandemic causing virus, SARS-CoV-2, which emerged in 2019. With each outbreak the focus has been on producing a vaccine to individual coronaviruses. This has posed a challenge, due to the ability of coronaviruses to mutate rapidly and quickly. Within the last two years, there has been new interest in a "pan-coronavirus" vaccine that could protect not only against a wide range of coronaviruses but also any newly emerging coronaviruses. We previously published that an inactivated whole virion vaccine candidate for SARS-CoV-2 (SolaVAX) was able to protect against SARS-CoV-2 infection in an animal model (Golden Syrian Hamsters). In this study, we sought to lay the groundwork for developing a pan-coronavirus vaccine with MERS, SARS-CoV-2, and SARS-CoV-1 and to determine if vaccination cross-protected hamsters from homologous and heterologous viral challenge. We observed vaccination with SolaVAX alone produced neutralizing antibodies against SARS-CoV-1 and reduced viral burden in the caudal lung lobe. Additionally, we observed the combination of SARS-CoV-2 and MERS vaccines produced neutralizing antibodies against both viruses, as well as significantly decreased viral burden in both the cranial and caudal lung lobes. Lastly, we are the first to demonstrate that Golden Syrian Hamsters can generate neutralizing antibodies against MERS. Overall, our study has laid the groundwork for pursuing a whole virion multivalent pan-coronavirus vaccine. This project was funded by a CSU CVBMS College Research Council grant from fiscal year 2022.

DVM Student/ Biomedical Sciences

1. A physiologically based toxicokinetic model to predict the disposition and fate of N, N-Dimethylformamide in animals and humans.

Ibrahim F Alshammari, Brad Reisfeld

N, N-Dimethylformamide, also known as dimethylformamide (DMF), is used extensively in the production of acrylic fibers, films, leathers, and pharmaceutical products. Because of this widespread usage, humans are often exposed to the compound, particularly in an occupational setting, where the compound enters the body primarily through the respiratory and dermal systems. Evidence suggests that DMF exposure can cause liver toxicity, which may include necrosis, fibrosis, cirrhosis, autoimmune hepatitis, and hepatoma. In addition, other adverse effects, such as renal toxicity, liver toxicity, digestive system dysfunction, teratogenicity, and potential carcinogenicity, have been associated with exposure to this compound. Though occupational exposure limits (OELs) for DMF have been set by several countries, these values have often lacked a firm underpinning based on the chemical's biodistribution and dose response in the body. Such information can often be provided by a suitable predictive model that has been developed using appropriate physiological and biochemical principles and is underpinned by relevant data. The focus of this study was to develop such a model and to test it against available literature data arising from both animal experiments and human biomonitoring studies. We anticipate that such a model, when fully verified, will have significant benefits in helping to characterize the hazards posed by DMF and in safeguarding the health of humans exposed to this chemical.

Graduate Student/ Environmental and Radiological Health Sciences

2. Evaluation of the validity of joint stress tests in dogs with and without musculoskeletal disease.

Sarah L Anderson, Felix M Duerr, Lindsay H Elam

Localizing the source of lameness in veterinary patients can be challenging. Flexion tests are one commonly utilized diagnostic tool in equine orthopedic exams to aid in the identification of the clinically affected joint. Flexion tests involve holding a joint in a stressed, flexed position for a period of time, followed by visual gait analysis to evaluate for any exacerbation of lameness following manipulation. Some canine practitioners utilize flexion testing, but its validity has yet to be researched in dogs. Additionally, stressing a joint in flexion exclusively can create issues with canine anatomy as each joint cannot be effectively isolated. Thus, depending on the anatomic region, the researchers in this study stressed joints in flexion (forelimb joints and all digits) or extension (hindlimb joints). Normal dogs and dogs with confirmed musculoskeletal disease underwent joint stress testing with visual and objective gait analysis via pressure sensitive walkway to determine the validity of joint stress tests as a diagnostic tool. The authors hypothesized that: 1) normal dogs would be unaffected by joint stress testing, 2) dogs with musculoskeletal disease would demonstrate a worsened lameness following manipulation of only the clinically affected joint, and 3) visual gait scoring would correlate with objective kinetic parameters. An understanding of the validity of joint stress tests in dogs may benefit practitioners in the localization of lameness to facilitate the efficient prescription of appropriate diagnostics and ultimately treatment.

DVM Student/ Clinical Sciences

3. Dietary rice bran and navy beans are digestible during chemotherapy and beneficially-modulate fecal and plasma metabolomes in dogs undergoing chemotherapy.

Juan Aragon, Madison Tipton, Jennifer Thomsen, Sangeeta Rao, Nora Jean Nelson, Kelsey Moreland, Kristen Weishaar, Johnathan Stockman, Jan Schudolski, Elizabeth P Ryan

Chemotherapy can cause adverse gastrointestinal events in dogs and people. Dietary rice bran and navy beans have been shown to improve metabolism, while also reducing diarrhea and gut inflammation. The overall objective herein is to assess the impact of chemotherapy treatment on gut dysbiosis in dogs with lymphoma, and we hypothesized that a rice bran and navy bean based diet switch during chemotherapy will positively impact gut microbial metabolism. Dogs undergoing chemotherapy for lymphoma provided stool and blood samples that were analyzed for targeted metabolite panel of phytosterols and bile acids, and gut microbiota. The study protocol (VCS-2016-078) recruited 35 client-owned dogs into a 15-week chemotherapy regimen with or without a 6-week dietary intervention with a complete and balanced diet containing 22.5% w/v navy beans and 5% rice bran. Plasma and fecal samples were profiled at five timepoints for targeted analytes. A two-way repeated measures analysis of variance compared metabolite abundances over time. Significance was defined as $p<0.05$. The study diet was well-tolerated by dogs, with only mild, self-limiting, vomiting, and diarrhea reported sporadically throughout the six-week intervention and without any differences from a control group that did not experience a dietary change. A dysbiosis index was calculated for each dog with varied responses. The pre and post chemo analysis revealed increases in gut dysbiosis. Blautia genus level log DNA (-0.02), and beta-sitosterol (-0.02) fold changes were observed between start and end of chemotherapy. Blautia is associated with preventing inflammation, and has shown protective effects against carcinogenesis. Beta-sitosterol also has cancer protective effects, and is present in rice bran. While preliminary, these findings suggest that canine diets rich in rice bran and navy beans may be beneficial to dogs with cancer and merit continued attention as nutrient-rich feeds to maintain gut health during chemotherapy.

Undergraduate Student/ Environmental and Radiological Health Sciences

4. Notable characteristics of the oral microbiome of domestic cats infected with Feline Immunodeficiency Virus in the presence and absence of antiretroviral therapy.

Laura Bashor, Chris Kozakiewicz, Mary Nehring, Elisa Behzadi, Craig Miller, Jeffrey Kim, Megan Conry, Scott Carver, Jennifer Rawlinson, Zaid Abdo, Sue VandeWoude

Feline immunodeficiency virus (FIV) infection of domestic cats is the analogue of HIV infection in humans. Both viruses induce oral dysbiosis in untreated cats and humans, and primary clinical signs of FIV in cats include gingivitis and periodontal lesions. Furthermore, a number of opportunistic microorganisms that are found in the saliva of patients with HIV have been reported in the saliva of cats with oral disease. Despite the advent of highly effective combination antiretroviral therapy (cART) for HIV+ humans, oral disease manifestations still occur. To elucidate the mechanism underlying oral dysbiosis in the presence and absence of cART, we used 16S rRNA metagenomics analysis to investigate microbial communities in the oral cavities of three experimental groups of domestic cats: FIV positive receiving placebo (FIV+placebo), FIV positive receiving cART (FIV+cART), and healthy control cats (FIV-) (N=6/group). All cats were randomly assigned to groups, with 3 intact females and 3 neutered males per group. FIV-infected cats began treatment with placebo or with cART four weeks post-inoculation (Week 5) and gingival biopsies were collected from the three treatment groups at Weeks 0, 5, 11 and 24 and subjected to DNA extraction, 16S sequencing, and taxonomic classification. The sequencing error rate calculated by sequencing a mock microbial community standard was 0.000465%. Median sequencing depth was 59,523 reads per sample (N=78 samples). Data were clustered into operational taxonomic units (OTUs) and normalized using cumulative sum scaling. Characteristic oral phyla *Proteobacteria, Bacteroides, Firmicutes, Fusobacteria* and *Actinobacteria* were observed. Rarefied OTU richness was not significantly different among treatment groups; however, there was significantly lower richness in week 0 compared to the following weeks. This study provides unique temporal characterization of the FIV+ feline oral microbiome with and without antiretroviral treatment, revealing similarities to and key differences from the HIV+ human oral microbiome.

Graduate Student/ Microbiology, Immunology and Pathology

5. Are wild armadillos a reservoir for *leprosy bacilli* in Ecuador?

Lauren S Bennett, Ritika R Janapati, Charlotte Avanzi, Daniel Romero-Álvarez, Alaine Warren, Emily Cisneros-Vásquez, Melanie Cabezas-Moreno, Jacobus H de Waard, Carlos Bastidas-Caldes, Daniel Garzon-Chavez, Manuel Calcopiña, Mary Jackson

Leprosy is a neglected tropical infectious disease that causes both skin lesions and nerve damage. The disease is caused by both *Mycobacterium leprae* and *Mycobacterium lepromatosis*. The mechanism of leprosy transmission is unknown, but it is assumed that human-to-human transmission is the main route of infection. Nevertheless, *M. leprae* has been found in wild armadillos, in different countries of the Americas where the animal is endemic. In the US, zoonosis transmission has been reported in the past. This raises public safety concerns for regions where armadillos are endemic, and where humans and armadillos come into close contact. Ecuador is a country where armadillos are found in the wild and humans are infected with leprosy bacilli. However, leprosy bacilli have never been found in wild armadillos in this country. As a result, the objective of our research was to investigate the presence of *M. leprae* and *M. lepromatosis* in wild armadillos in Ecuador and to an extent to study the connection between humans and armadillos bacterial strains. Using DNA extractions and quantitative PCR, we have tested 82 tissue samples from 36 armadillos from 10 provinces and seven tissue samples from human patients from Ecuador for both *M. leprae* and *M. lepromatosis*. Our results show that 31/82 of the Ecuadorian armadillo samples (22/36 armadillos from eight provinces) and 6/7 human samples tested positive for *M. leprae*, and none tested positive for *M. lepromatosis*. Genotyping of the strains is ongoing. From these results, it can be concluded that *M. leprae* is present in the Ecuadorian armadillo population. Furthermore, our research suggests that regions with endemic armadillos should begin taking measures for public safety, such as testing humans for leprosy.

Undergraduate Student/ Microbiology, Immunology and Pathology

6. A retinal contribution to opioid-induced sleep/wake disturbances.

Nikolas Bergum, Jozsef Vigh

Melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) mediate the entrainment of mammalian sleep-wake rhythms to environmental light. Interestingly, recent mouse studies from our lab revealed that ipRGCs express μ-opioid receptors (MORs), the primary molecular target for opioid analgesics. Furthermore, MOR agonists can directly inhibit ipRGC firing, which could prevent ipRGCs from regulating sleep-wake rhythms in response to light. In humans, opioid metabolites can be detected within eye following opioid use. However, it remains unclear whether opioids accumulate in the mouse retina following systemic exposure. To confirm that morphine reaches the mouse retina following systemic delivery, we collected tissue (retina and serum) from the adult male mice at different time points over a 24-hour period following 20 mg/kg intraperitoneal morphine injection(s). Morphine levels in serum and retina were measured using tandem liquid chromatography-mass spectrometry. Importantly, results from this study show that systemically administered morphine accumulates in the mouse retina. Additionally, we implanted mini-telemetry devices into mice to assess how chronic morphine alters their sleep-wake behavior. To establish the role of ipRGCs in opioid-induced perturbations in sleep-wake behavior, we performed these experiments in wildtype mice along with mice lacking MORs exclusively in ipRGCs (McKO). Results from these studies reveal that McKO exhibit decreased morphine-induced locomotion compared to controls, which implicates MORs expressed by ipRGCs as a mediator of opioid-induced sleep-wake alterations. Taken together, these findings support the idea that opioid that accumulate in the eye persistently activate MORs on ipRGCs, altering the ability of ipRGCs to transmit light information to the brain's sleep-wake circuitry. This alteration in photic input to the brain could underlie some of the sleep/wake problems associated with long-term opioid use.

Graduate Student/ Biomedical Sciences

7. Mutation to the Synaptotagmin Calcium Binding Pocket Produces Congenital Myasthenic Syndrome-like Symptoms in Drosophila.

Andrew Bollegar, Morgan Litchford, Vincent Elias, Noreen Reist

Neuronal communication is mediated by activity-dependent synaptic transmission. Depolarization of an active nerve terminal results in calcium influx that triggers the fusion of neurotransmitter-filled synaptic vesicles with the plasma membrane. This fusion event depends upon synaptotagmin, the presynaptic calcium sensor, binding calcium to initiate fast and synchronous vesicle fusion events. Synaptotagmin is a vesicle protein containing two calcium binding pockets, C2A and C2B, that coordinate calcium via 5 highly conserved negatively-charged resides, predominantly aspartate residues. An amino acid substitution within the C2B domain of synaptotagmin 2, the isoform found at the neuromuscular junction, has been identified in a patient with congenital myasthenic syndrome (CMS). Specifically, aspartate 301 has been replaced with a glutamate (D1E); the effect of this substitution on synaptotagmin function has not been previously examined. In this study we sought to determine whether a homologous aspartate to glutamate substitution in Drosophila would cause CMS-like deficits in the fly, thereby indicating a role for this substitution in CMS etiology. To mimic expression in the human patient, we expressed a syt-C2B-D1E mutation in synaptotagmin heterozygotes – one wild type gene and one mutant gene. To measure the physiological effect of the D1E mutation, we completed a series of electrophysiological and behavioral analyses. To ensure any deficits were a result of the mutation and not due to disruptions in protein expression levels, we conducted Western analyses. We found that evoked transmitter release and overall activity levels were decreased in syt-C2B-D1E mutants compared to controls. These findings are consistent with the decrease in neuromuscular transmission and overall weakness seen in the CMS patient. Our results indicate that the C2B-D1E substitution induces CMS-like symptoms in Drosophila and support the hypothesis that this synaptotagmin substitution is involved in etiology of congenital myasthenic syndrome.

Graduate Student/ Biomedical Sciences

8. Chinese water dragons and goldfish as hosts for maintaining *Burkholderia pseudomallei*.

Anneliese Bruening, Linzy Jauch, Richard Bowen

Burkholderia pseudomallei is the cause of melioidosis, a prominent bacterial disease in Australia and Southeast Asia. In addition to causing severe human disease, this bacterium also has an extremely broad host range for many domestic and wild animal species such as goats, pigs, and sheep. Infection of *B. pseudomallei* occurs through contact with contaminated soil or water. There have been a few documented cases of iguanas that have been infected with *B. pseudomallei*, as well as an incident of transmission of *B. pseudomallei* to a human from a contaminated fish tank that contained imported tropical fish. Although infection of ectotherms is possible, it has been an understudied area for this bacterium. The purpose of these pilot studies was to examine the persistence of *B. pseudomallei* in fish tank water and its effects on goldfish and Chinese water dragons. We hypothesized that *B. pseudomallei* would colonize the fish tank water and fish, but the fish would not show overt disease and that the lizards would become infected regardless of the route of inoculation. We infected two groups of water dragons with *B. pseudomallei*, one orally and one subcutaneously, and performed necropsies to analyze the tissues for signs of infection. The fish tank was contaminated by the inoculation of *B. pseudomallei* into the water and was sampled routinely for culture. *B.* pseudomallei was isolated from the fish tank for several days but cleared rapidly. The water dragons were found to be highly susceptible to infection and developed characteristic abscesses, especially prominent in the liver. Individuals owning these species should be aware of the risk of possible infection of these reptiles or fish, especially if imported from endemic areas.

DVM Student/ Biomedical Sciences

9. Quantitative assessment of genes involved in early lymphopoiesis using droplet digital PCR (ddPCR) to distinguish immature lymphoid neoplasms in the dog.

Thomas G Burnett, Robert C Burnett, Janna A Yoshimoto, R Adam Harris, Anne C Avery

The DNA nucleotidylexotransferase (*DNTT*) gene encodes an enzyme involved in random addition of nucleotides at the junction of rearranged immunoglobulin and T cell receptor gene segments. *DNTT* expression is restricted to normal and neoplastic pre-T and pre-B lymphocytes and is used in human diagnostics to identify immature lymphoid neoplasms. Currently, markers of immature hematopoietic tumors are limited in veterinary diagnostics and a commercialized cross-reactive DNTT antibody is not available for veterinary species. We sought to develop a quantitative assay for measuring *DNTT* gene expression using droplet digital PCR (ddPCR). Total RNA was extracted from peripheral blood samples obtained from 5 clinically healthy dogs and 4 dogs with CD34+ acute leukemia(s) submitted to the Clinical Hematopathology laboratory for immunophenotyping. Post first strand cDNA synthesis, ddPCR was performed to measure gene expression of *SDHA* (HEX-labeled), *DNTT* (FAM-labeled), and *CD34* (FAM-labeled). *SDHA,* a housekeeping gene, was used as a reference to determine the ratios of gene expression for *DNTT* or *CD34*. *CD34* gene expression was measured to serve as a positive control. The proportion of CD34+ cells identified by flow cytometry was correlative with *CD34* gene expression ($r^2 = 0.60$). All acute leukemia samples overexpressed *DNTT* and *CD34*, compared to normal controls (p value < 0.05). The ranges of gene expression ratios for both *DNTT* and *CD34* were narrow for normal samples (*CD34* range = 0.0 – 0.01, *DNTT* range = 0.0-0.01), but considered wide for the acute leukemia samples (*CD34* range = 0.02 – 1.3, *DNTT* range = 0.7-7.9). This data provides evidence that genes involved in early lymphocyte development can be quantitatively measured using ddPCR in dog samples. Assessment of *DNTT* expression in other hematopoietic malignancies is warranted to further evaluate the specificity of this assay.

Undergraduate Student/ Microbiology, Immunology and Pathology

10. Canine osteosarcoma as a translational model for advancing solid tumor CAR T cell therapy.

Jennifer W Cao, Jessica Lake, Renata Impastato, Lyndah Chow, Jade Kurihara, Dylan Ammons, Ashley Yingst, Michael Verneris, Steven Dow

Canine Osteosarcoma (OS) has been used as a translational model for pediatric OS due to similar presentation, molecular markers and clinical progression with high rates of metastasis to the lungs. The checkpoint molecule B7-H3 is upregulated in human and canine OS and correlates with poor prognosis, increased metastasis and decreased tumor infiltrating lymphocytes in both species. CXCL8 a chemokine that binds to CXCL2 is secreted in high amounts by a proportion of both human and canine OS which correlates to poor prognosis and greater metastatic potential. Chimeric Antigen Receptor (CAR) T cell therapy allows for the targeting of a specific surface antigens to generate an adaptive immune response. Despite being able to achieve complete remission in patients with B cell malignancies, clinical trials in solid tumors have not shown the same favorable outcomes. Immune suppression by myeloid cells within the tumor microenvironment (TME) and CAR T cell homing from the circulation to the tumor are challenges unique to solid tumors. This study aims to develop metastatic canine osteosarcoma as a translational model to evaluate enhancing the efficacy of CAR T cell therapy by immune suppressor cell depletion and increasing CAR T cell signaling by dual valent B7-H3-CXCR2 CAR. We found that dual valent CAR T cells were activated by canine B7H3 positive tumors with increased activity against high CXCL8 secreting tumors. CAR T cell activity was evaluated by secreted proinflammatory cytokines, direct tumor killing and migration to CXCL8 secreting tumor cells. Repurposed drugs losartan and propranolol in combination significantly decreased the infiltration of immune suppressive TAMs within xenograft canine OS tumors in SCID-beige mice.

DVM/PhD Student/ Clinical Sciences

11. Examining the Vital Vascular Network: Evaluation of the Microvasculature and Endothelial Glycocalyx in a Canine Hemorrhagic Shock/Resuscitation Model.

Jenna H Cardillo, Kristin Zersen, Julien Guillaumin

The Endothelial Glycocalyx (EGC) role in normal and impaired perfusion needs further investigation. Sublingual videomicroscopy by sidestream dark field imaging (Glycocheck™) allows analysis of microcirculation and perfused boundary region (PBR), an EGC thickness surrogate. Our goal was to investigate the microcirculation and EGC in a canine hemorrhagic shock model. Seven dogs were anesthetized. Blood was removed until mean arterial pressure (MBP) reached 40mmHg or 60% blood volume was removed. Microcirculatory variables red blood cell flow (Flow), total vessel density (TVD), capillary blood volume relative and absolute (CBV_{rel}, CBV_{abs}), and PBR were evaluated at baseline (T1), hemorrhagic shock (T2), and post-resuscitation (T3). Normality was tested using the Kolmogorov-Smirnov test. Impact on Flow, TVD, CBV_{rel}, CBV_{abs} and PBR was tested with analysis of variance for repeated measures with Bonferroni correction. Median age was 5.0 years (3-5). Mean body weight was 8.8±1.6 kg. Mean blood withdrawn was 35±12 ml/kg. MBP was 72±4 mmHg, 45±6 and 72±7 mmHg at T1, T2, T3, respectively. Flow was 271.6±38.0 µm/s, 177.1±17.0 µm/s, and 305.9±50.9 µm/s, at T1, T2, T3, respectively (p=0.039). TVD was 230.4±30.4 mm/m², 441.9±246.9 mm/m², and 240.9±30.7 mm/m² at T1, T2, T3 respectively (p=0.4). CBV_{rel} was 1.2±0.06 10^3µm³, 1.3±0.04 10^3µm³, and 1.3±0.06 10^3µm³ at T1, T2, T3, respectively (p=0.16). CBV_{abs} was 15.7±2.6 10^3µm³, 25.5±11.7 10^3µm³, and 14.6±2.9 10^3µm³ at T1, T2, T3, respectively (p=0.423). PBR was 2.2±0.1 µm, 2.3±0.09 µm, and 2.3±0.1 µm, at T1, T2, T3 respectively (p=0.89). Our hemorrhagic shock model induced reversible decrease in Flow. PBR was unchanged. Type-II error is possible.

Resident/ Clinical Sciences

12. Got Stim? Check the Brainstem.

Emily A Castellanos, Stuart A Tobet

The vagus nerve (VN) is a major component of the autonomic nervous system. It influences multiple components of visceral sensation, motor activity, and homeostasis. This nerve passes through the neck into the thorax and abdomen, and to the ear as its auricular branch. Electrical stimulation of the vagus auricular branch has been proposed as a non-invasive alternative neuromodulatory therapy for treating disorders, such as depression. Unlike the invasive method of cervical vagal nerve stimulation where surgery is done to stimulate the entire nerve, activation of the auricular branch stimulates a small portion of the VN. The cervical branch of the VN synapses through the nodose ganglia, directly going to the caudal portion of the nucleus of the solitary tract (NTS), while the auricular branch of the vagus synapses through the jugular ganglion where there are afferent inputs to the NTS. To begin mapping the chemoarchitecture of the likely auricular vagus targets in the brainstem, mice brains labeled with initial neuropeptide targets: calcitonin gene-related peptide (CGRP), Glutamate Decarboxylase 67 (GAD67), and Tyrosine Hydroxylase (TH). The distribution of each substance was seen in various brainstem nuclei involved in autonomic regulation, like the NTS. Ongoing studies are examining other neurochemical markers along with mapping cFOS activation as a function of auricular vagal nerve stimulation and the underlying chemoarchitecture. Therefore, this study reinforces previous literature of cellular components in the brainstem and will establish activity of the auricular branch of the vagus nerve.

Graduate Student/ Biomedical Sciences

13. Investigating T cell responses to an orally delivered probiotic vaccine platform.

Christian Cherry, Ben Swartzwelter, Kayl Ecton, Callie Lang, Allison Vilander, Gregg Dean

The development of vaccines against emerging pathogens is critical to the health of humans and animals. Desirable features of a vaccine platform include rapid engineering, inexpensive manufacturing, logistically simple storage and distribution, and induction of robust, durable immune responses. Most pathogens infecting animals and humans enter through mucosal tissues. The mucosal immune system is highly capable of controlling local infections, and mucosal vaccines effectively induce both systemic and mucosal immune responses. Our group has focused on the use of the probiotic organism *Lactobacillus acidophilus* (LA) as an orally delivered vaccine platform. LA is an ideal mucosal vaccine platform because it survives the gastric acid and bile, accesses immune inductive sites, and interacts with critical pattern recognition receptors. We have engineered LA to express antigens of pathogens relevant to humans and animals and shown induction of antigen specific immune responses. While our previous studies indicate effective generation of antibody-mediated immunity, little is known about T cell induction by LA vaccine constructs. T cells are responsible for clearing virus infected cells, inducing antibody responses, and driving the immune response through the production of various cytokines. Here, we develop a model to evaluate T cell responses to an orally delivered LA vaccine construct using flow cytometry to identify changes in critical T cell subsets following LA vaccination. This study provides important information for the development of future probiotic and/or orally delivered vaccines against pathogens infecting humans and animals. A better understanding of T cell response induction by this vaccine platform will improve rLA vaccine design.

DVM/PhD Student/ Microbiology, Immunology and Pathology

14. The Detection of *Plasmodium* Species in Anopheles Vectors in Burkina Faso, Africa to Determine the Role of *Plasmodium* Species in Malaria Epidemiology.

Kathryn L Coffin, Anna-Sophia Leon, Molly Ring, Paula Lado, Emanuel Sougue, Greg Pugh, Sunil Parikh, McKenzie Colt, Brian Foy

Malaria is a vector-borne disease transmitted by mosquitoes of the genus *Anopheles* caused by *Plasmodium* parasites. It affects individuals worldwide, particularly those within the African region. RIMDAMAL II is a clinical trial designed to determine the efficacy of adding ivermectin mass drug administrations to the standard malaria control measures with the aim of reducing the incidence of uncomplicated malaria episodes in children. The trial occurred in Burkina Faso, one of the countries with the highest malaria incidence, and lasted from 2019 to 2020. During RIMDAMAL II, *Anopheles* mosquitoes were captured, preserved, and sent to our lab for analysis. The majority of mosquitoes were identified as *An. coluzzii, An. funestus* s.s, and *An. gambiae* s.s. Three different *Plasmodium* sporozoites species were detected in head and thorax samples of the mosquitos: *P. falciparum* (78/2477 in *An. gambiae* sl and 6/216 in *An. funestus* complex), *P. ovale* (24/2477 in *An. gambiae* sl, and 14/216 in *An. funestus* complex), and *P. malariae* (10/2477 in *An. gambiae* sl and 6/216 in *An. funestus* complex). Coinfections of *P. ovale* and *P. falciparum* were also detected in *An. gambiae* sl (2/2477). The most prevalent *Plasmodium* species detected in *An. gambiae* sl. Mosquitoes was *P. falciparum*. However, in *An. funestus* mosquitoes, a high sporozoite rate of *Plasmodium* minor species (*P. ovale* and *P. malariae*) was observed. The detection of high rates of minor malaria species signifies that malaria epidemiology in Burkina Faso might be more complicated than initially thought.

Undergraduate Student/ Microbiology, Immunology and Pathology

15. Blood serum-derived extracellular vesicles as a potential peripheralization mechanism for Chronic Wasting Disease in White-tailed deer.

Jesse Cole, Erin McNulty, Audrey Sandoval, Amy Nalls, Joseph Westrich, Candace Mathiason

Chronic Wasting Disease (CWD) is a rapidly spreading, invariably fatal protein misfolding or prion disease of cervid species (deer, elk, moose and reindeer), that has expanded in strain designation, geographic location, and host range. CWD is the most efficiently transmitted of all the prion diseases and is currently detected in captive and free-ranging cervid populations in 30 U.S. States, 4 Canadian Provinces, Europe, and Asia. Transmission of CWD has been largely attributed to horizontal transmission by direct animal-to-animal contact with bodily secretions (saliva, blood, urine and feces), and by indirect contact with the infectious agent shed in these products to the environment. Prion diseases manifest as a posttranslational modification of the host-encoded cellular prion protein (PrP^{c}) as it converts to an aberrantly folded, partially proteinase-resistant disease associated form (PrP^{Sc}). Extracellular vesicles (EVs) are nano-sized vesicles (30-150nm) known to be released from virtually all cell types and have been demonstrated to facilitate intercellular communication via transport of RNA, lipids, and proteins to other cells. Currently, *in vitro* studies have suggested that EVs may facilitate dissemination of both PrP^{c} and PrP^{Sc} in other prion-infected species. To begin to unravel mechanisms associated with CWD peripheralization in the host, we investigated whether blood serum-derived extracellular vesicles can be a potential transport mechanism of PrP^{c} and PrP^{Sc}. Here, EVs were isolated from blood serum collected from experimentally CWD-infected white-tailed deer. Nanoparticle tracking analysis (NTA) was performed to quantify the size distribution and concentration of the EV isolates. Western blot and real-time quaking induced conversion (RT-QuIC) were used to assess the presence and seeding activity of the EV isolates, respectively. These studies will provide the basis for continued studies determining CWD peripheralization in the host and permit further investigation of EVs as a potential biomarker for CWD diagnostic testing.

DVM/PhD Student/ Microbiology, Immunology and Pathology

16. Mosquito tracking through larval barcoding.

Kaleb A Davis, Natalie Wickenkamp, Julius Stuart, Ashlyn Chen, Arielle Glass, Quinn Hitchcock, Robert Fathke, Will Schlatmann, Christopher Snow, Rebekah C Kading

Monitoring of mosquito dispersal is a strong area of current interest for researchers as this information is vital for the control of mosquito borne pathogens. Currently this information is gathered using mark-release-recapture methodology through the application of fluorescent dyes to the mosquito's surface. This method can be unreliable and negatively impact mosquito behavior, survivability and is limited by the number of fluorescent dyes available. To address these shortfalls, we have developed a novel method of mosquito marking. The objective of this study was to field-validate this novel mosquito tagging technique in the context of seasonal West Nile virus (WNV) surveillance in Fort Collins, Colorado. This novel method involves the use of nano-porous microcrystals which readily adsorb synthetic DNA oligonucleotide barcodes. These DNA-loaded microcrystals are ingested by larval stage mosquitoes when they are placed in their larval habitat effectively marking individual mosquitoes with the DNA barcode. These barcodes are readily scalable and as such can be tied to information such as dose date and location. In our field trial 14 bins which mimic a natural larval habitat were placed in 4 locations to be colonized. These were then dosed with micro-crystal on a weekly basis. Testable samples of field caught mosquito homogenate were supplied through the existing West Nile Virus surveillance network and tested for barcode presence via. Polymerase Chain Reaction. Analysis of the barcodes with Next Generation Sequencing will allow us to determine the point of larval origin and to estimate age as well. This information can then be applied to vector control efforts allowing for more targeted control of the target populations. In our field trials we have demonstrated that these crystals can both be introduced and recovered from wild mosquito populations in a real-world setting.

Undergraduate Student/ Microbiology, Immunology and Pathology

17. Glial inflammation in aging canines.

Alice Droeger, Amelia Hines, Lisa Mulligan, Breonna Kusick, Amanda Latham, Brittney MacQuiddy, Stephanie McGrath, Julie A. Moreno

Tracking human-canine interactions through the centuries shows how canines have been experiencing parallel lifestyles beside their guardians. These companions are enabling a closer look at neurobiological aging processes. When observing aging of humans and canines, both display gliosis which is increased reactivity and inflammatory signaling of both astrocytes and microglia within the central nervous system. An increase of microglia and astrocyte activation has also been noted to increase susceptibility to neurodegenerative diseases; such as Alzheimer's disease (AD) and its comparable canid-disease called canine cognitive dysfunction (CCD) syndrome. The correlating pathology of aging, as well as the similar lifestyle shared between these two species suggests that the canine acts as an innovative translational comparison between the biological aging process and both progressive neurodegenerative diseases: AD and CCD. By using immunohistochemistry (IHC) to analyze various age groups of young and old canines, we hypothesize that both astrocytes and microglia numbers and inflammation will increase in older canines. We find that in the frontal cortex of older canines there is increased astrogliosis measured by S100b, as well as an increase in microglia reactivity detected by Iba1, in comparison to younger canines. These findings will further our understanding of aging and neuroinflammation in both canines and humans. Further directions for this study will include looking at misfolded proteins such as phosphorylated tau and amyloid beta in canines with CCDS.

DVM Student/ Environmental and Radiological Health Sciences

18. Role of the CD40/Th40 inflammatory pathway in canine autoimmune diseases.

Caity Erickson, Rae Isdale, Gisela Vaitaitis, Dan Waid, David Wagner, Craig B Webb, Tracy L Webb

Both humans and canines present with a variety of conditions having an autoimmune etiology. A number of these diseases appear similar between species, and advancements in the diagnosis and treatment of these conditions may have significant translational implications. Current treatments often involve non-specific immune suppression with drugs that have significant side-effects. Ideally a therapeutic target would be identified that could be specifically disrupted at the molecular level with minimal side-effects. The CD40 receptor on effector T-cells (Th40) is a central component of the autoimmune process in a variety of diseases and therefore is potentially a critical target for molecular intervention. Wagner et al. have designed a peptide, derived from the CD40 ligand (CD154), that has proven to be both safe and effective as a CD40 receptor modulator in a murine model of Type 1 diabetes mellitus and is currently under investigation in Type 1 diabetic dogs. This study seeks to determine the degree of CD40 expression on CD4+ T cells in dogs with autoimmune disease compared to healthy controls in order to identify those diseases most likely to be responsive to this novel therapeutic. Clinical cases of naturally-occurring, untreated canine immune-mediated disease as well as two groups of control animals (healthy dogs and dogs with osteoarthritis, a non-immune-mediated, chronic inflammatory disease) are being recruited into the study. Whole blood is collected and processed for determination of CD40 expression on CD4+ T cells (Th40 level). To date, 19 control dogs, including 4 dogs with osteoarthritis, have been enrolled. Preliminary data show that control dogs have significantly lower Th40 levels than diabetic dogs ($p<0.0001$), osteoarthritis may not be associated with increased Th40 levels, and sex and age may not affect Th40 levels. Patient enrollment for all groups is ongoing.

DVM Student/ Clinical Sciences

19. Omacetaxine reduces c-MYC expression and demonstrates antitumor effects in osteosarcoma.

Kristen B Farrell, Douglas H Thamm

Omacetaxine mepesuccinate (OMX), also known as homoharringtonine, is a protein translation inhibitor approved for use in chronic myeloid leukemia. Recent studies suggest OMX may be effective against other cancer types with high rates of protein translation, and may be successful against protein targets that are difficult to inhibit by reducing their translation rates. Using both canine and human osteosarcoma (OS) cell lines, we have investigated the efficacy of OMX against osteosarcoma and expression of c-MYC. OMX provides significant growth inhibition of OS cells with IC_{50} values in the low nanomolar range. OMX also reduces migration and invasion capabilities of OS cells. Strikingly, we observed reduction in levels of c-MYC protein in most canine and human OS cell lines after OMX exposure. This reduction was both dose and time dependent. We also investigated the efficacy of OMX against OS in an orthotopic mouse OS model. OMX is a promising candidate for treatment of OS in both dogs and humans, and potentially additional cancers dependent on c-MYC.

Post-doctoral Fellow/ Clinical Sciences

20. The use of cannabidiol as adjunct therapy for refractory idiopathic epilepsy in 51 dogs: a double-blinded crossover design.

Brooke T Gallagher, Aaron J Rozental, Daniel L Gustafson, Breonna R Kusick, Isabella Corsato Alvarenga, Sangeeta Rao, Lisa R Bartner, Stephanie McGrath

Approximately 30% of dogs with idiopathic epilepsy are refractory to treatment. Recent studies have suggested cannabidiol (CBD) may be an effective anticonvulsant in dogs with idiopathic epilepsy, but more evidence is required. The objective of this study was to evaluate the effect of adding CBD to conventional antiepileptic drugs (AEDs) on seizure control. Fifty-one client-owned dogs diagnosed with idiopathic epilepsy of tier II confidence were enrolled in this study. The study was conducted as a double blinded placebo-controlled crossover design, where 12 dogs were orally administered 5mg/kg/day of a highly purified CBD oil, and 39 dogs were orally administered 9mg/kg/day. Dogs were randomly assigned to receive either CBD suspended in hemp seed oil or vehicle for three months, and then switched to the opposite oil following a one-month washout period. Total number of seizures and seizure days (clusters over a 24-hour period) were recorded. Bloodwork was performed periodically throughout the trial. Data were analyzed as repeated measures over the course of three months on each oil using the generalized linear mixed model procedure from statistical analysis software (SAS v 9.4). There was no statistically significant effect on seizure frequency at the 5mg/kg/day dose ($P > 0.10$). At the 9mg/kg/day dose, dogs on CBD had a 3.31% increase in total number of seizures from baseline, while dogs on placebo had an increase of 30.72% ($P=0.0360$). There was a 24.1% reduction in seizure days for dogs receiving CBD compared to a 5.8% increase in dogs receiving placebo ($P=0.0017$). Liver enzymes (alkaline phosphatase and alanine aminotransferase) increased with both doses of CBD administration. CBD oil may be effective in controlling seizure days compared to placebo when administered orally at dose of 9mg/kg/day. This supports further studies investigating the role of CBD in the treatment of idiopathic epilepsy in dogs.

DVM/MPH Student/ Clinical Sciences

21. Comparison of C-reactive protein measurement on three assays and following storage.

Amanda Garrick, Jennifer Hawley, Tracey Wangler, Russell Moore, Michael Lappin

C-reactive protein (CPR) is a pentameric protein made by hepatocytes, the production of which is stimulated by various inflammatory cytokines. CRP concentrations are commonly used to detect inflammation and monitor systemic inflammatory disease syndromes. There are multiple methods of measuring CRP in serum, and there is no clear consensus on the most sensitive and specific assay for canine samples. The utility of CRP measurement in samples stored over time is also contended as this peptide is thought to degrade and lose diagnostic relevance when stored for extended periods. The objectives of this study were to assess the degradation of CRP when stored at -80° C as well as to compare the results of three different commercially available CRP assays. Sera were originally analyzed on a commercially available ELISA titrated for use with canine samples (Abcam). The sera were stored for approximately 14 weeks at -80° C until assayed in this study. After being thawed at room temperature, the sera were assayed again in the ELISA as well as in 2 other assays (Randox Canine CRP and Gentian CRP-G) being evaluated in the Clinical Pathology Laboratory. Of the 20 samples re-tested in the same ELISA, 18 returned with an increased CRP concentrations following storage, with elevations ranging from 24 to 2000%. While the CRP concentrations determined by the three different methods varied greatly for some samples, results of the assays were generally grouped within ranges. The causes of the increases in CRP concentrations after storage or the variation amongst the 3 assays are unknown. The results of these experiments suggest that if CRP measurement is used in the management of clinical cases, the assay used in serial monitoring should be consistent.

Resident/PhD Student/ Clinical Sciences

22. Retrospective investigation of the correlation between clinical cause of death and necropsy cause of death due to malignant neoplasia in a tertiary academic hospital population.

Molly K Gasparini, Audrey Ruple, Douglas H Thamm, Brittney Sanfacon, Olivia Uzan, Kate Vickery

The purpose of this retrospective study was to evaluate if the clinical cause of death (cCOD) correlated with the necropsy cause of death (nCOD) in dogs that died or were euthanized due to clinical concerns related to malignant neoplasia. The cCOD was defined as the clinical reason for humane euthanasia or patient death while the nCOD was defined by the final necropsy report. Clinical records and necropsy reports of all dogs that died or were euthanized and received a post-mortem examination at CSU Veterinary Teaching Hospital between 2016-2019 were reviewed. cCOD was determined by review of the medical record. Necropsy reports were reviewed and determined if nCOD was related to malignant cancer, other systemic causes, or unknown causes. Correlation of malignant cancer as a cCOD with nCOD was evaluated. 526/1,129 (46.5%) dogs had a cCOD of neoplasia, of which 457/526 (86.8%) had a necropsy which confirmed malignant cancer. 69/526 dogs (13.1%) had a necropsy which did not correlate with cancer as a cCOD. Irrespective of age and weight there was a high correlation of malignant neoplasia as a clinical and necropsy cause of death.

Resident/ Clinical Sciences

23. Peripheral high-dose *Mycobacterium tuberculosis* infection induces neuroinflammation and reduces the integrity of the blood-brain barrier.

Charlize E Geer, Amanda S Latham, David F Ackart, Amelia D Hines, Randall J Basaraba, Julie A Moreno

More than ten million people fall ill with tuberculosis (TB) every year, a disease caused by infection with *Mycobacterium tuberculosis* (Mtb). TB continues to be a global health threat, especially with the rise of antibiotic resistance. Mtb is intensely immunogenic, and respiratory infection leads to production of pro-inflammatory cytokines and activation of peripheral immune cells. In rare cases, Mtb crosses the blood brain barrier (BBB) and manifests as a more deadly form of disease known as tuberculosis meningitis (TBM), but data shows that patients are predisposed for neurodegenerative disease and cognitive deficiencies even without a diagnosis of TBM. Our preliminary data also shows that guinea pigs peripherally infected with aerosolized Mtb demonstrate gliosis and neurotoxicity compared to uninfected controls. We hypothesize that in a high dose guinea pig model, the peripheral immune response to Mtb impacts the central nervous system, including reducing the integrity of the BBB and causing neuroinflammation, without dissemination of bacteria to the brain. Dunkin Hartley guinea pigs were infected by aerosol with a high dose of hypervirulent Mtb. After fifteen days, the animals were euthanized and immunofluorescent staining was used to characterize BBB integrity and biomarkers of neuroinflammation. Through staining for Collagen IV, we found a weakened BBB in several of the high-dose guinea pigs compared to uninfected controls. Additionally, activation of the complement cascade and increased glial cells were identified in infected animals. These findings demonstrate that CNS damage in TB patients may occur due to a strong peripheral immune response crossing the BBB and pushing resident glial cells into a pro-inflammatory state.Through these findings, we will obtain a better understanding of how peripheral Mtb infection alters the brain.

Undergraduate Student/ Environmental and Radiological Health Sciences

24. The Effects of Prostatic Extracellular Vesicles on Canine Frozen-Thawed Semen Function and Viability.

Alexandra M Hafey, Mindy A Meyers, James K Graham, Fiona K Hollinshead

Reproductive success after artificial insemination (AI) with frozen-thawed (FT) semen is highly variable in dogs. Even the most effective freezing methods result in impaired sperm function which negatively impacts fertility. Cryoinjuries to sperm include damaged plasma membranes, increased membrane fluidity, and premature acrosome exocytosis which results in reduced longevity and viability. It has been shown that addition of prostatic fluid (PF) to FT canine sperm had a positive effect on motility and resulted in larger litter sizes and improved conception rates after AI. Extracellular vesicles (EVs) are nanoparticles secreted by all cells that mediate cell communication via their cargo. EVs produced by prostatic cells may play a physiological role in canine sperm function. In this study our specific aim was to reverse the effects of cryoinjury to FT sperm with the addition of EVs isolated from PF collected from young dogs. Frozen-thawed semen from 3 young and 3 old dogs were extended in capacitating media without EVs (Tx 1), with EVs (Tx 2) and with PC12 (Positive Control) and incubated at 37°C. Samples were removed at 0, 1, 3, 6 and 24h, assessed for sperm motility using microscopy/CASA, and sperm viability (PI), acrosome integrity (FITC-PNA), and membrane fluidity (MC540) using flow cytometry. To assess sperm function, a perivitelline membrane (PV) binding assay was developed for canine sperm. We found that the addition of EVs to FT canine semen reduced both membrane fluidity and the proportion of sperm that bound to the PV membrane. In conclusion, the addition of PF EVs to FT canine semen improved longevity and function. Therefore, addition a of PF EVs may improve the reproductive performance of FT canine semen.

DVM Student/ Biomedical Sciences

25. Osteosarcoma exosome priming of alveolar macrophages promotes formation of a pre-metastatic niche.

Laurel A Haines, Eric P Palmer, Sophi J Schofield, Kathryn E Cronise, Daniel P Regan

Osteosarcoma (OS) is the most common primary malignant tumor of bone and has a high incidence in children and adolescents. This disease frequently progresses to a highly fatal metastatic form that frequently affects the lungs. Treatment options for individuals with metastatic OS have not significantly improved in nearly four decades beyond protocols that employ highly toxic chemotherapies. The development of novel therapies is limited by our understanding of the underlying mechanisms driving tumor growth in the lungs. It has been suggested that prior to circulating tumor cell arrival, resident cells at a metastatic site are "primed" to support tumor cell seeding and outgrowth by factors secreted by the primary tumor. These factors promote a tumor-permissive microenvironment known as a "pre-metastatic niche". Among these secreted factors are nano-sized extracellular vesicles called exosomes which are known to elicit tumor-promoting changes in tissue-resident cells in several other metastatic cancer types. However, the role of exosomes in modulating the lung microenvironment during OS is not well understood. Our data shows that OS exosomes display a specific tropism for the lung, supporting their hypothesized role as early drivers of pre-metastatic niche formation. We hypothesize that resident alveolar macrophages (AMs) are a target of OS exosomes and that primed AMs subsequently orchestrate tumor-permissive immunological and structural changes in the lungs. To investigate this, we evaluated OS exosome biodistribution in mice using intravital imaging, multi-parameter flow cytometry, and immunofluorescence. We also investigated the effects of OS exosomes on the lung microenvironment in mice and in primary human donor-derived AMs using RNA sequencing, multiplex cytokine analysis. We show that OS exosomes can be taken up by resident AMs and elicit distinct changes in tumor-promoting cytokines. Our findings demonstrate a novel role for AMs as drivers of pre-metastatic niche formation and identify potential therapeutic targets.

DVM/PhD Student/ Microbiology, Immunology and Pathology

26. Leishmania major inactivation using riboflavin and ultraviolet light.

Jack Hall, RJ Leverett, LM Hartson, RP Goodrich, IK Ragan

Leishmaniasis is a vector-borne, parasitic disease that currently has no licensed vaccine for use in humans. The World Health Organization estimates over 350 million people are at risk of infection and about 700,000-1 million new cases and 20,000-30,000 deaths are reported annually. L. major is a cutaneous strain of the disease. This study seeks to employ Mirasol, a pathogen reduction technology, to produce a killed whole parasite vaccine. Mirasol uses riboflavin and ultraviolet light to reduce pathogen load in blood products. Based on previous work with other pathogens, we hypothesized that 1J/ml would inactivate a parasite load of up to 1e6 (or 10^6) parasites/ml in media, using the Mirasol device. By exposing aliquots of L. major to increasing concentrations of energy from the Mirasol device, then checking these samples for regrowth of parasites in one week intervals, the efficacy of parasite killing by the Mirasol device may be determined. By completing a kinetic curve we have demonstrated the 1J/ml dose completely inactivated all parasites and no parasite replication has been seen for 7 days. Successful inactivation of L. major parasites could be a forerunner of producing a killed whole parasite vaccine against leishmaniasis..

DVM Student/ Biomedical Sciences

27. Local immune responses to radiation therapy and myeloid cell-targeted therapy in naturally occurring canine sinonasal carcinoma.

Cullen Hart, Thomas Lee, Braden Burdekin, Daniel Regan, Steven Dow, Mary-Keara Boss

Sinonasal carcinoma (SC) is an aggressive cancer. We are conducting a canine SC trial to test whether stereotactic body radiotherapy (SBRT) and myeloid cell-targeted drugs (propranolol, losartan) will decrease myeloid cell-associated immunosuppression in the tumor microenvironment compared to SBRT alone. Canine SC patients were randomized to 1) SBRT or 2) SBRT + propranolol and losartan (SBRT-PL). Tumor biopsies and nasal lavage samples were collected pre-treatment and 2-weeks post-SBRT. Hematoxylin and eosin (H&E) pathologic review, immunohistochemical (IHC) quantification of tumor infiltrating immune cells, and flow cytometric analysis of immune cells collected via nasal lavage were performed. From H&E slides (n=2/group), tumors treated with SBRT alone had increased immune cell infiltration at 2-weeks compared to pre-treatment, while the tumors treated with SBRT-PL showed minimal to no change in immune cell infiltration. With IHC quantification (n=1/group), the immune cell densities of the tumor treated with SBRT were 7.33% macrophages, 3.97% regulatory T cells (TRegs), 1.56% T cells, and 0.04% B cells; the tumor treated with SBRT-PL yielded 14.16% macrophages, 1.07% TRegs, 0.78% T cells, and 0.03% B cells. The percentages of immune cells collected via nasal lavage for the dog treated with SBRT were 94.25% neutrophils, 0.25% macrophages, 2.46% monocytes, 0% Tregs, 0.87% CD8 T cells, 0.01% CD4 T cells; the dog treated with SBRT-PL had 49.50% neutrophils, 47.6% macrophages, 0.33% monocytes, 0% TRegs, 0.06% CD8 T cells, 0.37% CD4 T cells. From this preliminary data, tumors treated with SBRT had increased immune cell infiltration at 2 weeks compared to those treated with SBRT-PL. SBRT-PL-treated tumor had a greater density of macrophages and lower densities of TRegs and T cells compared to the SBRT-treated tumor. From nasal lavage at 2-weeks, the dog treated with SBRT-PL had lower percentage of neutrophils and greater percentage of macrophages compared to the dog treated with SBRT.

Graduate Student

DVM Student/ Environmental and Radiological Health Sciences

28. Effects of long-term cannabidiol administration in dogs.

Annie Hess, Isabella Corsato Alvarenga, Stephanie McGrath

Interest in the use of cannabidiol (CBD) to treat epilepsy, inflammation, anxiety, and other conditions in veterinary patients is increasing. However, the long-term tolerability of CBD supplementation in dogs remains unknown. Based on previous research, chronic administration of CBD is expected to cause mild gastrointestinal events, as well as an elevation in alkaline phosphatase (ALP). Cannabidiol in a carrier oil was administered at a 0 (control, just carrier oil), 5 and 10 mg/kg doses to 18 dogs (n=6) in a complete randomized design to determine the effects of long-term CBD administration (ongoing study). Monthly physical and blood exams were conducted to monitor dog health. Adverse events including abnormal fecal scores were recorded. Liver enzymes data were analyzed as repeated measures over time using the GLIMMIX procedure in SAS (v 9.4). Dogs receiving the 10 mg/kg dose had higher ALP values than the placebo, and the ALP values of the 5 mg/kg CBD were similar to the extremes ($P < 0.05$). Other blood parameters were within normal reference ranges and the dogs appeared clinically healthy. Additionally, all treatment groups had abnormal fecal scores, with dogs dosed with 10 mg/kg CBD having the most episodes of loose stool. The elevation of serum ALP values in dogs administered CBD without changes in other liver enzymes may indicate that no observable hepatocellular damage is occurring. However, further studies investigating hepatocellular integrity in response to CBD administration are needed to fully understand CBD's impact on hepatocellular activity.

DVM Student/ Clinical Sciences

29. Modulation of embryonic oxidative stress response to mitigate oxidative damage during the preimplantation period and beyond.

Emma A Hessock, Ahmed Gad, Nico G Menjivar, and Dawit Tesfaye

The use of technologies such as *in vitro* fertilization (IVF) has rapidly increased over the past twenty years both in the livestock and human industry. Current IVF practices produce inferior embryos compared to *in vivo* derived with increased exposure to oxidative stress (OS) postulated as a major contributor to this inferiority. Previous studies by our lab have identified nuclear factor erythroid 2-related factor 2 – kelch-like ECH-associated protein 1 (NRF2-KEAP1) as a pathway differentially expressed between *in vivo* and *in vitro* exposed embryos with a role in protecting the embryo during suboptimal conditions. We hypothesize that activation of NRF2 during the bovine preimplantation period through genetic suppression of KEAP1, a NRF2 inhibitor, or supplementation of quercetin, a pharmacological NRF2 activator, will lead to increased embryo survival under induced OS and additionally diminish the effects of OS on methylation and gene expression of the embryos. Three methods will be used to increase NRF2 activity in the bovine preimplantation embryos: CRISPR-Cas9 mediated KEAP1 knockout, siRNA KEAP1 knockdown, and pharmacological NRF2 activator quercetin. Bovine embryos will be produced from cumulus-oocyte complexes subjected to typical *in vitro* maturation conditions and *in vitro* fertilization using sexed semen. Embryos will be cultured at 20% oxygen tension and subjected to one of the three methods or its corresponding control treatment. Day 8 blastocysts will be collected and used for different analyses to determine reactive oxygen species accumulation, mitochondrial activity, DNA methylation, and transcriptome profile. We believe increasing NRF2 activity through either disruption of the KEAP1 gene activity or pharmacological activation will promote survival of preimplantation embryos under OS conditions and reveal genes and regions of the genome which may be protected by these approaches and play a role in mitigating long term impacts of OS during the preimplantation period on fetal growth and overall offspring health.

Graduate Student/ Biomedical Sciences

30. Effects of short and long term frozen storage of recombinant hamster prion protein in real time quaking induced conversion assay.

Parker Hogan, Nathaniel Denkers, Candance Mathiason, Edward Hoover

Real-time quaking induced conversion (RT-QuIC) is a prion amplification assay currently used as a novel diagnostic tool for human and animal transmissible spongiform encephalopathies. Compared to ELISA and immunohistochemistry, the gold standards of chronic wasting disease (CWD) prion detection, RT-QuIC has demonstrated a 10^5 fold greater sensitivity. A major hurdle in bringing RT-QuIC to the forefront of prion research diagnostics is the generation and storage of bulk substrate, i.e. recombinant hamster prion protein (rhaPrP), for the assay. Deep freeze storage of our in house generated rhaPrP has successfully been validated, yet the effects of long term storage and a single freeze/thaw cycles on the proteins use in the RT-QuIC assay has not been explored. The purpose of this study was to establish how stable and consistent thawed rhaPrP was after short (3-4 months) or long (18-22 months) term storage by assaying the protein neat or pre-treating with centrifugation or filtration. Additionally, we analyzed the use of iron-oxide beads (a prion concentrating method) to further assess protein stability in RT-QuIC. Amyloid formation rates (reaction rate) from CWD positive and negative brain homogenates were then compared to never frozen rhaPrP. Preliminary results on the frozen protein, regardless of duration, revealed no significant difference (Mann-Whitney test), between the never frozen data rates and the post thaw rates. The use of centrifugation and more notably filtration post thaw reduced false positive rates in negative controls without decreasing the sensitivity of the RT-QuIC assay. These findings indicate that rhaPrP, when frozen, is currently stable for up to 2 years and that filtration post thaw maintains the consistency and sensitivity of the assay. Ongoing experiments are being conducted to provide insight on the reproducibility of these results.

Undergraduate Student/ Microbiology, Immunology and Pathology

31. A New Way to Think about Recovery.

Dakota D Hunt, Sam DieCecco

One of the characteristics of addiction is a strong attentional bias for drug cues. Attentional biases for drug cues play an important role in individuals with drug-seeking behavior and recurrent relapse. In non-clinical research, arbitrary stimuli has been shown to immediately capture attention when associated with rewards. This investigation explains how attentional biases for arbitrary reward-associated stimuli predict drug dependence. This research claims that the attentional components of addiction are reflective of a normal cognitive process and promotes reward-seeking behavior. In essence, after a few dozen trials of an associative learning task, any individual can demonstrate some of the most noticeable attentional features of a drug-addicted person. When an arbitrary stimulus is repeatedly paired with a reward in an associative learning task, it can acquire an attention capturing quality resembling that of drug cues, similarly the drug cue can overpower the individuals intentions to ignore it and focus on something else (arbitrary reward). In this way, an addicted individual can be trained to associate arbitrary rewards with drug-seeking behavior to avoid drug use.

Undergraduate Student/ Other

32. Incidence of suspected blood transfusion reactions in the Veterinary Teaching Hospital.

Rae Isdale, Kris Kofron, Kaci Shaw, Sarah Shropshire, Terri Ward, Michael Lappin

Blood transfusions are frequently administered as life-saving treatments for a variety of disease syndromes resulting in anemia in dogs. Our community-based blood donors are screened for common infectious disease agents, blood typed, and the blood is collected aseptically in anti-coagulants for storage until needed. When indicated, major and minor cross-matching prior to administration is also performed in attempt to lessen transfusion reactions. Blood transfusion reactions are occasionally suspected and when reported to the committee, the cause of the reaction is investigated thoroughly. The objective of this study was to report the incidence of suspected blood transfusion reactions in dogs seen at the Veterinary Teaching Hospital over a 16-month period. A medical record search was performed to identify dogs administered a whole blood product to correlate with those suspected to have a transfusion reaction. In the time-period studied, 470 dogs received at least one blood transfusion and of these dogs, a suspected reaction was recorded in the medical record for 6 dogs (1.3%). In the time period studied, reported transfusion reactions were uncommon. The medical records are in review to provide further specific information concerning the likely causes of the suspected reactions.

Staff/ Clinical Sciences

33. Nonspecific stimulation of mucosal immunity and protection against severe COVID-19 disease in hamsters.

Linzy Jauch, Anneliese Bruening, Richard Bowen

The Δ *capB* strain of *Francisella tularensis* is a highly attenuated bacterium that has been used as a vector for producing several vaccines against Tier 1 select agents *Bacillus anthracis, Yersinia pestis, Burkholderia pseudomallei*, and *Francisella tularensis.* Previous studies involving the Δ *capB* vector indicated that this vector by itself afforded some non-specific protection against severe COVID-19 disease. The aim of this study was to test the hypothesis that intranasal administration of the Δ *capB* vector would stimulate a non-specific mucosal immune response that and provide respiratory resistance to a variety of pathogens, including SARS-CoV-2. Golden Syrian hamsters were inoculated intranasally with Δ *capB* at various time intervals prior to intranasal challenge with SARS-CoV-2. Efficacy in providing protection was based on measurements weight loss, virus shedding, virus titers in lung and turbinates, and lung pathology. Plaque assays were conducted on daily oral swabs and samples of nasal turbinates and lung tissue upon necropsy to assess viral load. Significant reduction in viral titers of Δ *capB*-treated hamsters was not demonstrated, but treatment with this vector seven days prior to challenge significantly reduced weight loss, thereby offering non-specific immune protection against severe disease. The data suggests that successful non-specific stimulation of mucosal immunity may provide the respiratory system with resistance against a variety of pathogens and has the potential to be utilized in future production of inexpensive intranasal vaccinations..

DVM Student/ Biomedical Sciences

34. Investigation of four methods to identify infectious ulcerative keratitis in horses; standard microbiology culture, real-time polymerase chain reaction (RT-PCR), next-generation DNA sequencing (NGDS), and corneal cytology.

Katrina EV Jones, Michael R Lappin, Joshua B Daniels, Michala de Linde Henriksen

Diagnosis of infectious ulcerative keratitis in horses is currently reliant on cytology and aerobic bacterial culture. Prompt identification and diagnosis are necessary to prevent the progression of the disease. The aim of this study was to determine the diagnostic utility (speed and specificity) of four diagnostic methods for bacterial and fungal ulcerative keratitis in the horse. The four diagnostic tests to be evaluated were aerobic bacterial and fungal culture, corneal cytology, real-time polymerase chain reaction (RT-PCR) for Aspergillus fumigatus and Candida albicans, and next-generation DNA sequencing (NGDS). Ten client-owned horses were involved in this study, all were presented to the comparative ophthalmology service at CSU-VTH for infected ulcerative keratitis from July 2021 to July 2022. A complete ophthalmic examination, aerobic bacterial and fungal culture, corneal cytology, NGDS (MicroGen Vets LLC), and samples collected and stored (-80C freezer) for later RT-PCR analysis, were performed for all horses. Fisher's exact test and one-way ANOVA were used as the statistical analysis method, and a P-value < 0.05 was statistically significant. Results demonstrated that 60% (n=6) had infectious organisms noted on cytology, 50% (n=5) had positive growth on aerobic bacterial culture, 10% (n=1) had positive growth on fungal culture (Penicillium sp.), and 30% (n=3) had positive identification of infectious organisms on NGDS. No significant differences were found between culture versus NGDS, culture versus cytology, nor NGDS versus cytology (all P-values>0.05). Only one case was positive for fungal culture, but this case was negative on the RT-PCR. A significant difference was found between collection time until result, with cytology being the fastest, followed by culture, followed by NGDS (all P-values<0.05). This is the first study to evaluate four different methods for diagnosing infectious ulcerative keratitis in horses. Based on the results of the current study, cytology and aerobic culture were upheld as superior diagnostic methods.

DVM Student/ Clinical Sciences

35. Waste not want not: Piloting a clinical waste audit at a university veterinary teaching hospital.

Caroline Kern-Allely, Malea McGimsey, Tiera McAdam, Valerie Cortes, Stacey Baumgarn, Gregg Griffenhagen, Colleen Duncan

Waste is one of the core pillars of sustainability. Considerably less is known about the amount and type of waste generated in the delivery of veterinary care compared to human medicine. The objective of this project was to develop, and pilot, a waste audit protocol for veterinary medicine that could be adapted to a variety of veterinary settings and assist clinics to meet their waste reduction targets. We conducted a multi-day observational review of the Colorado State University Veterinary Teaching Hospital small animal surgery and anesthesia units to determine items used during routine surgeries. Metrics included total weight, number of bags and individual counts for specific items of concern (e.g. syringe casings) as well as items with sustainable alternatives (e.g. surgical gowns). Frequencies and percentages of waste by waste audit material category were calculated. Over the three-day period, we collected 41 bags of non-recyclable waste and 4 bags of expected recyclable waste generated during 26 surgeries. Overall, 66.7% of expected recyclables were truly recyclable with 33.3% misplaced non-recyclable waste. Of the 158.3 kg of waste collected, 18.4% were "un-sortable or contaminated" trash. The most common type of waste was non-reusable fabrics (40.4%, 63.5 kg), followed by plastics (33.2%, 52.6 kg). Despite waste being a top sustainability issue in veterinary medicine, there is a need for better education on how waste is managed within veterinary practice to optimize existing resources through behavior modification. Education can address the observed improper sorting of non-recyclable and recyclable waste, and use of high waste impact items instead of reusable alternatives. To meaningfully address (rethink, reduce, reuse, recycle, and research) waste management in veterinary medicine, we need to better understand its footprint.

DVM Student/ Microbiology, Immunology and Pathology

36. Connection: Osteoarthritis and Tuberculosis.

Heidi Kloser, Karen Dobos, Brendan Podell, Julie Moreno, Lizzy Creissen, Taru Dutt, Pablo Maldonado, Kristina Tran, Amanda Hitpas, Kelly Santangelo, Marcela Henao-Tamayo**

**Co-corresponding authors*

Osteoarthritis (OA) is an inflammatory disease of the joints that burdens 273 million people globally. It is a multifactorial disease with many risk factors including: injury, genetics, age, sex, and obesity. However, no known direct/systemic infectious causes have been identified. Tuberculosis (TB), primarily a lung disease caused by *Microbacterium tuberculosis* (Mtb), newly affects over 10 million people per year. TB can exhibit extrapulmonary disease and is associated with systemic inflammation. We hypothesize that this systemic inflammation may be a contributing factor to OA. To explore this, we examined mice and guinea pigs infected with TB for accelerated signs of OA. Animals were infected with Mtb via aerosolization. TB-infected mice were compared against age-matched controls for behavior/mobility measures using overhead enclosure monitoring with ANY-maze software. Guinea pig joints were examined via histopathology. Mice that were severely burdened with TB exhibited decreased mobility in measures of speed, time mobile, and distance; correspondingly, they exhibited increased time in their security hut. Other mice that were infected with Mtb but did not experience as high of a burden had decreasing trends in distance traveled and speed. Guinea pigs that had been infected with Mtb had accelerated signs of OA in the knee joints when examined via histology; specifically, increased synovitis and loss of proteoglycan were observed in comparison to control animals. Our work is the first to investigate if TB influences the development of OA. Findings from this pilot study suggest that TB is associated with altered animal behavior/mobility, as well as acceleration of OA pathogenesis. Next steps will include: examining the prevalence of non-pulmonary TB in the joints and; determining whether direct or indirect influences of systemic inflammation from infection contribute to OA-like conditions in these animal models.

Graduate Student/ Microbiology, Immunology and Pathology

37. Sphingolipid biosynthesis pathways are altered during flavivirus infection.

Hannah Laurence, Laura St. Clair, Michael Spedding, Francis Platt, Carissa Drake, David Priestman, Paul Soma, Rushika Perera

Flaviviruses (FVs), which include dengue, Zika, and West Nile viruses, are growing global health threats that lack effective vaccines for prevention or antivirals for treatment. Upon infection, flaviviruses have previously been shown to alter the morphology of host cell membranes suggesting that FVs affect host lipid metabolism. Ceramide, a molecule that belongs to a group of bioactive signaling molecules called sphingolipids, forms both a structural component of host cell membranes and is also utilized in cellular signaling pathways. Ceramide is produced either through de novo synthesis, or through degradation of complex sphingolipids in the salvage and sphingomyelinase pathways. Alterations in sphingolipid metabolism have been associated with pathology resulting from flaviviral infections. In addition, certain components of the pathways have been shown to bind to flaviviral non-structural proteins in affinity purification-mass spectrometry analysis. We hypothesize that flaviviruses alter expression of enzymes in sphingolipid synthesis pathways to confer an advantage over the host. We employed siRNA knockdown in human hepatoma (Huh7) cells of multiple key enzymes involved in ceramide production followed by infection with dengue virus, serotype 2, and assessed the effects on viral replication using plaque assays. Knockdown of enzymes in the salvage pathway, including glucocerebrosidase 1 and glucocerebrosidase 2 (GBA1, GBA2) showed opposing effects on viral replication, with GBA1 being anti-viral and GBA2 being pro-viral, and knockdown of UDP Glycosyltransferase 8 (UGT8) being antiviral. In addition, 3-Ketodihydrosphingosine Reductase (KDSR), an enzyme from the de novo synthesis pathway, was shown to have an antiviral effect. Confocal microscopy is being used to assess co-localization of KDSR, UGT8, GBA1, and GBA2, with nonstructural proteins 3 and 4A of dengue and Zika viruses. These studies will help deepen our understanding of the mechanisms flaviviruses employ to gain an advantage over the host cell during infection. Current results will be presented.

Resident/Post-doctoral Fellow/ Microbiology, Immunology and Pathology

38. The functional impact of ubiA mutations associated with chronic *Mycobacterium abscessus* infections – implications for host adaptation.

Elena Lian, Juan M Belardinelli, Zuzana Palčeková, Mary Jackson

Mycobacterium abscessus is an environmental mycobacterium found ubiquitously in the environment that has recently emerged as a human pathogen. To better understand the transition of *M. abscessus* from opportunistic to emerging pathogen, unraveling the mechanisms facilitating host adaptation is key. One gene of interest is *ubiA*. In *M. abscessus* isolates from chronically infected patients, *ubiA* was found to be mutated at a frequency higher than expected by chance, indicating *ubiA* may help *M. abscessus* adapt to the human host. The encoded enzyme, UbiA, catalyzes the synthesis pathway for the sole arabinose donor used by mycobacteria to make two structures essential for the integrity of the mycobacterial cell envelope: arabinogalactan (AG) and lipoarabinomannan (LAM). We hypothesize *ubiA* is under evolutionary pressure to alter the cell envelope and the consequent interactions between the mycobacterium and the host, thereby facilitating *M. abscessus* adaptation to and persistence in the human host. For this study, four isogenic strains expressing clinically relevant *ubiA* mutations were generated in *M. abscessus* subspecies *abscessus* ATCC 19977. We assessed the biochemical and physiological consequence of the selected *ubiA* mutant strains to evaluate the impact of the mutant on the AG and LAM structure and mycobacterial growth, respectively. Current results indicate a loss/reduction of an epitope in LAM from certain *ubiA* mutant strains which has implications for potential immune evasion in infection models. Additionally, at least two of the four *ubiA* mutants have physiological consequences, with one consequence being an unexpected enhancement in biofilm formation. The results to be presented indicate the functional impact of the *ubiA* mutations on *M. abscessus* may alter infection dynamics in favor of the mycobacterium. This work was supported by the Cystic Fibrosis Foundation

Graduate Student/ Microbiology, Immunology and Pathology

39. Implantation and early placentation in the mare: the role of kisspeptins in trophoblast invasion.

Linda Jennifer Lott, Jennifer S Palmer, Samuel R Fisher, Rao Veeramachaneni, Christianne Magee

The invasion of trophoblast cells and subsequent formation of endometrial cups is a crucial step in equine fetal development. Yet, much remains to be elucidated about this process in the context of kisspeptins. Equine placental invasion is similar to that of humans, in which kisspeptins and their receptor have been shown to regulate the invasion of trophoblast cells. As the cells penetrate the maternal endometrium, the response resembles that of metastatic lesions, which kisspeptins control. During this intrusive process, maternal and fetal communication is vital in order to procure a successful implantation. Consequently, in humans, a change in kisspeptin concentrations has been correlated with early complications during pregnancy. Therefore, understanding the role of kisspeptins during equine trophoblast invasion may provide a novel application in assessing compromised pregnancies. Using immunohistochemistry, samples of embryos and uterine biopsies from six pregnant mares at 30-, 36- and 40-days post ovulation, will be stained and compared to non-pregnant mare samples. This will provide information on the maternal-fetal interface: prior, during and after invasion of the endometrium. Localizing kisspeptins and their receptor, relative to the invading trophoblast cells, would establish their role in regulating invasion in the horse. The results of this study will therefore not only provide key information about equine fetal development but provide an opportunity for clinical application as well.

DVM Student/ Biomedical Sciences

40. Emergent prion disease in Swedish moose causes remarkably rapid disease in mice.

Diana C Lowe, Julianna Sun, Sehun Kim, Jenna Crowell, Emma Raisley, Bailey Webster, Glenn Telling

Chronic Wasting Disease (CWD) is a neurodegenerative disease of cervids (deer, elk, moose, reindeer and red deer) caused by prions, which are aberrantly folded forms of the normal, host-encoded prion protein (PrP). CWD has a long incubation period and is extremely contagious, constituting a growing endemic situation for cervids in North America. The zoonotic risk of CWD transmission to humans is uncertain, but animal prion diseases have shown a potential to cross the species barrier, illustrated by the outbreak of mad cow disease in the United Kingdom in the mid-1980s. In addition, CWD has recently emerged in Northern Europe and recent studies demonstrate, that it constitutes a sporadic, unstable type of CWD, with distinct properties from its North American counterpart, posing an unpredictable threat to wildlife in the region and potentially to humans. To study the characteristics of this new strain, we inoculated moose brain material in a mouse model of CWD and followed the kinetics of disease until onset of clinical signs. Collected brain tissues were analyzed by western blot, histology features by immunohistochemistry and conformational stability by the presence of resistant PrP^{Sc} after guanidine hydrochloride treatment. Survival distributions were compared using the long-rank mantel-cox test and a non-linear least-squared fit to compare conformational stabilities using the Prism analysis software. We show that Swedish moose CWD presents unique transmission properties, diagnosing clinical signs at ~90 days, the fastest progress to disease ever reported for a model of CWD. Other properties like conformational stability and histological features, were remarkably different from North American CWD and other strains in Europe, demonstrating the growing diversity of emergent CWD strains. Future studies will determine additional properties of this CWD strain which can shed light on the mechanisms of evolution of prion diseases in the wild and their zoonotic potential.

Graduate Student/ Microbiology, Immunology and Pathology

41. Improving the reproductive success in the Black-Footed Ferret using new minimally invasive Assisted Reproductive Techniques.

Emily Lugo, Fiona Hollinshead, Greg Burns, Barbara Wolfe

Assisted reproductive technologies (ARTs), such as semen banking and artificial insemination, have helped bring the black-footed ferret (*Mustela nigripes*, BFF) population back from the brink of extinction. At present, electroejaculation and laparoscopic artificial insemination are the most commonly used ART methods for the propagation of this endangered species. While both procedures are successful, they are invasive and require special equipment and sterile facilities. There is a clear need to build upon the foundational success of these methods and optimize the ART approach in BFFs so that these procedures can be more easily carried out in field settings. Recent ART developments in cats and dogs include transcervical insemination and the collection of semen via urethral catheterization and pharmaceutical induction of ejaculation. We are utilizing domestic ferrets as a model to explore the efficacy and success rates of these ART procedures in ferret species with the aim of improving the cost efficiency, field friendliness, and success of ART in black-footed ferrets. By introducing techniques that are easier to conduct and less invasive, this work aims to advance the recovery of the black-footed ferret by more rapidly increasing the population size and genetic diversity of this North American icon.

DVM Student/ Clinical Sciences

42. A Retrospective Review of Chemotherapy-Related Extravasation Events in Dogs and Cats.

Elise Martens, Rachel Hritz, Craig Clifford, Christine Mullin, Corrine Camero, Kai-Biu Shiu, Catherine Chan, Chelsea del Alcazar, Carol DeRegis, Lindsay Donnelly, Bryan Marker, Katarzyna Purzycka, Kate Vickery

There is a paucity of information in the veterinary literature pertaining to the treatment and outcome of chemotherapy extravasation injury. The aim of this retrospective, descriptive study was to evaluate outcomes and complications resulting from extravasation of chemotherapy in dogs and cats. Retrospective review of records from multiple contributing institutions were evaluated. Dogs or cats with a suspected extravasation event from chemotherapy administration were included. Information obtained included: signalment, tumor type, drug extravasated, method of drug administration, treatment for extravasation, and side effects. 20 dogs and 3 cats were included. The most common cancer type was lymphoma (10). The most common drug extravasation was doxorubicin (8), followed by carboplatin (5). Eighteen extravasations occurred at a specialty hospital. According to VCOG-CTCAE criteria, 6 animals had a grade III (4 carboplatin, 2 vincristine) and 4 animals had a grade IV (3 doxorubicin, 1 carboplatin) skin toxicity. Amputation would have been required in two dogs, but once client elected humane euthanasia instead. Both of these dogs received doxorubicin at their primary veterinarian, and both were treated with dexrazoxane. 11 animals received treatment with a neutralizing agent. At least 19 animals had short-term side effects, which included: erythema, edema, swelling, ulceration, necrosis, cellulitis, or bruising. 13 animals had no long-term side effects. Of animals with long-term side effects, they were mild and consisted of alopecic, scarring and a permanent scab. Most extravasation injuries were moderate in grade, with the majority being grade II or III.

Resident/ Clinical Sciences

43. Characterization of Retroviral Elements in Jamaican Fruit Bats.

Brittany N Martin, Sue VandeWoude, Tony Schountz, Coby A McDonald

Bats are a diverse group of mammals recognized for their role as zoonotic reservoir hosts that transmit numerous pathogenic viruses in the apparent absence of clinical disease. One hypothesis explaining this phenotype is that endogenous retroviruses (ERVs) have conferred viral tolerance. ERVs are retroviral elements integrated into host genomes as proviruses, which are vertically transmitted through the germline. Specifically, ERVs can introduce variation in gene expression, resulting in heritable immunological changes. For this reason, immunity derived from ERVs may play a vital role in bat immune system evolution, relating to virus-tolerant phenotype. While a handful of retroviral infections and ERVs of bats have been defined, retroviral sequences in the Jamaican fruit bat, *Artibeus jamaicensis*, have not been studied. Building on prior studies that identified retroviral transcripts in cells derived from clinically normal colony-reared *A. jamaicensis*, we develop a qPCR assay to measure proviral copies in host DNA samples. Housekeeping gene GAPDH was used as a diploid control. Our results demonstrated that squirrel monkey gamma retrovirus-like ERVs were present in high abundance in normal spleen DNA. Based on GAPDH Ct values, we estimated a range of 6-25 copies of ERV (mean = 12 copies), indicative of the number of ERV genomes per *A. jamaicensis* cell. Further analysis will include quantifying ERV in an array of host tissues, and evaluation of ERV mRNA to assess gene expression in different cell types. These preliminary findings will provide pilot information for future studies evaluating impact of bat ERV on susceptibility to infectious diseases.

DVM Student/ Microbiology, Immunology and Pathology

44. Don’t cry wolf on Echinococcosis: retrospective analysis of a neglected zoonosis in US patients with regional canine spillover risk.

Treana C Mayer, Mary Ellen (Ellie) Krienke, Andrés F Henao-Martinez, Susan VandeWoude

Echinococcosis is a zoonotic parasitic disease found in people and animals with potentially fatal complications. Both a globally neglected disease and regionally emergent in the US, wild and domestic canids serve as definitive hosts responsible for transmission to other species, including people. This disease risk complicates wolf reintroduction efforts despite our limited epidemiologic understanding in the US. Our objective is to begin addressing the gaps in our knowledge of Echinococcus spp. transmission to US human patients. Retrospective analysis was conducted with de-identified diagnosis codes for human echinococcosis from 2002-2022 in a federal multi-healthcare network database (TriNetX). Analyses included Kaplan-Meier survival and specific parasite species cohort comparisons with descriptive summaries of demography and co-morbidities, and odds ratios for interventions (surgery, antiparasitics, cyst aspiration) and complications (anaphylaxis, sepsis, etc.) Over 36,000 US patients were diagnosed with echinococcosis in the past twenty years. Most cases had an unspecified parasite species, primarily affecting pediatric patients (average age 16y +/- 10y) in the South/Southeast US, with very few interventions or medical complications reported. Patients with specific parasite diagnoses (N=500) had higher rates of mortality (11.7% at 10y), surgical (8.8%) and medical (18.2%) interventions, and complications (2%-17.2%), with demographic differences noted. Our findings are contrasted with reported case series in other countries. This study revealed a higher-than-expected burden of echinococcosis in the US, confirming it is an under-recognized disease. Greater than 99% of all human cases occurred outside current wolf ranges, suggesting other canid hosts may be more important reservoirs. Future studies will address gaps regarding the origin of unspecified infections, with targeted surveillance of domestic dogs in high-risk areas.

Post-doctoral Fellow/ Microbiology, Immunology and Pathology

45. Evaluation of the nutritional content in senior vs adult dog food diets.

Cynthia Melgoza, Camille Torres-Henderson

There is limited information regarding how the nutritional needs of senior pets differ from those of adult pets. Currently the Association of American Feed Control Officials (AAFCO) does not have any specific requirements for diets labeled as senior dog food. As a result, diets formulated for senior dogs can vary depending on the philosophy of the pet food manufacturer. This prompted our study to evaluate nutritional content differences between senior dog food and adult dog food. The caloric density, protein, fat, fiber, moisture, ash and mineral content of diets labeled for senior dogs were compared to diets intended for adult dogs. 31 diets labeled for senior dogs and 30 diets labeled for adult dogs were randomly selected from local pet stores and sent to Midwest Laboratories for complete proximate analysis including minerals. Diets nutritional content was variable between brands. 11 of 61 diets submitted did not meet AAFCO requirements on a dry matter in at least: phosphorus, potassium, magnesium or calcium. Fat on a g/1000 kcal basis was significantly lower (p-value: 0.0003) in diets labeled for seniors than diets labeled for adults. Carbohydrates on a g/1000 kcal basis were significantly higher (p- value: 0.0132) in diets labeled for senior dogs than diets labeled for adult dogs. Crude fiber was numerically higher in senior diets than adult diets with a p-value of 0.0501. No other significant differences were found in crude protein or other minerals. Diets marketed for seniors are significantly different from adult diets in fat and crude fiber, but senior diets are very variable in nutrient content between brands. Veterinarians should not make general statements to switch dogs to any senior diet and should ensure that a specific brand's senior diet meets the appropriate nutrient profile that is best for the patient.

DVM Student/ Other

46. The potential role of behavioral testing in the diagnosis of canine cognitive dysfunction.

Lisa P Mulligan, Julie A Moreno, Evan L MacLean, Breonna Kusick, Stephanie McGrath

Canine Cognitive Dysfunction (CCD) is a disease akin to Alzheimer's disease in humans that strikes the aging population. The prevalence of CCD ranges from 14-35% of senior dogs. It presents with reduction in activity, decreased social interactions, disruption in sleep-wake cycles, increased wandering and disorientation, and house-soiling. Currently, there is unfortunately no definitive antemortem test to diagnose CCD. Owner completed surveys, the Canine Dementia Scale (CADES) and Canine Cognitive Dysfunction Rating (CCDR) scales, are commonly used to identify dogs with apparent cognitive impairment, but there are no objective behavioral tests that have been validated to aid in diagnosis. Interestingly, recent studies utilizing cognitive testing have demonstrated feasibility in older dogs. Therefore by using a battery of behavioral tests to evaluate memory and executive function in dogs with and without signs of cognitive dysfunction, we aim to assess their ability to accurately diagnose CCD and to correlate the findings with biochemical markers in blood and CSF. Dogs in multiple age groups with and without signs of CCD will undergo serial neurological exams, blood and CSF collection, surveys, and behavioral testing every three to six months for two years. To assess for potential biomarkers, levels of glial fibrillary acidic protein (GFAP), amyloid-b neurofilament light chain protein and phosphorylated tau protein will be measured in the blood and CSF. In summary, the availability of simple, objective testing may enable earlier diagnosis and allow for monitoring of response to treatment and progression of disease. Further, advances in the diagnosis of CCD may catalyze advances in Alzheimer's disease research.

DVM Student/ Clinical Sciences

47. Longitudinal SARS CoV-2 identification in human stool and relationships with systemic immune activation markers.

Nicole Natter, Maddie Tipton, Kristen Otto, Abby Veath, Pankaj Trivedi, Emily N Gallichotte, Emily Fitzmeyer, Michael C Young, Gregory Ebel, August Luc, Jim Huang, Carol Wilusz, Taru Dutt, Stephanie M LaVergne, Kim McFann, Julie Dunn, Elizabeth Ryan

According to the WHO severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) has infected over 640 million since its emergence in December of 2019. Diagnostic tests for SARS-CoV-2 typically involve nasopharyngeal (NP) and saliva biospecimen analysis alongside wastewater testing for community spread. While mechanisms for viral clearance by the immune system is active research, a recent meta-analysis revealed that the persistence of SARS-Cov-2 detection in stool for months after infection is less understood. A cohort of adults from Northern Colorado with confirmed SARS-CoV-2 infection (n=152), and adults without history of infection (n=27) provided written informed consent to providing stool samples over the course of six months to one year following infection (NCT04603677). Of 185 participants enrolled, 152 adults provided at least one stool sample. 44.1% of adults, including one control participant, had at least one positive detection for SARS-CoV-2 via ddPCR or real-time PCR in the stool after their initial NP pcr diagnostic test. 8.6% of adults had more than one, persistent positive stool detection. Of these participants 48.5% tested positive 31+ days post initial infection with ddPCR values between 0.001 and 358.55 and real-time PCR cycle threshold values between 22.252 and 37.68. SARS-CoV-2 persistence in the gut is hypothesized to be associated with low-grade inflammation that can be measured by peripheral blood mononuclear cells and whole blood immune cell activation markers by flow cytometry. A pilot analysis suggests a higher SARS-CoV-2 PCR detection rate in stool for adults who are overweight and obese when compared to normal weight, and a higher number of females were identified with positive SARS-CoV-2 PCR in stool when compared to males. We conclude that intestinal colonization of virus merits further investigations with respect to changes to microbiota, persistent immune activation, and differences in variants for involvement during disease progression, post vaccination, and in cases of re-infection.

Undergraduate Student/ Environmental and Radiological Health Sciences

48. Triple Drug Immunotherapy for Treatment of Metastatic Osteosarcoma: Understanding Immune Mechanisms of Action.

Alyssa Nelson, Lyndah Chow, Jade Kurihara, Steven Dow

Cancer results from multiple acquired genomic changes and an environment of chronic inflammation. Identifying treatment for cancer poses a difficult challenge due to the heterogeneous interactions within the tumor microenvironment, composed of cancer and immune cells that are continually evolving and adapting to escape elimination. However, manipulation of myeloid-derived suppressor cells (MDSCs), a regulatory subset of monocytes and neutrophils, may offer therapeutic potential. Prior dual-drug immunotherapy trial using Losartan and Toceranib demonstrated anti-tumor activity in dogs with metastatic osteosarcoma (OS). By performing comparative transcriptome analysis of responses to a triple drug immunotherapy targeting MDSCs, distinct genes involved in neoplasia growth, angiogenesis, and metastasis were identified in blood leukocytes and cancer cells. This study investigates the effectiveness of the addition of a third drug, Ladarixin, which blocks neutrophil recruitment through CXCR1 and CXCR2 signaling. In combination, this triple drug immunotherapy may synergistically inhibit recruitment of MDSCs resulting in decreased immune suppression and clinical benefit.

DVM Student/ Microbiology, Immunology and Pathology

49. CBD protects UV stress in a skn-1 dependent pathway in *C. elegans*.

Margaret Neuheardt, Abdullatif Alsulami, Stephanie McGrath, Julie A Moreno

Alzheimer's disease is a neurodegenerative disease that progresses with age and can disrupt memory and general brain function. One of the characteristics of the disease is the presence of misfolded proteins, particularly amyloid-b and tau hyperphosphorylation. These cause neuronal stress and induce the accumulation of reactive oxygen species (ROS), which have damaging effects on neurons. This research aims to use various strains of the model organism *C. elegans* to understand the mechanism of cannabidiol (CBD) at the cellular level. CBD is a non-psychoactive phyto-cannabinoid has been previously shown to be neuroprotective, but the mechanism remains unknown The nuclear factor erythroid 2-related factor (Nrf2) is a transcription factor that regulates antioxidant responses, which is predicted to counteract the oxidative stress produced by ROS. The SKN-1 gene, which is the ortholog of Nrf in *C. elegans*, encodes for three different isoforms: skn-1a, skn-1b, and skn-1c. Skn-1c is a known transcription factor conserved with Nrf2, whereas the function of skn-1b remains partially unknown. We hypothesize that mediated survival extension is accomplished through activating skn-1. This effect extends the lifespan through the ROS scavenging ability of CBD. We are currently testing this hypothesis in skn-1 mutant worms, through applying ultra violet (UV) exposure to CBD treated and untreated worms. We have found that CBD does improve lifespan through the mechanism of ROS scavenging. Compared to a known ROS scavenger, NAC, CBD elongates the lifespan of the worms.

Undergraduate Student/ Environmental and Radiological Health Sciences

50. Examination of *in vitro* Proliferation Properties of Bone Marrow Derived Mesenchymal Stem Cells Harvested from Osteoporotic Sheep.

Paloma Orozco, Katie Bisazza, Lindsey Burton, and Jeremiah Easley

Osteoporosis is a disease that causes weak and brittle bones due to an alteration in homeostasis caused by an increase in bone resorption and decrease in bone density. Mesenchymal stem cells (MSCs) are important key players in bone modeling and remodeling as they have the ability to self-renew and differentiate into not only osteoblasts, but also adipocytes and chondrocytes. Although the majority of MSC research studies utilize cells isolated from rodents to investigate their role in osteoporosis, ovine MSCs could potentially be a more translational model due to shared Haversian bone remodeling characteristics with humans, which rodents lack. The overall objective of this study was to compare MSC viability, proliferation, and cell differentiation properties between osteoporotic and healthy sheep to potentially establish sheep as a model for future investigative studies of progenitor cell role in osteoporosis. Bone marrow aspirates (BMA) were collected from the iliac crests of four (n=4) osteoporotic ewes that had previously undergone ovariectomy (OVX) and corticosteroid administration, as well as from four (n=4) intact control ewes. BMA was harvested at baseline and 3-months post-OVX, when the last round of high-dose steroids was completed and was processed to isolate MSCs. The viability and proliferation properties of each cell line were assessed through MTT assay and cell counts to determine population doubling time. The same cell lines will then be used to compare tri-lineage cell differentiation properties between healthy and osteoporotic MSCs. Preliminary trials to determine optimal seeding density for each assay indicated that ovine MSCs should be seeded at densities of 5,000 cells/well, 100,000 cells/well, and 10,000 cells/well for MTT, population doubling, and differentiation, respectively.

DVM Student/ Biomedical Sciences

51. Comparison of mercury concentrations in grey seals (*Halichoerus grypus*) from two United Kingdom colonies.

Brittany Padayachee, Patti Pomeroy, Sean D Twiss, Amy Bishop, Lorrie Rea

In the United Kingdom, two grey seal (*Halichoerus grypus*) colonies are experiencing divergent population trends. One colony, located in North Rona (NR), has shown a decline in population numbers while another colony, located in Isle of May (IoM), has remained stable or even shown an increase in population. The cause of these patterns is currently unknown. Wildlife exposure to high mercury concentrations during fetal development has been shown to cause neurological deficits resulting in abnormal behaviors throughout life. Thus, we hypothesize that by comparing NR grey seal colony to the IoM colony, mercury can potentially be contributing to the NR colony's population decline. To examine this, we assessed median mercury levels in hair of seals from both colonies. Since mercury has a high affinity for the protein present in hair, this technique provides a non-invade method for longitudinal tracking of mercury levels in the same individuals. Overall, in 2012, the NR colony displayed a 1.29-fold increase in mercury levels when compared to the IoM colony in the same year. Additionally, mercury levels in the NR colony increased by 3-fold from 2009-2012. Notably, two seals in the 2012 NR colony had mercury levels that measured above the known toxicologic level for pinnipeds (>20ppm). The increased mercury levels observed in the 2012 NR colony could provide an explanation for the decline in population numbers. Samples from the NR and IoM colonies are being analyzed at additional timepoints to get a better indication of trends throughout the years. One drawback of this study is that mercury exposure in seals is believed to be from diet. As a result, variations in foraging ecology between the two colonies could explain the observed differences in mercury levels. To counteract this, analyses are ongoing to quantify stable isotopes present in the hair samples.

DVM Student/ Other (UAF)

52. Retrospective evaluation of large granular lymphocyte (LGL) lymphoma in dogs.

Ashley S Parker, A Russell Moore, Jenna H Burton

Large granular lymphocyte (LGL) lymphoma is not well-described in dogs, however this disease tends to have an aggressive clinical course in both cats and humans with frequent involvement of the liver, spleen and intestinal tract. The aim of this study was to characterize the clinical and diagnostic findings and outcome of dogs with LGL lymphoma. Cases were identified by a Colorado State University (CSU) Clinical Pathology database search and included if they had a cytological diagnosis of LGL lymphoma and received treatment at CSU. Medical records were retrospectively reviewed and data abstracted included signalment, clinical signs, results of blood work and staging diagnostics, treatment, and outcome, including progression free survival (PFS) and overall survival (OS). Nine dogs were included in this study. All were substage b at presentation and the most common clinical signs were hyporexia and lethargy, followed by vomiting, diarrhea, and weight loss. Common lab work abnormalities included elevated liver enzymes, hyperbilirubinemia, and hypoalbuminemia, and common abdominal ultrasound findings included gastrointestinal wall thickening and liver changes followed by splenic changes and abdominal lymphadenopathy. Five dogs were treated with chemotherapy, two dogs received prednisone alone, and two dogs were euthanized at diagnosis. For the seven dogs that received treatment, PFS was 24 days (range, 2 to 27) and OS was 26 days (range, 6 to 45). Dogs with LGL lymphoma frequently are substage b with visceral organ involvement. LGL lymphoma in dogs follows an aggressive clinical course with a poor prognosis despite treatment.

Resident/ Clinical Sciences

53. Nanocrystals as a potential drug delivery mechanism for intranasal vaccination.

Brielle H Patlin, Alec A Jones, Christopher D Snow, Stuart A Tobet

There are a variety of different vaccination methods that vary in time of release and location. One of the best methods of vaccination for respiratory diseases, intranasal vaccination, faces a variety of challenges and is only sporadically approved. This vaccination method is nasal drip vaccination which immunizes nose and throat membranes as well as lung. Challenges with this vaccination method are the inability to deliver RNA to the necessary tissues and the absence of a slow-release mechanism. One way to overcome these challenges is to utilize a mesoporous protein crystal scaffold that can deliver nucleic acids. Fluorescently labeled crystals can be visualized separately from fluorescently labeled guest 'contents' in lung tissue. These crystals slowly release nucleic acids when introduced to precision cut lung slices. Crystals were not cytotoxic and did not trigger an immune response from the tissue. Furthermore, the crystals can be loaded with lipopolysaccharide (LPS), a component of the bacterial cell wall that can trigger an immune response. Administration of fluorescently labeled LPS in crystals, compared to a soluble LPS control, demonstrated release from crystals via generation of B cells in lung slices. This result was verified by observation of fluorescence loss in crystals exposed to tissue over five days. These results demonstrate the potential of porous protein microcrystals as a delivery vehicle for intranasal respiratory vaccines.

Graduate Student/ Biomedical Sciences

54. Role of Triacylglycerol during dengue virus replication in *Aedes aegypti*.

Samantha M Pinto, Elayne D Burshek, Oshani Ratnayake, Camryn S Guenther, Chasity Trammell, Suad Elmegerhi, Paul S Soma, Venugopal Pujari, Dean Crick, Rushika Perera

Dengue viruses (DENVs) are flaviviruses, transmitted by the bite of an infected *Aedes aegypti* mosquito. We have previously shown that infection of these mosquitoes with DENV, serotype 2 caused significant changes in the lipidome of the mosquito. Further studies using liquid chromatography-mass spectrometry revealed a specific increase in Triacylglycerol (TAG) levels in *Ae. aegypti* mosquitoes fed with an infectious blood meal containing DENV2. A similar trend was also observed with Zika Chikungunya virus infections. We hypothesize that TAG levels increase in response to virus infection and may play a significant role in viral replication, dissemination and transmission in the vector. Alternately, TAG levels may influence the immune response to infection. To test this hypothesis, we have inhibited TAG synthesis as well as TAG hydrolysis in the mosquito host using three chemical inhibitors followed by infection with DENV2. The current study determined the half maximal inhibitory concentration (IC50) of the chemical inhibitor to be used in order to inhibit TAG synthesis without killing the vector. The results of TAG analyses and assays developed will be presented.

Undergraduate Student/ Microbiology, Immunology and Pathology

55. Biofeedback Application to a Military Training Task.

Maggie Read, Benjamin Clegg

The proliferation of multitasking within many jobs, specifically real time military tasks, often creates situations prone to high cognitive workload. Physiological responses to increased cognitive workload can include changes in heart rate, affecting an individual's heart rate variability. Biofeedback balances the sympathetic and parasympathetic nervous systems, which has been found to increase performance. Previous studies have found that modulating physiological responses, specifically heart rate, can increase the retention of working memory. The current study explored whether a simple breathing intervention to induce heart rate variability biofeedback might successfully mitigate some of the adverse consequences of high cognitive load in multitasking. In the current study, a wearable device was used to monitor and collect heart rate, as well as offer a basic type of heart rate variability biofeedback to all participants via a blinking screen. This preliminary study (n=11) collected both heart rate and performance data from two groups, while completing a military simulation. The military simulation platform was constructed to greatly increase cognitive load via multitasking at one stage of the experiment. A treatment group (n=6) had a 4-7-8 breathing technique taught to them as biofeedback and were compared to a control group (n=5). The treatment group showed overall increased performance versus the control group, despite there being only a small overall increase in heart rate associated with the high workload portion of task. This research suggests that biofeedback can increase performance during a high cognitive workload task, when linked to an approach to reduce physiological effects.

Undergraduate Student/ Other

56. Evaluating Zika viral proteins capacity for CXCL10 induction in human placental cells.

Hennio Rubio, Joseph Westrich, Tyler Bettencourt, Erin McNulty, Amy Nalls, Candice Mathiason

Zika virus (ZIKV) was first identified in 1947 in the Ziika forest, Uganda. As a flavivirus, ZIKV is principally spread through mosquito vectors and had relatively low impact for the first 60 years after its discovery. The first major outbreak of Zika occurred on the western pacific Yap Island in 2007, followed by an outbreak in the Americas in 2015. It was during this latter outbreak, ZIKV was identified to be causally associated with negative birth outcomes, most notably neurological diseases and microcephaly in babies born to infected mothers. Although ZIKV has been shown capable of crossing the placental barrier, another factor potentially contributing to negative outcomes is the cytokine CXCL10. CXCL10 is an inflammatory that has pro-apoptotic properties. CXCL10 has been shown to be highly upregulated in the serum of ZIKV patients with negative birth outcomes. Furthermore, CXCL10 has been implicated in being causative for neuronal apoptosis during fetal development. Currently, the mechanisms of ZIKV mediated CXCL10 induction remain unknown. We have previously shown CXCL10 induction is highly correlative to ZIKV burden in human placental cell lines (JEG3). To evaluate the mechanism of induction, we aim to establish separate expression plasmid vectors containing the ten ZIKV proteins. As ZIKV NS5 has been shown to directly interact with proteins known to induce CXCL10 we hypothesize this protein is involved. Lastly, we have shown different ZIKV stains have differential ability to induce CXCL10, thus we aim to determine if specific viral proteins have differing capacity to induce CXCL10.

Undergraduate Student/ Microbiology, Immunology and Pathology

57. RT-QuIC detection of amyloid seeds in brain tissues harvested from aged canines.

Samantha Scherner, Amy Nalls, Julie Moreno, Candace Mathiason

The World Health Organization predicts that Alzheimer's disease (AD) and AD-related diseases (ADRD) will be the second leading cause of death in the United States within the next decade. Early diagnosis has proven to be difficult as most patients seek care only once the disease has progressed. Development of *in vitro* assays to detect amyloid presence in biological samples early in disease stage is paramount. Canine cognitive dysfunction syndrome (CCD) is a well-recognized neurodegenerative disease in older dogs and serves as an ideal naturally occurring surrogate for AD/ADRD in humans. Here we employ the *in vitro* amyloid conversion assay, real time-quaking induced conversion (RT-QuIC), to detect low concentrations of misfolded proteins associated with CCD. Misfolded proteins present in tissues and fluids are amplified by RT-QuIC when the misfolded protein coerces a recombinant substrate, in this case amyloid beta 1-42 (Aβ1-42), into the misfolded shape. Real time readout is achieved by the intercalation of a fluorescent dye, Thioflavin T, between the growing amyloid fibrils. We aim to use Aβ1-42 to amplify amyloid fibrils present in canine brain, CSF and serum extracellular vesicles. The use of human Aβ1-42 protein substrate requires the optimization of the RT-QuIC assay to ensure positivity and negativity when applied to biological samples. Our preliminary findings indicate that RT-QuIC amyloid seeding presence in canine brain is higher in older dogs and correlates with Aβ detection by immunohistochemistry. Future directions include assessment of additional canine brain samples and extension of the assay to canine extracellular vesicles harvested from CSF and serum. Findings from this project will feed translational studies to assess human AD patient samples. An *in vitro* biomarker for AD and CCD will help further the development of therapies for these and other neurodegenerative disorders.

Undergraduate Student/ Microbiology, Immunology and Pathology

58. Colostrum Depletion in the Post-partum Mare.

Mason Schumaker, Emily May, Brittany Middlebrooks, Patrick McCue

It is vital that a newborn foal ingest high quality colostrum via its dam or through supplementation shortly after parturition. This is due to there being no transplacental transfer of maternal antibodies from the mare to the fetus in utero. Newborn foals acquire IgG following ingestion of colostrum in the first 24 hours of life. Repeated bouts of nursing by the newborn foal decrease the IgG concentration of antibodies in the mammary fluid over time. The goal of this project was to evaluate the depletion of IgG concentration in mammary fluid over a 48-hour period post-partum. Colostrum samples were collected from mares immediately after foaling and subsequently every 2 hours up to 12 hours post-partum. Additional samples were collected at 24 and 48 hours post-partum. Samples were analyzed using a Brix refractometer and also evaluated for the depletion of IgG antibodies through the use of single radial immunodiffusion (SIRD) assay. Utilizing the results of these two tests can help better understand when colostrum IgG concentrations decline to baseline levels. This knowledge is helpful in determining when to allow a foal considered to be at risk of neonatal isoerythrolysis to nurse from their dam and for when the best time to collect and store high quality colostrum for future use.

DVM Student/ Biomedical Sciences

59. Not All Models Of Maternal Stress Are Created Equal.

Julietta A Sheng, Robert J Handa, Stuart A Tobet

Exposure to adversities or stressors during fetal life influences fetal neurodevelopment and increases risk for neuropsychiatric diseases in adulthood. To determine the generality of stress effects on offspring, we are evaluating the impact of different models of maternal stress on hypothalamic development and related behaviors in mice. These models include fetal exposure to synthetic glucocorticoids (GC) to mimic a maternal corticosterone stress response, maternal nutritional stressors [caloric restriction (CR) versus maternal high fat diet (mHFD)], and maternal immune activation (MIA). Prenatal exposure to synthetic glucocorticoid, dexamethasone (DEX), resulted in decreased neonatal body weights ($p < 0.02$) and reduced social interaction behavior in male ($p < 0.05$) and female ($p < 0.05$) offspring. Maternal CR resulted in decreased body weights and social interaction behavior in males ($p < 0.02$) and females ($p < 0.05$) and increased anxiety-like behavior ($p < 0.05$) and acute stress response ($p < 0.01$) only in males. Maternal HFD resulted in altered body weight gain in male ($p < 0.02$) and female ($p < 0.05$) offspring with decreased anxiety-like behavior in a female-biased manner ($p < 0.02$). As mHFD may induce an immune inflammatory response in the mother (Ortiz-Vallardes et al., 2021; Neurosci Biobehav Rev 129:218) we are currently testing the impact of a direct agonist of a Toll-like receptor to mimic an immune response. Given that viral stressors stimulate GC release (Silverman et al., 2005; Viral Immunol 18:41) and immune stress therapy is often synthetic GC (e.g., DEX), it will be important to test an immune stress crossed with DEX in a factorial design. These studies will help determine if late gestation exposure to anti-inflammatory DEX can partially or fully recover alterations in fetal neurodevelopment influenced by MIA. Supported by ORWH-U54-MH118919.

Graduate Student/ Biomedical Sciences

60. Targeting neuroinflammatory pathways using Nanoligomers™ reduces glial inflammation and protects the brain from spongiosis and neuronal loss in prion disease.

Payton Shirley, Sydney J Risen, Sean Boland, Sadhana Sharma, Grace Weisman, Amelia D Hines, Arielle JD Hay, Vincenzo Gilberto, Stephanie McGrath, Anushree Chatterjee, Prashant Nagpal, Julie A Moreno

Neuroinflammation is a key factor in the development of neurodegenerative diseases, including prion disease. There are currently no effective treatments to halt pathogenesis and progression, which includes accumulation of misfolded proteins and glial inflammation, followed by irreversible neuronal death. We hypothesized that downregulation of two key neuroinflammatory targets would be neuroprotective. To test this, we utilized Nanoligomers™ in a prion-diseased mouse to assess the impact on glial inflammation, spongiosis, and neuronal loss. The four brain regions; hippocampus, cortext, thalamus and cerebellum were examined to assess the impact on glial inflammation and spongiosis. To assess the impact of neuronal loss the hippocampus brain region was examined. Prion-diseased mice treated with Nanoligomer™ showed decreased microglia and astrocyte inflammation in all four brain regions assessed. Spongiotic change, formation of vacuoles as the prion disease advances, was significantly reduced and therefore protected against in all brain regions assessed in mice treated with Nanoligomer™. Within the hippocampus neuronal numbers were notably protected when mice were treated with the Nanoligomer™. Nanoligomer™ treatment is independent of the misfolding of the PrP^{Sc} and inhibits the inflammatory pathways, ultimately preventing neuronal death and slowing the progression of neurodegenerative diseases.

Undergraduate Student/ Environmental and Radiological Health Sciences

61. Investigation of NCAM1 as a potential biomarker of feline infectious peritonitis..

Justin Silvey, Alora LaVoy, Ben Curtis, Gregg Dean

Feline infectious peritonitis (FIP) is a devastating infectious disease of felids. The etiologic agent, feline infectious peritonitis virus (FIPV), is thought to arise from a genetic mutation of the innocuous feline enteric coronavirus (FECV). The current gold standard for FIP diagnosis is immunohistochemistry (IHC) for Feline Coronavirus (FCoV) antigen on biopsy/autopsy tissues. Given the invasive nature of this test and the poor condition of the patient, most IHC diagnoses are made postmortem. Traditional virological and serological methods are rarely able to diagnostically differentiate FECV from FIPV. Proteomic analysis is a powerful tool which can measure/compare large sets of plasma proteins to identify diagnostically relevant biomarkers. We previously used the SOMAscan® assay to quantify over 1000 different plasma proteins between FIP and non-FIP cats and identified 18 proteins which best differentiated the two groups. The SOMAscan® assay is designed for human proteins and each protein-specific aptamer must be validated for recognition of the feline homologue. The purpose of this study was to validate the specificity of one of those 18 proteins, neural cell adhesion molecule 1 (NCAM1), using mass spectrometry (MS). Briefly, streptavidin plates were coated with SOMAmer; feline plasma samples were added to the wells, and then the SOMAmer®:protein complexes were released from the plate by UV photocleavage. Protein identification was confirmed by gel electrophoresis, excision, and MS. Future studies will validate additional SOMAmers® on their respective feline proteins followed by testing their diagnostic potential to identify cats with FIP.

DVM Student/ Microbiology, Immunology and Pathology

62. Proprietary probiotic for lessening pruritus in atopic dogs.

Megan Slaughter, Lia McCoy, Jennifer Hawley, Michael R Lappin

As a leading cause of disease in dogs, inhalational allergies require lifelong medical management, including immunotherapy (oclacitinib or lokivetmab) and glucocorticoids. Therefore, finding a dietary supplement to decrease the dose required for any of these medications is advantageous. A randomized 12-week trial with a proprietary probiotic and a placebo group was performed in dogs with stable atopic dermatitis without evidence of pyoderma. Owners were trained to assign a Pruritus Visual Analog Score (PVAS) weekly and select biomarkers were measured at Week 0, 4, 8, and 12. A decrease in PVAS of 2 points compared to Week 0 was considered a treatment success. Positive response rates were 40% (4 of 10 dogs) in the probiotic group and 57.1% (4 of 7 dogs) in the placebo group. None of the biomarkers measured, including IL13 and IL4, correlated to a treatment response in either group. The results of this study indicate that either the dose of the probiotic is too low or that this probiotic does not have the same effect in dogs as it does in humans. Because 4 of 7 dogs supplemented with the placebo improved as compared to baseline, there is evidence of either owner bias in the PVAS scoring or a decrease in environmental allergens during the course of the trial for some dogs.

DVM Student/ Clinical Sciences

63. Investigating Transtyretin's presents within immortalized ovine trophoblast cell lines.

Aimee M Snow, Anna Donovan, Quinton Winger

Transthyretin (TTR) is a thyroid hormone (TH) binding protein that is produced by trophoblast cells and it transports thyroxine (T4) to the placenta. TTR may also help T4 across the placenta. Immortalized ovine trophoblast (iOTR) cells are a trophoblast cell line that may be a good *in vitro* model for testing TTR function. T4 plays an important role in the activation, differentiation, and maturation of the fetal central nervous system (Patel et al.). Maternal nutrient restriction models of intrauterine growth restriction (IUGR) pregnancies showed a decrease in thyroxine (T4) levels in both maternal and fetal circulation (Adu-Gyamfi et al.). The cause of the reduction of TH in IUGR pregnancies has not been elucidated. We hypothesize that transthyretin is playing a larger roll in thyroid hormone transport then previously thought, and in the ovine placenta its functionality may be dependent on a lower oxygen environment (3%). Based on the experiments conducted and data collected it is confirmed that TTR is found in iOTR cells. This is the first time that TTR has been found in iOTR cells. The final cell count experiment did not show a difference in the numbers of cells grown in either 3% or 21% oxygen. Lastly, the iOTR cells exposed to 3% oxygen showed higher expression of TTR than cells expose to 21% oxygen.

DVM Student/ Biomedical Sciences

64. Alterations in the ocular surface immunome and microbiome in horses with corneolimbal squamous cell carcinoma.

Taylor Snyder, Maggie Williams, Lyndah Chow, Steven Dow, and Kathryn L. Wotman

Squamous cell carcinoma (SCC) is the most prevalent ocular tumor encountered in equine ophthalmology. Response rates vary widely among the different treatment modalities suggesting the presence of multiple factors that may regulate tumor responses, including the microenvironment of the ocular surface. Therefore, understanding the immune and microbial constituents of the ocular SCC tumor microenvironment (TME) using next generation sequencing technologies should help with the development of more targeted therapies. The goal of this study therefore was to evaluate the ocular microbiome and the ocular immunome between eyes with corneolimbal SCC and clinically normal eyes in the same animal to identify important differences. The study will also use *in vitro* assays to help elucidate the impact of the ocular surface microbiome and secreted factors on tumor immune responses. Ocular surface gene expression will be evaluated on 2 normal eyes and 2 corneolimbal eyes using next generation RNA sequencing, while microbiome analysis will be performed using 16S bacterial rRNA and internal transcribed spacer (ITS) gene sequencing respectively. Correlations between ocular surface immunomes and microbiomes in healthy versus affected eyes will be determined using appropriate statistical analyses. Use of these next-generation sequencing technologies will provide greater ability to identify new connections between the microbiome and surface immunome of the eye, and how ocular cancer may impact those connections. Furthermore, this research will provide important translational insight across species because the horse serves as an excellent spontaneous model for ocular squamous cell carcinoma in humans.

DVM Student/ Clinical Sciences

65. Surveillance of companion and exotic animals for SARS-CoV-2 and evaluating transmission potential within veterinary medicine.

McKenzie N Sparrer, Natasha F Hodges, Tyler Yamashita, Izabela Ragan, Tracy L Webb, Treana Mayer, Molly Carpenter, Candace K Mathiason, Christie E Mayo

Human cases of COVID-19 in the United States have exceeded 97 million, and with 70% of households owning a pet, millions of companion animals have potentially been exposed to SARS-CoV-2 through their owners. SARS-CoV-2 has been reported to infect both wild and domestic animal species after contact with infected humans, and transmission from infected animals to humans has been reported in rare cases after close contact. Veterinary professionals have an increased likelihood of exposure to zoonotic diseases like SARS-CoV-2, so improved understanding of the transmission risk of this virus in animals and the veterinary community is warranted to better protect their health. Our goal was to estimate the prevalence and seropositivity of SARS-CoV-2 in companion and exotic animals to assess transmission risk in a veterinary healthcare setting. We collected oral swabs, nasal swabs, and paired residual blood samples when available from domestic mammal species seen at the CSU Veterinary Teaching Hospital. Oral and nasal samples were tested using reverse-transcription polymerase chain reaction (RT-PCR) to detect nucleic acid while serum was tested for neutralizing antibodies against SARS-CoV-2 using a plaque reduction neutralization test (PRNT). SARS-CoV-2 RNA was detected by RT-PCR definitively in three of 176 domestic dogs and in one of 85 domestic cats. Using PRNT at 80% cutoff, 12.79% of domestic dogs (n=11/86) and 12% of domestic cats (n=3/25) had neutralizing antibodies detected against the virus. No exotic animals had evidence of SARS-CoV-2 detected by either RT-PCR (n=63) or PRNT (n=6). Next steps include variant analysis using next generation sequencing on oral and nasal samples with detected viral RNA. This study provides insights regarding SARS-CoV-2 infection rates of domestic and exotic pets, contributing to our understanding of its transmission risk in a veterinary setting. Study supported by NAHLN Farm Bill grant. Student supported by NIH Trainee grant.

DVM Student/ Microbiology, Immunology and Pathology

66. A single residue of the prion protein controls disease pathogenesis in deer and elk.

Julianna Sun, Sarah Kane, Sehun Kim, Jenna Crowell, Bailey Webster, Emma Raisley, Diana Lowe, Glenn Telling

Prions are infectious proteins causing fatal neurodegenerative diseases in humans and animals. Contagious transmission of chronic wasting disease (CWD), a prion disease affecting deer, elk, and other cervids, has caused disease in wild and captive animals in 26 States and 3 Canadian provinces. The primary sequence of the prion protein (PrP) plays a role in determining susceptibility and disease pathogenesis in both intra and interspecies transmissions of prion disease. North American deer or moose PrP encodes glutamine at residue 226 (Q226), North American elk PrP encodes glutamate (E226). To assess the effects of this difference on CWD pathogenesis, we created gene targeted (Gt) mice in which the murine PrP coding sequence was targeted and replaced with CervidPrP-Q226 or CervidPrP-E226, referred to as GtQ and GtE mice. Previous studies showed that GtQ and GtE mice were susceptible to North American CWD, and that time to disease onset was faster in GtE mice. To fully understand the mechanism underlying this difference, we conducted a longitudinal analysis of disease in GtE and GtQ in which mice were intracerebrally inoculated with elk CWD prions. Mice were collected every 15 days until terminal disease. Brain extracts were analyzed for PrP27-30 and glycoform ratio profiling by western blotting, disease-associated PrP by immunohistochemistry and histoblotting, titer determination by the cervid prion cell assay (CPCA), and the appearance of PrPSc using mAb PRC7 in ELISA format. Our analysis reveals primary structural differences at residue 226 of CerPrP have pronounced effects on the outcomes of disease in Gt mice infected with North American CWD prions. Since North American deer and moose express CerPrPC-Q226 and elk express CerPrPC-E226, this study lends insight into the natural pathogenesis of CWD in these species.

Post-doctoral Fellow/ Microbiology, Immunology and Pathology

67. Effect of alpha-casozepine supplementation on stress-associated findings in cats.

Maya Swiderski, Rachael Isdale, Kristine Kofron, Megan Slaughter, Kara Maslyn, Cassie McDonald, Imani Jones, Michael Lappin

Visits to the veterinary clinic are extremely stressful experiences for cats. Methods to decrease stress in cats primarily involve the usage of pheromones and/or medications such as gabapentin. For short-term stress management in cats, use of a natural substance with stress-relieving properties could be preferred over drug usage. The objective of this pilot study was to evaluate if a nutraceutical containing the natural stress-relieving substance alpha-casozepine (AC; Zylkene; Vetoquinol) could alter physical parameters including mean arterial blood pressure, heart rate, respiratory rate, body temperature, select behavioral stress scores, and serum cortisol concentrations. In this study, twelve healthy cats were randomly assigned to two equal groups. Once daily for five days, the cats in the experimental and control groups were fed canned food with or without the AC product, respectively. Twelve hours following the final feeding, the cats were taken in groups of three to measure routine bloodwork and physical parameters following a 12-minute drive to the veterinary clinic. The experiment was repeated ten weeks later with an additional twelve cats to increase statistical power. No significant differences were present in any of the measured parameters. This suggests that the protocol used was ineffective at altering the measured physical parameters and behavioral stress scores.

DVM Student/ Clinical Sciences

68. Intestinal Neuroimmune Involvement in Parkinson's Disease Development.

Hayley N Templeton, Casey P McDermott, Ronald B Tjalkens, Julie A Moreno, Stuart A Tobet

Accumulating evidence suggests that Parkinson's disease (PD) pathology can arise in the gut. A hallmark of PD is the neuronal accumulation of misfolded α-synuclein (α-syn) proteins. The enteric nervous system (ENS) facilitates bidirectional communication between the brain and the gut. Insults to the ENS have been shown to trigger the formation and progression of α-syn pathology from ENS to central nervous system. Rotenone is a pesticide known to induce α-syn aggregation providing a model for PD-like pathology in mice. The goal of this study was to gain insight into how rotenone contributes to accumulation of α-syn aggregate pathologies in the gut and identify cellular mechanisms of aggregate formation and uptake. Mice received intraperitoneal injections of rotenone once daily for 14 days. For immunohistochemistry, 50 µm thick section were cut from sections of ileum and colon. Cell counts in ileum were analyzed via the crypt-villus axis and colon analyzed from crypt to lumen. Rotenone treated tissue showed striking alterations in UEA-1 positive (Goblet) cells, calcitonin gene related peptide (CGRP) neurons, and ACK2 immunoreactive (mast) cells. Rotenone treated tissue had fewer Goblet cells in the top half of the crypts that were more fluorescent. Goblet cells secrete mucus to help prevent pathogen infiltration. These results suggest that rotenone reduces Goblet cell number and perhaps prevents mucopolysaccharide secretion. CGRP neurons and mast cells showed higher immunoreactivity near enteric neurons when treated with rotenone. Together, these results indicate the intestines as a possible origin of PD pathogenesis. Advancing understanding of aggregate formation and uptake in the gut will help identify causal relationships between their dysregulation and the development of PD pathologies.

Graduate Student/ Biomedical Sciences

69. The Effect of Water Bowl Material and Volume on Downstream Biofilm Formation in Canines.

Brodie M Thomas, Traci L Kinkel

Periodontitis, or gum disease, is the most common disease in dogs, affecting over two-thirds of the canine population. This disease is caused by an overgrowth of bacteria on the surface of the teeth, otherwise referred to as a biofilm. Symptoms of gum disease include bleeding or inflamed gums, loose of missing teeth, weight loss, bloody saliva, and irritability. Thus, this disease can have a huge impact on quality of life in canines. The purpose of this research project was to evaluate the effect of both the material and volume of water of dog's water bowls on the formation of biofilms. Samples were opportunistically collected over the course of three days, in which a canine companion drank from a provided water bowl. To test our hypothesis that water bowl material alters the abundance of bacteria within the water dish, we utilised the following experimental design. First, three separate water bowl materials were used: plastic, ceramic and stainless steel. To evaluate biofilm formation in these containers, 10mL of tap water and 200uL of our inoculum was added. The CFU/mL of each bowl material was calculated over the course of seven days, using an OD reading. This led to results that corroborate stainless steel displays reduced bacterial growth within the water dish, whilst plastic and ceramic materials display log level increases in bacterial growth, respectively. This work ultimately will help pet owners properly manage their water bowls to prevent bacteria accumulation in the water, leading to biofilm formation within their pets mouths.

Undergraduate Student/ Microbiology, Immunology and Pathology

70. Epidemiology of canine B cell leukemia and lymphoma.

Mary Jo Thometz, Emily D Rout, Julia D Labadie, Anne C Avery

Lymphoproliferative disorders affect many dogs across the U.S., but the frequency, clinical signs, and breeds associated with various subtypes are not well characterized. This study aims to identify genetic predispositions and other traits associated with various B cell neoplasms in the blood and lymph nodes. Data on 22,201 B cell neoplasms were obtained from Colorado State University's Clinical Hematopathology database from 1/1/2015 to 5/1/2022. Odds ratios comparing breed-specific risk for each B cell subtype were determined using population data from Banfield Pet Hospital (n = 9,928,122 unique dogs) and the Dog Aging Project (n = 33,172 unique dogs) and data from 16,857 nodal large cell B cell lymphoma, 1,959 nodal small cell B cell lymphoma,and 3,385 small cell B cell leukemia cases were analyzed. Compared to mixed breed dogs, the odds ratios suggest breed trends associated with the different subtypes. Large breed dogs have greater odds of developing nodal large B cell lymphoma and many small breed dogs have decreased odds. Conversely, many small breed dogs have greater odds of developing small cell B cell leukemia while large breed dogs have decreased odds. A mix of both large and small breed dogs were identified to have greater odds of developing nodal small cell B cell lymphoma. Researchers and practitioners can use this information to better recognize and diagnose these disorders in their canine patients, and to identify breed-specific genetic risk factors for different types of B cell neoplasms.

DVM/MPH Student/ Microbiology, Immunology and Pathology

71. Omega-3 fatty acid metabolite Resolvin D1 regulates the pulmonary immune response to agricultural dust.

Alissa N Threatt, Logan S Dean, Melea Barahona, Carly S Chesterman, Riley Anderson, Madelyn Gloe, Casey P McDermot, Sydney Risen, Gabriela Ramirez, Tara M Nordgren

Agricultural dust exposure causes chronic pulmonary diseases such as asthma and chronic obstructive pulmonary disease (COPD) in agriculture workers. There are currently no treatments for these diseases; however, there is evidence that increased omega-3 (ω-3) fatty acid tissue concentrations may slow the progression of chronic pulmonary diseases. This project explores the effects of the ω-3 fatty acid docosahexaenoic acid (DHA) metabolite Resolvin D1 (RvD1) in regulating recovery from lung inflammation, using *in vitro* cultured macrophages and an *in vivo* mouse model of repetitive dust exposure. Mouse alveolar macrophages (MH-S line) were cultured and stimulated with RvD1 with or without organic dust extract (DE). Supernatant and cells were collected at various timepoints over 24 hours and inflammatory mediator release was assessed at protein and transcript levels. Wild type mice were intranasally (IN) installed with sterile saline or 12.5% DE for 5 days per week for 3 weeks and treated with RvD1 intraperitoneally (IP) once weekly. Animals were sacrificed 5 hours or 3 days after the last DE installation. Bronchoalveolar lavage fluid was collected for cytokine and cell infiltrate analyses and lungs were harvested for histopathology and transcript evaluation. Two-way or three-way ANOVA analyses were performed to determine significance in all studies. MH-S cells co-exposed with DE and RvD1 exhibited increased production of pro-inflammatory and pro-resolution cytokines and altered kinetics, leading to enhanced inflammation resolution. Animal studies revealed increased immune cell infiltration in dust-exposed animals with altered cell types in RvD1-treated animals. These data suggest RvD1 accelerates the immune response to agriculture dust, resolving inflammation and initiating repair pathways more rapidly than non-RvD1-treated animals. These investigations assist in identifying functions of RvD1 in regulating the pulmonary immune response to organic dust, implicating its utility in possible therapeutic applications.

Graduate Student/ Environmental and Radiological Health Sciences

72. The prevalence of increased serum cobalamin in cats with disease.

Phabpim Thumarat, Camille Torres-Henderson

Serum cobalamin is a parameter that has been used extensively in cats to monitor the dysregulation of gastrointestinal tract. Low cobalamin has been associated with distal intestinal disease whereas increased serum cobalamin is not believed to have clinical significance in veterinary medicine. The goals of this retrospective study were to determine the prevalence of increased serum cobalamin in cats presented to Colorado State University and evaluate whether there a was an association of high cobalamin and clinical diseases in cats that had a biopsy or necropsy performed. This was a retrospective observational design study. Medical records of cats referred to Colorado State University between July 2018 and September 2021 were reviewed. Cats that had serum cobalamin measured at the time of their first referral were included for further investigation. 255 cats met the inclusion criteria. The reference interval of cobalamin was 290-1500 ng/ml, however; the laboratory did not report a numerical value for serum cobalamin that was >1000. Based on this we established the definition of increased serum cobalamin in cats as >1000ng/ml. Of the 255 cats, 122 had increased in serum cobalamin without a history of cobalamin supplementation (47.8%).The cats were further categorized based on whether they had a histopathologic diagnosis. Of the 255 cats evaluated, 82 had a biopsy or necropsy. Of these 82 cats, 45 (54.9%) had serum cobalamin >1000 and 30 (36.6%) had cobalamin within the reference range. Of the cats that had a biopsy or necropsy performed and had cobalamin >1000, 25 (55.5%) were diagnosed with lymphoma, 8 (17.8%) were diagnosed with IBD. In conclusion, there was a relatively high percentage of cats with hypercobalaminemia (47.8%) which differs from other studies looking at the prevalence of increased cobalamin in cats.

DVM Student/ Clinical Sciences

73. Serum metabolome and proteome reveals small molecules and metabolic pathways associated with COVID-19 disease severity and risk of development of post-acute sequalae of COVID-19.

Madison Tipton, Bridget A Baxter, Taru Dutt, Nicole Natter, Robert V Gerbasi, Lisa M Bramer, Luke Busot, Maya Jones, Elizabeth P Ryan

SARS-CoV-2 infection was shown to increase COVID-19 disease severity in people with chronic metabolic conditions. Metabolomics and proteomics are tools utilized for hypothesis generation and was applied herein to elucidate the impacts of SARS-CoV-2 infection on disease severity and future risk of developing post-acute sequalae of COVID-19 (PASC). The major objective of this pilot study was to longitudinally examine serum from 53 adults enrolled in the Northern Colorado Coronavirus Biobank (NoCO CoBIO). Adults had mild (n=16), moderate (n=14), and severe (n=11) disease and no history of infection (n=12). Twenty adults reported having pre-existing chronic conditions. Metabolomics was performed by gas chromatography-mass spectrometry and protein identification was completed after undergoing immunodepletion, protein digestion, TMT-labeling, and basic-RP-fractionation. A total of 145 metabolites and 1982 proteins were identified. Statistical analysis using R version 4.1.0 with the *pmartR* package (PMC6750869) revealed significant differences in 28 compounds including, but not limited to, maltose, 2-hydroxybutyric acid, and pseudo uridine. Maltose was reported in a separate study as depleted in COVID-19 and was different between those with mild vs moderate ($p=0.0001$) or severe ($p=7.78E^{-07}$) disease at 3 months after infection. 2-hydroxybutyric acid was different between those with mild vs moderate ($p=0.0079$), or severe ($p=2.40E^{-09}$) and moderate vs severe disease ($p=0.0484$) 3 months after infection. Pseudo uridine has implications for differences in vaccine platforms and had the largest fold change (0.57) between mild vs moderate disease ($p=0.0009$) at 6 months after infection. Pathways significantly impacted from proteomics analysis included neutrophil degranulation ($p=2.14E^{-24}$), response to elevated platelet cytosolic Ca2+ ($p=2.80E^{-21}$), platelet degranulation ($p=7.18E^{-20}$), and platelet activation signaling and aggregation pathways ($p=5.57E^{-18}$). These omics analysis revealed key immune and metabolic pathways and distinct differences between disease severity. Our results illuminate possible suites of biomarkers for disease severity and future directions will focus on pathways associated with risk of developing PASC.

Staff/ Environmental and Radiological Health Sciences

74. Educational Healing Gardens: Intersection of Human, Animal, and Environmental Health at Veterinary Medical Facilities.

Courtney L Trujillo, Sean Lewis, Rachel Fost, Alanna Gudmunson, Sam Hilty, Elizabeth A Patton, Alina Vise, Molly Carpenter, Treana Mayer, Danielle Scott, Colleen Duncan

The built and natural environment are critical determinants of health for animals and people. Despite this importance, our shared environment faces many concurrent and increasing threats. Communities trust veterinary professionals to provide health information, designating them as important educators regarding the intersection of environmental, animal, and human health. Designing an immersive landscape that incorporates this principle is a powerful educational tool for demonstrating how one's facility can promote multiple forms of welfare. Examples of such landscaping, termed "Healing Gardens" have recently been implemented into human health facilities and may have equivalent applications in veterinary practices. Our objective was to develop a resource guide for veterinary professionals to design their own educational and experiential healing gardens.

DVM Student/ Microbiology, Immunology and Pathology

75. Exploratory use of RT-QuIC to quantify and compare kinetics of amyloid formation in North American and Scandinavian CWD prions.

M.L. Tyer, Xutong Shi, Juliana Sun, Sehun Kim, Sylvie Benestad, Glenn Telling

Chronic wasting disease (CWD) is a transmissible, universally fatal neurodegenerative disease caused by the pathogenic misfolding of prion protein. CWD is endemic to North America (NA), and most recently has been identified in Scandinavian cervids. Interestingly, isolates collected from Scandinavian CWD cases have been shown to possess unique strain characteristics. Previous research from our group has shown Scandinavian CWD is etiologically distinct from its North American counterpart, which may account for these strain differences. Here, we seek to further characterize the kinetic properties of Norwegian CWD (NorCWD) isolates through real-time quaking-induced conversion (RT-QuIC) assay, a method for quantifying the rate a prion seed can form amyloid from a standard substrate. Our preliminary results show that Nor red deer and moose isolates display variable amyloid formation rates within technical replicates, with little discernible linear range over a dilution series. This is distinct from NA elk and mule deer, which display a predictable linear range over 10^{-4} to 10^{-7} titrations. Notably, the NorCWD isolates converted substrate at a faster rate than seeds taken from NA. Taken together, these results indicate that the distinct properties of NorCWD can be recapitulated using a standard *in vitro* assay with recombinant Syrian hamster PrP as a substrate. These preliminary data will guide the direction of our future research investigating features influencing NorCWD pathogenicity. *This work was part of a rotation project in the Telling Lab by M.L. Tyer who is the recipient of the NIH training grant award, T32GM144856. Research in the Telling Lab is supported by the following grants from the NIH: 1R01NS121682, 1R01NS109376 from the NINDS, and PO1-0011877A from the NIAID*..

Graduate Student/ Microbiology, Immunology and Pathology

76. Placement characteristics and flow rates of a novel manual intraosseous device versus an automatic device in canine and feline cadavers.

Olivia C Uzan, Julien Guillaumin, Kelly E Hall, Claire D Tucker, Liz S Guieu

Intraosseous (IO) catheterization enables rapid access to systemic circulation in critical patients. A battery powered automatic IO device (AIOD) utilized in veterinary practice is reliable in facilitating IO catheter placement. The AIOD has a limited lifespan (700 uses) due to its non-rechargeable battery, is expensive, and requires specific needles. A new manual IO device (MIOD) has been developed for human use. The battery-free MIOD is cost effective, has a longer lifespan (over 10,000 uses) and an adapter that is compatible with needles from other manufacturers. The goal of our study was to compare placement characteristics (number of attempts, time, and success of placement) and flow rates achieved with the AIOD versus the MIOD when operated by novice users. We hypothesized that users would perform faster IO catheterization with the AIOD compared to the MIOD, but other characteristics would be similar between devices. Six veterinary students performed 72 catheterizations in the humeri and tibias of 12 dog and 6 cat cadavers. The user, cadaver, device, and site of placement were randomized. Flow rates were determined by 3-minute infusions. Novice users had limited successful placements (~50%) using both devices. In dogs, no difference was found between devices for the median time for successful placement (33s AIOD, 45s MIOD, $p=0.156$), success rates (50% AIOD, 46% MIOD, $p=0.775$), and flow rates (1.62 L/hr AIOD, 2.03 L/hr MIOD, $p=0.566$). Median time to tibial placement was faster with the AIOD (35.6s AIOD, 124.7s MIOD, $p<0.001$) but similar between devices for humeral (32.3s AIOD, 47.4s MIOD, $p=0.110$). In cats, success rates were similar between devices (17% AIOD, 25% MIOD, $p=1.000$), but limited placements prevented further analysis. This is the first study to examine the use of the MIOD in animals, providing valuable preliminary data for future IO studies and potential direct application in the clinical setting.

DVM Student/ Clinical Sciences

77. Detection of gastrointestinal parasites in fecal samples from dogs attending dog-parks in New York State..

Camden Venator, Ben Wakschlag, Rae Isdale, Hailey Davis, Valeria Scorza, Christian Leutenegger, Michael Lappin

Dogs attending dog parks can harbor parasites that could be zoonotic. The objective of this study was to assess the prevalence of gastrointestinal parasites in dogs attending two dog parks in New York State. Positive *Giardia* spp. samples (immunofluorescent assay) were analyzed in 5 PCR assays to confirm the positive results and determine the assemblage. Positive *Cryptosporidium* spp. samples were confirmed and speciated by PCR assay. Fecal samples from dog parks in Amherst NY (n = 25) and Ithaca NY (n = 32) were tested. One sample from the Amherst dog park tested positive for *Ancylostoma caninum* by fecal flotation. *Giardia* spp. (8 samples from Amhearst; 4 samples from Ithaca) and *Cryptosporidium* spp. (3 sample from Amherst; 2 samples from Ithaca) were found in some samples. All *Giardia* spp. positive samples were confirmed by SSU rRNA-qPCR, and one sample typed as *Giardia duodenalis* assemblage C. *Cryptosporidium* DNA was not amplified by the PCR assay. Currently, the 11 *Giardia* samples that could not be identified by PCR assemblage sequencing techniques are being evaluated by meta sequencing to determine whether these results are from new variants.

Undergraduate Student/ Clinical Sciences

78. Using a novel high-volume throughput process to establish optimal energy dose for Zika virus inactivation.

Alina Vise, Lindsay Hartson, Raymond Goodrich, Andrew Andraski, John Mizia, Matt Willman, Izabela Ragan

The current accepted methods for whole virus inactivation for vaccine production is cumbersome, time-consuming, and environmentally hazardous. We attempted to use a safer, well-established method for pathogen inactivation using the photochemical interaction between riboflavin and UV light which has recently been employed for vaccine product development using whole inactivated viruses (SolaVAX technology). Currently, the SolaVAX manufacturing process is limited to small batch production. For large-scale production that can be used for making human clinical trials material, a novel photochemical prototype device was developed to show capability of a high-volume throughput process able to inactivate viruses in a fraction of the time. Based on previous Zika testing, we believe that a 1.0J/ml dose would be sufficient to inactivate Zika. To test this novel method with our prediction, a 500ml volume of riboflavin (vitamin B2) and PBS containing Zika virus (strain PRVABC59) (4-5x10^6 pfu/ml) was passed through a highly UV-transparent coil within the photochemical device, exposing the solution to incremental doses of UV energy (0.5J/ml, 1.0J/ml, and 1.5 J/ml). The three inactivated samples were tested by plaque assay. At 1.5J/ml dose, the Zika virus titer was reduced by 6 logs and showed no plaque formation on assay. Our results show the successful replication of riboflavin and UV light inactivation of whole virus particles in a high-volume throughput system, but at a higher energy dose than predicted. Next, we will use this same process to replicate data in Encephalomyocarditis and Vaccinia viruses. Future developments of this device will allow for efficient large-scale whole virus vaccine manufacturing.

DVM Student/ Biomedical Sciences

79. Effects of dietary omega-3 and -6 polyunsaturated fatty acids on muscle mitochondrial function in old mares.

Megan L Wilford, Kyle Fresa, Giovana D Catandi, Raul A Gonzalez-Castro, Elaine M Carnevale

Sarcopenia or age-related muscle wasting is common in geriatric horses and results in weakness and decreased muscle mass. Equine aging is associated with decreased oxidative capacity of skeletal muscle, which results in negative effects on mitochondrial function. In sedentary, older people, dietary supplementation with omega-3 (N3) polyunsaturated fatty acids (PUFA) improved muscle mass and grip strength and stimulated muscle protein synthesis. Lipid supplementation is frequently used in older horses to increase bodyweight; however, effects on muscle function have not been adequately studied, and lipid type is usually not considered. We hypothesized that dietary supplementation of lipids will increase mitochondrial function in skeletal muscle of older mares and that N3 PUFA will have more positive effects than omega-6 (N6) PUFA. Mares will receive either N3 or N6 PUFA: N3 (n=6, mean age of 22.5±2.9 years, 120 ml flaxseed oil daily) and N6 (n=6, mean age of 23.1±2.8 years, 120 ml corn oil daily). Muscle biopsies will be collected from the trapezius before and after 6 weeks of supplementation, allowing mares to serve as their own controls. Muscle samples will be permeabilized and evaluated for maximum oxygen consumption rate (OCR) and reactive oxygen species (ROS) production using the Oroboros high-resolution respirometer (O2K). Data will be analyzed by two-way ANOVA. We expect that N3 and N6 will increase muscle OCR in the old mares; however, we anticipate that N3 will be more effective than N6 at increasing OCR with less of a concomitant increase in ROS. Our findings could support dietary supplementation for the treatment and prevention of sarcopenia in older mares and have translational implications for aging humans.

DVM Student/ Biomedical Sciences

80. Correlations between DTI and NODDI parameters in the healthy aging white matter.

Jake Lonergan, Jordan Carroll, Miles Hopkins, Grace Hiner, Samantha Umland, Raghuram Kakinada, Neveah Newton, Nick Stirbis, Andrea Mendez Colmenares, Aga Z Burzynska

Human white matter (WM) contains mostly myelinated axons, whose properties determine the speed of transmission of neural signals within the brain. The aging WM has been studied predominantly using diffusion tensor imaging (DTI) which estimates the magnitude and directionality of water diffusion. Fractional anisotropy (FA) is a measure of the directional dependence of diffusion and is used as a proxy of WM microstructural integrity. Considering DTI lacks specificity for axonal or myelin integrity, the current study explored the use of a more advanced diffusion MRI technique, called Neurite orientation dispersion and density imaging (NODDI, Zhang et al., 2012). NODDI allows voxel-wise estimation of intra-cellular volume fraction (Vic) reflecting axonal density, orientation dispersion index (ODI), and isotropic volume fraction (Viso). The aim of this study was to explore the associations between DTI, NODDI, and age in a sample of cognitively and neurologically healthy adults (age 20-80, n=30). Our analyses focused on the genu corpus callosum (GCC) as the WM region known to be most vulnerable to aging. As expected, we observed a significant negative association between GCC FA and age ($r(1,29)=-.72$, $p<.001$), as well as between GCC Vic and age ($r(1,29)= -.76$, $p<.001$). GCC Viso and age were found to be moderately positively correlated ($r(1,29)= .39$, $p= .04$), however, no relationship was observed between GCC ODI and age. GCC FA was also strongly associated with GCC Vic ($r(1,29)= .89$, $p<.001$), and was negatively associated with GCC Viso ($r(1,29)= .42$, $p= .02$). Taken together, the relationship between age, DTI, and NODDI parameters point towards alterations in WM composition and microstructural integrity in the aging brain. Thus, NODDI offers a promising tool to dissociate age differences in fiber orientation from axonal integrity.

Undergraduate Student/ Other

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