23RD ANNUAL RESEARCH DAY

SCIENTIFIC PROCEEDINGS

JAN. 22, 2022 | LORY STUDENT CENTER

COLLEGE OF VETERINARY MEDICINE
AND BIOMEDICAL SCIENCES

COLORADO STATE UNIVERSITY

Our 23rd 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!

2022 CVMBS Research Day
Organizing Committee

Kelly Santangelo
Faculty Co-Chair – Microbiology, Immunology, and Pathology

Adam Chicco
Faculty Co-Chair – Biomedical Sciences

John Kisiday
Faculty Co-Chair – Clinical Sciences

Aimee Oke
Committee Coordinator – CVMBS Dean's Office

Theresa Rulon
Committee Coordinator – CVMBS Dean's Office

CONTENTS

RESEARCH DAY 2022

SCHEDULE OF EVENTS

10-10:45 a.m.	Poster Set up Oral Presentation	LSC Grand Ballroom AB Set up in assigned rooms
11 a.m.	Opening Remarks	LSC Theater
11:10-11:50 a.m.	ZOETIS RESEARCH EXCELLENCE AWARD WINNER – Dr. Angela Bosco-Lauth	LSC Theater
11:50-Noon	Break	
Noon-4 p.m.	ORAL PRESENTATIONS: Basic/Translational Science	Rm 1: LSC 308-10
Noon-4 p.m.	ORAL PRESENTATIONS: Basic Science	Rm 2: LSC 304-06
Noon-4 p.m.	ORAL PRESENTATIONS: Clinical Science	Rm 3: LSC 302
Noon-4 p.m.	ORAL PRESENTATIONS: Clinical/Translational Science	Rm 4: LSC 300
Noon-1:45 p.m.	POSTER SESSION I JUDGING: Odd-Numbered Posters	LSC Grand Ballroom A/B
1:45-2 p.m.	Break	
2-3:45 p.m.	POSTER SESSION II JUDGING: Even-Numbered Posters	LSC Grand Ballroom A/B
4-6 p.m.	Social Hour and Awards	LSC Grand Ballroom

DEPARTMENTAL ABBREVIATIONS

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

CONGRATULATIONS AGAIN TO 2021 CVMBS RESEARCH DAY WINNERS!

ORAL PRESENTATIONS

First Basic	Christina Geldert, D.V.M. Student, Other, "Dietary supplementation with phytochemicals improves diversity and abundance of honey bee gut microbiota." Mentor: Arathi Seshadri
Second Basic	Holly Stewart, Postdoctoral Fellow, CS, "Development of an experimental model of bone marrow lesions using the rat femoral condyle." Mentor: Chris Kawcak
Third Basic	Samantha Labb, Graduate Student, ERHS, "Separation of americium in higher oxidation states from curium for nuclear waste recycling." Mentor: Ralf Sudowe
First Clinical	Carly Gregory, D.V.M. Student, CS, "Evaluation of outcome associated with feline trauma: Veterinary Committee on Trauma (VetCOT) registry study." Mentor: Kelly Hall
Second Clinical	Ken Fukushima, D.V.M. Student, CS, "Effect of a commercially available synbiotic on mycophenolate associated diarrhea." Mentor: Michael Lappin

POSTER PRESENTATIONS

First	Bridget Ecklund, D.V.M. Student, MIP," NOD2 Expression by Mucosal CD11c+ Cells is Required for a Humoral Immune Response Against the Lactobacillus Acidophilus Vaccine Vlatform." Mentor: Gregg Dean
Second	Cameron Pearce, Graduate Student, MIP, "Characterizing Nanoparticle Localization in M. tuberculosis Infected Lungs." Mentor: Mercedes Gonzalez-Juarrero
Third	Amy Fox, Graduate Student, MIP, "Bactcountr: A Tool for Calculating Colony Forming Units." Mentor: Marcela Henao-Tamayo
Golden Pipette Award	Clinical Sciences

INTERCONNECTED IMPACTS

Researcher honored as a steward for animal and human health

By Rhea Maze

Angela Bosco-Lauth with her 23-year-old horse Maverick

ANGELA BOSCO-LAUTH ALWAYS WANTED TO BECOME a veterinarian. When an outbreak of West Nile virus hit northern Colorado as she was beginning her undergraduate studies, the trajectory of her career completely changed.

"I am a horse owner so I was pretty worried about it," said Bosco-Lauth, an assistant professor in the Department of Biomedical Sciences. It was then that she was introduced to Biomedical Sciences Professor Richard Bowen, who became her mentor throughout her education and with whom she now shares a lab space. "I was excited to see that his work would impact my horse's life. I decided that instead of doing veterinary school, which I ended up doing later, I wanted to become a researcher so that I could help keep animals and people safe."

Bosco-Lauth went on to earn both a Ph.D. in microbiology

and a Doctor of Veterinary Medicine degree from Colorado State University before launching her career as a researcher, educator and clinician. She was awarded the 2021 Zoetis Award for Veterinary Research Excellence for her innovative work and will deliver the keynote address at the 23rd annual College of Veterinary Medicine and Biomedical Sciences Research Day event.

PANDEMIC PIVOTING

"Most of what I do is research into zoonotic infectious diseases," Bosco-Lauth said. "And for the past year-and-a-half, that work has focused almost exclusively on SARS CoV-2."

When COVID-19 first emerged, Bosco-Lauth and Bowen teamed up and immediately began looking at host susceptibility in different animals including cats and dogs. "Every time there is a new and emergent pathogen, we jump on it quickly because we have the ability to do some really unique animal modeling," Bosco-Lauth said.

They quickly found cats to be highly susceptible and began discussing targeting vaccine efforts in domestic pets as well as continuing to understand the virus's impacts on wildlife species. Their ongoing SARS-CoV-2 work includes human vaccine and therapeutic efforts, animal vaccines, and looking at host susceptibility and transmission.

"Every time we think we have it under a control, a new variant pops up so now we are continuing to work on understanding what this means for different animals and for people's pets," Bosco-Lauth said. "There are still a lot more questions than answers at this point." This work has led to coverage in several national news organizations, including two New York Times articles.

A ONE HEALTH APPROACH

Bosco-Lauth is fueled by research that links human and animal health. Working to solve the complex health challenges that arise where human and animal environments overlap is a constant challenge in today's world. Bosco-Lauth is especially passionate about mitigating harmful impacts to wildlife. "Wildlife conservation is one of the most important things to me," she said. "The impact humans have on wildlife species is tremendous."

Of the many research efforts Bosco-Lauth is currently working on, one she is most excited about is an interdisciplinary collaborative project called BROADN (Biology Integration Institutes: Regional OneHealth Aerobiome Discovery Network) that will look at how microbes in the air move and interact. "We are investigating if there is such a thing as an airborne microbiome and if so what it means for human, animal and ecological health," she said.

Another of Bosco-Lauth's passion projects is her ongoing involvement in community-based West Nile virus research, which involves looking for antibodies in backyard chicken eggs. "This was the most fun thing I did all summer, and it is neat to be able to circle back to this kind of community-based work since West Nile virus is the disease that got me into science in the first place," she said.

Other projects underway in Bosco-Lauth's lab include studying wildlife as a potential source of a tickborne virus called severe fever with thrombocytopenia syndrome, which emerged in the U.S. a few years ago and can cause severe disease in humans; developing a vaccine for rabbit hemorrhagic disease; looking at feral swine infectious disease surveillance and response to anthrax vaccination; and studying RNA virus surveillance in ticks and Usutu virus infection in birds.

In addition to conducting research, teaching veterinary courses and mentoring students, Bosco-Lauth also serves as the attending veterinarian for the Animal Reproduction and Biotechnology Laboratory and the Animal Disease Laboratory.

"Dr. Bosco-Lauth is a rising star and shows exceptional promise as a research scientist," said the late Biomedical Sciences Professor and interim Department Chair Bob Handa in her Zoetis award nomination letter. "In addition to her research prowess, she's an outstanding teacher, mentor and clinician. She is clearly at the forefront of research in her field and is on an accelerated trajectory in all aspects of academics."

"This award is well-deserved," said College of Veterinary Medicine and Biomedical Sciences Associate Dean for Research and Microbiology, Immunology and Pathology Professor Mark Zabel. "Dr. Bosco-Lauth's work is important, impactful and very high quality."

SESSION 1: Basic/Translational Science

Early-stage research, Noon–3:45 p.m. | LSC 308-310

Time	Presenter	Topic	Dept.
12:00	Altina, Noelia	SARS-CoV-2 viral RNA biology and its impact on the infected cells | J Wilusz	MIP
12:15	Andretsos, Chris	Assessing the Role of Lung Fibroblasts in Modulating Osteosarcoma Chemotherapeutic Response | D Regan	MIP
12:30	Flory, Savannah	Vaccines "au natural": novel inactivation method using UV + riboflavin preserves influenza surface antigen expression when compared to traditional methods of vaccine preparation | R Goodrich	MIP
12:45	Granier, Shelby	Comparison of four freezing extenders on post-thaw motility of stallion spermatozoa | P McCue	CS
1:00	Hernandez, Adam	Restricted antimicrobial use among dairy production systems following implementation of the Veterinary Feed Directive: Impact evaluation through a Systematic Review | S Rao	CS
1:15	Hogan, Parker	The effect of short and long term frozen storage of recombinant hamster prion protein in RT-QuIC | C Mathiason	MIP
1:30	Whitcomb, Luke	Glucocorticoid receptor signaling is required for acclimation of skeletal muscle to hypobaric hypoxia | A Chicco	BMS
1:45	BREAK		
2:00	Bonilla, Andres	Mechanical compression as a model of disc degeneration disease | J Easley	CS
2:15	Jardine, Cydney	Virulence determinants and class I integron-encoded antimicrobial resistance among Salmonella enterica serovar Typhimurium of swine origin | S Rao	CS
2:30	Jauch, Linzy	Veterinarian's choices and practices of antimicrobial drug treatments among golden retrievers within the United States | S Rao	CS
2:45	Lowry, Brandon	Exploring the effects of standard two-dimensional anatomical models and virtual reality models on visuospatial skills | T Clapp	BMS
3:00	Palmer, Eric	Establishing the impact of osteosarcoma-derived exosomes in promoting lung metastasis | D Regan	MIP
3:15	Stromberg, Sophia	Plasma fatty acid quantification in adults with mild, moderate and severe COVID-19 disease | E Ryan	ERHS
3:30	VanZeeland, Emily	Elucidating sex differences in the anterior cruciate ligament response to injury using a novel mechanical rupture model | J Easley	CS

SESSION 2: Basic Science

Advanced-stage research, Noon–3:45 p.m. | LSC 304-306

Time	Presenter	Topic	Dept.
12:00	Ali, Malik Zohaib	Preclinical in vivo assessment of replacing linezolid for spectinamide-1599 in the Nix-TB regimen | M Gonzalez-Juarrero	MIP
12:15	Baxter, Bridget	Vitamin D2/D3 levels in the Northern Colorado Coronavirus Biobank: A biorepository for acute and convalescent patients (NoCO-COBIO) | P Ryan	ERHS
12:30	Charley, Phillida	Innate Immune Interactions of Bat Cells Infected with Middle East Respiratory Syndrome Coronavirus | T Schountz	MIP
12:45	Dutt, Tara Shikha	NTM exposure via drinking water enhance protective efficacy of BCG: An important role of humoral immunity | M Henao-Tamayo	MIP
1:00	Gilfillan, Darby	Probiotic puzzle: host-microbiome response to rotavirus vaccination using recombinant Lactobacillus | Z Abdo	MIP
1:15	Asma, Omar	Impact of maternal omega-3 fatty acid intake on ovine placental and fetal tissue metabolism | A Chicco	BMS
1:30	Parsons Aubone, Agata	Mitochondrial respiration in human placental cell lines | A Chicco	BMS
1:45	BREAK		
2:00	Kang, Young-Jin	Dentate granule cell birth date-specific upregulation of feedback inhibition in a mouse model of posttraumatic epilepsy | B Smith	BMS
2:15	Kincade, Jessica	Transient and Persistent BVDV Infections and a Unique Post-natal Phenotype | T Hansen	BMS
2:30	Lee, Sang-Hun	Vulnerability of cholecystokinin-expressing GABAergic interneurons in the unilateral intrahippocampal kainate mouse model of temporal lobe epilepsy | B Smith	BMS
2:45	Liebig, Bethany	CD146 expression by adult equine chondrocytes is not a strict indicator of suppression of stimulated lymphocyte proliferation | J Kisiday and L Goodrich	CS
3:00	Locklear, Taylor	Retrospective review of causes of mortality for zoo giraffe and okapi (1991-2020) | M Sadar	CS
3:15	Moskaluk, Alexandra	Two novel species of Arthroderma isolated from domestic cats with dermatophytosis in the United States | S VandeWoude	MIP
3:30	Williams, Katherine	Myoblast Exosome Production, Function, and MiRNA Cargo is Altered by Mechanical Stimulation: Therapeutic Implications for Skeletal Muscle Regeneration | N Ehrhart	CS

SESSION 3: Clinical Science

Early-stage research, Noon–3:45 P.M. | LSC 302

Time	Presenter	Topic	Dept.
12:00	Chang, Theodore	Clinical outcome of canine apocrine gland anal sac adenocarcinoma patients with non-surgical metastatic lymph nodes treated with stereotactic radiation therapy | S Larue	ERHS
12:15	Conry, Megan	Antiretroviral therapy decreases feline immunodeficiency virus RNA in saliva of infected cats | VandeWoude	MIP
12:30	Conway, Rachel	Behavioral and physiologic effects of a single dose of oral gabapentin in rabbits | M Sadar	CS
12:45	Denton, Matthew	The use of Trazodone as an Anxiolytic Prior to General Anesthesia in Dogs | G Griffenhagen	CS
1:00	Diaz, Devon	Imaging the cellular highway: microcirculation and endothelial glycocalyx in canine cardiopulmonary bypass | J Guillaumin	CS
1:15	Diaz, Amanda	Alpha-enolase as a biomarker for early diagnosis of acute kidney injury and chronic kidney disease in cats | M Lappin	CS
1:30	Fisher, Corey	Surgical interventions and outcome in a population of feline trauma patients | K Hall	CS
1:45	BREAK		
2:00	Goodman, Kathryn	Quantitative analysis of esophageal transit times in normal cats using contrast enhanced videofluoroscopy | E Randall	ERHS
2:15	Jones, Katrina	An increase in canine infected corneal ulcerations during the 2020 northern-Colorado wildfire season | M Henricksen	CS
2:30	Levy, Ivana	Get savvy about cavies: comparison of two portable lactate analyzers in guinea pigs (Cavia porcellus) | M Sadar	CS
2:45	Rice, Hannah	The use of increasing enclosure complexity to improve African elephant welfare at the Cheyenne Mountain Zoo | M Johnston	CS
3:00	Rogers, Tatiana	Retrospective Analysis Of Morbidity And Mortality Of Gas Anesthesia, Injectable Sedation, And Combined Protocols In Avian Species | M Sadar	CS
3:15	Worthington, Delaney	Investigating virtual versus hands-on learning interventions in promoting increased performance, confidence, and engagement in a large general microbiology course | J McLean	MIP
3:30	Young, Victoria	Association between feline hyperthyroidism and thoracic radiographic evaluation of cardiomegaly and pulmonary hyperinflation | A Marolf	ERHS

SESSION 4: Clinical/Translational Science

Early-stage research, Noon–1:45 p.m., Advanced-stage research, 2–3:45 p.m. | LSC 300

Time	Presenter	Topic	Dept.
12:00	Maldonado, Mikaela	Chiropractic treatment improves lameness and axial skeleton pain and stiffness in horses | K Haussler	CS
12:15	Marsh, Jordan	Phenotypic assessments of metabolic status and potential for dietary supplements to mitigate insulin resistance in the obese mare | E Carnevale	BMS
12:30	Maslyn, Kara	Evaluating the effects of telmisartan in healthy dogs as a preclinical model for Shar-Pei fever | M Lappin	CS
12:45	Sattar, Fouzia	Determinants of Antimicrobial Use in Poultry Sector in South Asia - A Systematic Literature Review | S Rao	CS
1:00	Slaughter, Megan	Evaluation for anti-erythrocyte and anti-platelet antibodies in healthy dogs administered lokivetmab | S Shropshire	CS
1:15	Weber, Annika	Rice bran in ready-to-use therapeutic foods for microbiota-targeted treatment of childhood malnutrition | E Ryan	ERHS
1:30	Weisburg, Ilana	Evaluating the confidence and competency of leadership and teamwork communication skills gained for the RECOVER BLS and ALS simulation CPR training course | K Hall	CS
1:45	BREAK		
2:00	Bisazza, Katherine	Comparison of Advanced Imaging Modalities to Assess Bone Mineral Density in the Sheep Model | J Easley	CS
2:15	Burton, Mollie	Pharmacokinetics of Gabapentin after Single, Oral Administration in Domestic Rabbits (Oryctolagus cuniculus) | M Sadar	CS
2:30	Fathke, Robert	Colorado Dairy Farmer Knowledge, Attitudes and Practices for Livestock and Human Infectious Disease Prevention | S Rao	CS
2:45	Kelley, Jennifer	Prognostic indicators for feline craniofacial trauma: a retrospective study of 130 cases | J Rawlinson	CS
3:00	Lederman, Jessica	Estrus suppression in the mare through the use of altrenogest releasing intravaginal rings | J Hatzel	BMS
3:15	Maison, Rachel	Serosurveillance for anthrax exposure in Texas feral swine: A potential biosurveillance tool for mapping risk | A Bosco-Lauth	BMS
3:30	Schlein, Lisa	Feverfew: Cheerful foliage or source of an anticancer compound? | D Thamm	CS

POSTER PRESENTATIONS

SESSION 1 | ODD-NUMBERED POSTERS | Noon-1:45 p.m.
SESSION 2 | EVEN-NUMBERED POSTERS | 2-3:45 p.m.

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

No.	Presenter	Title | Mentor	Dept.
1	Ammons, Dylan	Investigation of the canine immune landscape with single cell transcriptomics | Dow	CS
2	Barton, Madisen	Testing the validity of using a biochemical analytical assay for diagnosing canine cognitive dysfunction, as a translational model for Alzheimer's disease | McGrath	BMS
3	Bashor, Laura	SARS-CoV-2 in felids | VandeWoude	MIP
4	Bautista, Anna	Estrogen Receptor Beta in oxytocin neurons has an implicated role in HPA axis regulation | Handa	BMS
5	Berezin, Casey-Tyler	Endogenous opioid signaling in the retina modulates sleep/wake behavior | Vigh	BMS
6	Bergum, Nikolas	A novel target for opioid-induced sleep disorders | Vigh	BMS
7	Bisazza, Katherine	Using the Ovariectomized Ewe to Model Postmenopausal Osteoporosis Disease Progression | Easley	CS
8	Berry, Kailey	Reported neurocognitive symptoms post SARS-CoV-2 infection in adults and the relationship with IL-6, IL-8, and D-dimer inflammatory markers | Ryan	ERHS
9	Bonilla, Andres	Indirectly targeting the intervertebral disc nucleus pulposus tissue for a model of degenerative disc disease | Easley	CS
10	Bouse-Eaton, Cassidy	Verifying correlation between equine clinical examination parameters and inertial measurement unit data to optimize wearable inertial sensor development | Kawcak	CS
11	Burke, Bradly	Immunophenotyping immune cells of the Jamaican fruit bat | Schountz	MIP
12	Cardillo, Jenna	Point of care ultrasound measurement of caudal vena cava diameter and collapsibility index for predicting hypovolemic states in dogs | Cavanagh	CS
13	Carpenter, Molly	Evaluating Bluetongue Virus Reassortment in Culicoides sonorensis | Mayo	MIP
14	Castellanos, Emily	Transcutaneous Vagal Nerve Stimulation of the Auricular Branch in the Mouse | Tobet	BMS
15	Chino, Yuiko	A review of biodosimetry methods for wildlife mammals experienced radiation exposure | Johnson	ERHS

No.	Presenter	Title | Mentor	Dept.
16	Chornarm, Nida	Anti-erythrocyte antibodies detected by flow cytometry assay in 235 anemic client-owned dogs | Lappin	CS
17	Cooper, Sara	Spatial patterns of immune protection reveal granuloma heterogeneity among BCG vaccinated, Mycobacterium tuberculosis-infected mice | Podell	MIP
18	Coupannec, Maelle	A Radioanalytical Method For The Separation Of Radium From Hydraulic Fracturing Waste Water Using Ion Exchange Resin | Sudowe	ERHS
19	Daniels, Alyssa	Evaluation of factors associated with surgical site infection in equine proximal interphalangeal joint arthrodesis: 54 cases (2010-2019) | Hendrickson	CS
20	Davis, Hailey	Use of a commercially available electrolyte supplement in the management of acute diarrhea in shelter dogs | Lappin	CS
21	Donkoh, Jasmine	Dengue Virus Reprograms Macrophage Gene Expression and Metabolism | Rovnak	MIP
22	Dugan, Conner	Prevalence of Upper Respiratory Pathogens in Colorado Front Range Rescue Horses | Landolt	CS
23	Gary, Hadley	Intrapulmonary host-directed therapy using bioactive vitamin A for Mycobacterium tuberculosis infection in guinea pigs | Podell	MIP
24	Gonzalez-Castro, Raul	Cryopreservation of stallion sperm results in a loss of Phospholipase C zeta | Carnevale	BMS
25	Haberman, Jared	Influence of sex and BMI on changes to quality of life scores in adults from 3 months to one year post COVID-19 disease | Ryan	ERHS
26	Haines, Laurel	Osteosarcoma exosomes selectively home to the lung and elicit pro-metastatic changes in resident alveolar macrophages | Regan	MIP
27	Harris, Maccallister	Kinetics and effector function of lipid antigen CD1-restricted immunity in M. tuberculosis infected guinea pigs | Podell	MIP
28	Wist, Sara	Outcomes of horses undergoing ventral midline celiotomy for colic with dorsal mesenteric attachment abnormalities of the colon and cecum | Hassel	CS
29	Isdale, Rachael	Prevalence of FeLV proviral DNA in samples from client owned cats in a university blood bank | Lappin	CS
30	King, Connor	Spatial transcriptomics illuminates pathways correlated to immune control of Mycobacterium tuberculosis infection | Podell	MIP
31	Kuldell, Caroline	Differences in CD146 expression between neonatal and adult chondrocytes are not reflected in microRNA expression |Kisiday	CS
32	Labb, Samantha	Closing the Nuclear Fuel Cycle: Minor Actinide Separations | Sudowe	ERHS

No.	Presenter	Title | Mentor	Dept.
33	Larson, Blaine	Safety and analgesia of liposomal bupivacaine administered intra-abdominally and peri-incisionally for laparoscopic ovariectomy in mares | Pezzanite	CS
34	Lee, Rahmi	Co-activation of nicotinic acetylcholine receptors leads to improvement of brain rhythms and memory in AD | Kim	CS
35	Lee, Sera	Ocular penetration of oral acetaminophen in the horse | Hector	CS
36	Lian, Elena	Altering the mycobacterial cell envelope structure differentially affects cell surface properties in Mycobacterium abscessus – implications for host adaptation | Jackson	MIP
37	Linch, Mikaela	Development of a novel vaccine for Feline Enteric Coronavirus using recombinant Lactobacillus acidophilus | Dean	MIP
38	Lopes Sicupira Franco, Patricia Mara	Molecular Characterization of Giardia intestinalis isolates in Dogs from a Rescue Shelter in Northern Colorado | Lappin	CS
39	MacNeill, Blaire	Enrichment media improves detection of Streptococcus equi subsp. equi in environmental screening samples | Daniels	MIP
40	Maldonado Jr, Pablo	Defining specialized M1 phenotypes in Mycobacterium tuberculosis infection | Henao-Tamayo	MIP
41	Mankowski, Clara	FIV quick assay: a novel technique for diagnosis of feline immunodeficiency virus | VandeWoude	MIP
42	Martens, Elise	Rabacfosadine (TANOVEA) for the Treatment of Relapsed Multicentric Canine Lymphoma | Thamm	CS
43	Martin, Jason	WHO Let The Dogs Out: How Virtual Animal Anatomy Facilitated a Successful Transition to Online Instruction and Supported Student Learning During the Coronavirus Pandemic | Magee	BMS
44	McAdam, Tiera	Fecal bacterial microbiota and insights into antibiotic-induced perturbation of the microbiome in domestic ferrets (Mustela putorius furo) | Wolfe	CS
45	McGimsey, Malea	Ambient temperatures experienced by free-ranging giraffe across sub-Saharan Africa | Johnston	CS
46	McGimsey, Malea	Assessing northern sea otter (Enhydra lutris kenyoni) immune function in Alaska | Hollmen	Other
47	Mendex-Vazquez, Hadassah	Autism-associated-catenin G34S mutation promotes GSK3-mediated premature degradation and social deficits | Kim	BMS
48	Molli, Angela	Pharmacokinetics of oral ondansetron in dogs admitted to a tertiary referral hospital, a pilot study | Shropshire	CS

No.	Presenter	Title | Mentor	Dept.
49	Natter, Nicole	Longitudinal SARS CoV-2 identification in human stool and associated gut microbiota | Ryan	ERHS
50	Ojeda, Vivian	Intramedullary stents as a device to prevent post-radiation fractures in canine patients with osteosarcoma | Seguin	CS
51	Orluk, Julia	Temporal comparison of plasma total mercury in Steller sea lions (Eumetopias jubatus) of the Aleutian Islands | Rea	Other
52	Parker, Ashley	Early Failure of CHOP Protocol Indicates Poor Response to Rescue Protocol in Dogs with Lymphoma | Thamm	CS
53	Patlin, Brielle	Characterization of neuronal fibers, immune cells, and coronavirus in a murine organotypic lung slice | Tobet	BMS
54	Patterson, Hannah	Use of computed tomography and nuclear scintigraphy for diagnosis and staging of primary anterior uveal osteosarcoma in a client-owned rabbit | Sadar	CS
55	Pearce, Camron	Assessing the host immune response to Mycobacterium abscessus infection in cystic fibrosis | Gonzalez-Juarrero	MIP
56	Peraza, Jacqueline	Short-term Treatment of Keratoconjunctivitis Sicca (KCS – dry eye) in Three Dogs With Equine Interleukin-1 Receptor Antagonist Protein (IRAP) and its Effect on Clinical Parameters | Henriksen	CS
57	Perez, Luisanny	Evaluating B7-H3 as a tumor antigen target in canine osteosarcoma for CAR T cell therapy | Dow	CS
58	Priore, Maggie	SFTSV: Characterizing a novel bunyavirus in North American Wildlife | Bosco-Lauth	MIP
59	Ringdahl-Mayland, Beck	Clinical Outcome in Dogs with Appendicular Osteosarcoma Treated with Palliative Radiation Therapy With and Without Bisphosphonates | Tiffany	ERHS
60	Rinker, Cody	Repurposing -blockers as anti-cancer immunotherapeutics to target myeloid derived suppressor cells | Dow	MIP
61	Sanfacon, Brittney	The impact of TP53 missense and truncating mutations on protein function and tumor progression in canine osteosarcoma | Duval	CS
62	Sauerwein, Leah	One Health, One Cat at a Time | Duncan	MIP
63	Sauerwein, Leah	A retrospective analysis of traumatic brain injury (TBI) in canine trauma patients | Hall	CS
64	Sheng, Julietta	Maternal stress alters hypothalamic development | Tobet	BMS
65	Stewart, Joseph	Measuring Systemic Genomic Instability in Yeast | Argueso	ERHS

No.	Presenter	Title | Mentor	Dept.
66	Tarbutton, Jordan	Markers of endothelial injury in canine trauma patients: a pilot study | Hall	CS
67	Templeton, Hayley	Intestinal Model for Parkinson's Disease Development | Tobet	BMS
68	Timkovich, Ariel	Differential transcript expression in compensatory limbs following DMM or sham surgery in male and female mice | Santangelo	MIP
69	Tipton, Madison	Dried Blood Spots and Urine Metabolite Profiling reveals dietary biomarkers of increased cowpea consumption by young Children and Pregnant Women in Ghana | Ryan	ERHS
70	Tovar Lopez, Silvia	Multiple myeloma with aberrant CD3 expression in a red-lored Amazon parrot (Amazona autumnalis) | Sadar	CS
71	Weaver, Danielle	Neurotoxicity is seen in the aging cat brain by accumulation of misfolded proteins and glial inflammation | Moreno	ERHS
72	Webster, Meghan	Comparative phenotypic and transcriptomic analysis of synovial macrophages and tissue macrophages from healthy horses and horses with osteoarthritis | Pezzanite	CS
73	Westbrook, Madeleine	Evaluation of prednisolone pharmacokinetics and toxicity in dogs with lymphoma or immune-mediated disease | Lana	CS
74	Woyda, Reed	Campylobacter prevalence differs across and within broiler houses with re-used poultry litter | Abdo	MIP

O-1. SARS-CoV2 viral RNA biology and its impact on the infected cell.

Noelia H Altina, David G Maranon, John R Anderson, Loran B Anderson, Rushika Perera, Brian J Geiss, Jeffrey Wilusz

Our laboratory investigates fundamental knowledge gaps in the RNA biology of SARS-CoV-2. Specifically, we are interested in the biological implications of cap-proximal adenosine methylation of viral transcripts as well as the impact of viral RNA-protein interactions on host cell RNA biology. First, we obtained evidence suggesting that the adenosine at the 5′ end of SARS-CoV-2 mRNAs likely contains an 'm^6A_m' methylation modification. This is significant because all cellular mRNAs that initiate with an adenosine has the adenine (A) residue methylated, resulting in a similar 'm^6A_m' modification. Thus, we hypothesize that SARS-CoV-2 is modifying its mRNAs to prevent their detection as 'non-self' by the cellular innate immune response. We are currently testing this hypothesis and we are focused on the identification of the mechanism for SARS-CoV-2 m^6A_m RNA modification. Second, two cellular proteins, hnRNPA1 and PTBP, that regulate alternative splicing bind to the abundant viral mRNAs in related coronaviruses and become mis-localized to the cytoplasm during infection. Therefore, we hypothesize that SARS-CoV-2 mRNAs can sequester these splicing factors leading to significant changes in alternative mRNA splicing. RT-PCR analyses of the splicing patterns across select exons of hnRNPA1/PTBP-targeted pre-mRNAs confirm this hypothesis and suggest a new mechanism for the impact of SARS-CoV-2 infection on cellular RNA biology. Collectively, these studies collectively identify attractive targets for developing novel broad-spectrum anti-coronavirus drugs. This work was supported by NIH grant R21 AI158335.

Graduate Student / Microbiology, Immunology and Pathology

O-2. Assessing the Role of Lung Fibroblasts in Modulating Osteosarcoma Chemotherapeutic Response.

Chris G. Andretsos, Eric P. Palmer, Aaron Offermann, Daniel Regan

Osteosarcoma (OS) is the most common primary malignant tumor. Survival rates for patients with OS have not improved in over 30 years, entirely due to our inability to treat the ~40% of patients who develop disease recurrence, occurring almost exclusively in the form of lung metastasis. While OS tumor cell intrinsic mechanisms of metastasis and drug resistance have been elucidated, significantly less is known about how the OS tumor microenvironment (TME) may extrinsically promote these processes. One of the major cell types present in the tumor microenvironment are fibroblasts. In primary tumors, these cancer-associated fibroblasts promote tumor growth through the release of soluble factors such as cytokines and growth factors which drive angiogenesis, tumor cell survival and proliferation. However, whether resident fibroblasts in distant metastatic sites play a similar role in OS progression is unknown. Thus, we sought to determine if primary human donor-derived lung fibroblasts (LFs) facilitate OS cell chemoresistance to current OS standard-of-care. Utilizing 3D "lung-like" organotypic co-cultures of human OS tumor cells (n=3) and primary lung fibroblasts, we assessed the impact of LFs on human OS tumor cell proliferation and response to doxorubicin, cisplatin, and methotrexate chemotherapy. LFs exerted significant differences in tumor cell proliferation and drug response in a cell line- and drug-dependent manner. Furthermore, these LF-mediated phenotypic differences in OS cells were associated with changes in IL-6, IL-8, and CCL2 secretion in these cultures, cytokines all previously implicated in tumor cell survival and/or metastasis. Combined, this preliminary data provided a first step in identifying novel therapeutic targets for modulation of the lung microenvironment as a potential means to slow or prevent OS metastasis.

Staff / Microbiology, Immunology and Pathology

O-3. Vaccines “au natural”: novel inactivation method using UV+riboflavin preserves influenza surface antigen expression when compared to traditional methods of vaccine preparation.

Savannah E Flory, Izabela K Ragan, Lindsay M Hartson, and Raymond P Goodrich

Infectious diseases are within the top ten causes of death globally. Vaccines have proven to be an effective method for combating infectious diseases, such as influenza and coronaviruses, especially on an epidemic and pandemic scale. Chemical treatments are commonly used in vaccine preparation to inactivate whole pathogens by modifying nucleic acid and protein structures, which prevents the pathogen from replicating and being infectious. However, such methods use harsh chemicals and risk damaging key antigenic epitopes that are needed to stimulate a robust immune response. Our objective is to test the efficacy of a novel photochemical inactivation method, which uses riboflavin and UV light to irreversibly damage viral nucleic acid. We hypothesize that this method will enhance vaccine immunogenicity by preserving the quantity and quality of viral antigens while also decreasing vaccine production time and waste. Using influenza virus as a virus model, we compared the outcome of this inactivation method against traditional chemical methods (formalin, BPL), specifically in terms of the effect on preserving major antigenic proteins. Our work to date has found that UV+riboflavin inactivation preserves key influenza proteins needed for a robust immune response. This inactivation method could increase the immunogenicity and efficacy of influenza vaccines, while also decreasing vaccine risk, production costs, and production of harmful waste by-products. The knowledge gained from this study can then be applied to other infectious pathogens with significant public health impacts.

DVM Student / Microbiology, Immunology and Pathology

O-4. Comparison of four freezing extenders on post-thaw motility of stallion spermatozoa.

Shelby K. Granier, James K. Graham, Paula D. Moffett, Patrick M. McCue

Insemination of mares with frozen-thawed sperm is common practice in the equine breeding industry. Unfortunately, there is significant variability between stallions and semen extenders used to cryopreserve spermatozoa. Here, we aimed to evaluate post-thaw sperm motility for stallion semen cryopreserved in differing extenders, which included bases of Lactose EDTA (LE) and Modified French (MFR5) extenders both containing either 5 % glycerol or 2 % glycerol plus 3 % methylformamide (LE+ and MFR+). One ejaculate was collected from each of 20 stallions, and frozen in 4 aliquots of equal volume in each extender. For the analysis, one straw from each stallion, cryopreserved in each extender was thawed and evaluated for total and progressive sperm motility. Results indicate that post-thaw total and progressive sperm motility were significantly higher ($P<0.05$) for semen cryopreserved in the dual-component cryoprotectant extenders (LE+, 62.9 ± 12.5 % and 50.0 ± 13.8 %, respectively; MFR5+, 57.3 ± 11.9 % and 46.5 ± 11.6 %, respectively) than semen cryopreserved in extenders containing 5% glycerol (LE, 44.3 ± 18.3 % and 34.3 ± 19.1 %, respectively; MFR5, 43.6 ± 15.6 % and 34.0 ± 16.0 %, respectively). There was no statistical difference ($p>0.05$) in post-thaw total or progressive motility between the two extenders containing the dual-component cryoprotectant as well as the two extenders containing only 5 % glycerol. Of the 20 stallions evaluated, 9 stallions exhibited post-thaw total sperm motility that was ≥ 5% higher when frozen in LE+ extender compared to others, while 3 stallions were highest in MFR5+ and 1 when cryopreserved in LE. In conclusion, stallion semen freezing extenders that contained dual-component cryoprotectants yielded higher post-thaw total and progressive sperm motility than those extenders containing a single cryoprotectant.

Graduate Student / Clinical Sciences

O-5. Restricted antimicrobial use among dairy production systems following implementation of the Veterinary Feed Directive: Impact evaluation through a Systematic Review.

Adam Hernandez, Jessica Wilson, Sangeeta Rao

Antimicrobial use for livestock production including dairies in the United States (US) has been an area of concern due to an increasing threat of antimicrobial resistance (AMR) potentially related to the misuse and disuse of antimicrobials. Proliferation of AMR would be a hazard to the capacity of our food animal production and human health systems, as many of the antibiotics see use in both. To address this concern, the FDA created the Veterinary Feed Directive (VFD), an amendment to the Animal Drug Availability Act that went into effect on January 1st of 2017. The VFD imposes restrictions on the usage of antimicrobials in livestock production, particularly their addition to feed. Following implementation, the addition of antibiotics to livestock feed can only occur under the supervision of a licensed veterinarian. The goal of this study is to describe the potential impacts of the implementation of the VFD on the dairy industry in the US by identifying changes to production indicators, clinical outcomes, and economic impacts associated with restricted antimicrobial use.

A systematic review is being conducted as per PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines using three databases: PubMed, CAB Abstracts, and Medline, as well as searches conducted in Google Scholar. Searches are in progress using the keywords and Boolean operators: (dairy OR cow OR calf) AND (antibiotic or antimicrobial) AND (restrict OR ban) AND (milk OR production OR cost OR yield). After removing duplicates this search produced 513 documents. Literature was screened by title and abstract reducing the number of documents to 105. These documents will be assessed in their entirety. While no results are available at this time, it is expected that a number of production and economic indicators will be evident in the final set of literature and available for extraction and synthesis.

DVM Student / Clinical Sciences

O-6. The effect of short and long term frozen storage of recombinant hamster prion protein in rt-quic.

Parker J Hogan, Edward A Hoover, Candace K Mathiason, Nathaniel D Denkers

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 5-log 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 for the assay, the recombinant hamster prion protein (rhaPrP). Deep freeze storage of rhaPrP has successfully been validated, yet to date, 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. Therefore, the purpose of this study was to establish how stable and consistent rhaPrP was post early (3-4 months) or late (18-22 months) thaw cycles. Additionally, filtration and centrifugation were employed on the thawed protein prior to use as a means of determining the effect on sensitivity and to assist in decreasing false-positive signals. The rates of amyloid formation (reaction rate) from CWD positive and negative brain homogenates were used to compare the thawed to never frozen rhaPrP. Preliminary results on the freeze/thaw, regardless of duration, revealed no significant difference between the original data rates and the post thaw rates. Additionally, the use of centrifugation and specifically 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 studies continue to explore the reproducibility of these experiments.

Undergraduate Student / Microbiology, Immunology and Pathology

O-7. Glucocorticoid receptor signaling is required for acclimation of skeletal muscle to hypobaric hypoxia.

Luke A Whitcomb, Katelyn E Maher, Dorcas D Ka, Phillip T Zilhaver, Casey P McDermott, Ronald B Tjalkens, Katie J Sikes, Ryan W Maresh, Adam J Chicco

Hypobaric hypoxia (HH) encountered at high altitudes acutely impairs aerobic exercise capacity, which partially recovers following 1-2 weeks of acclimation to chronic HH. Persistent elevations in serum glucocorticoids occur during HH exposure, but their role in these acute and chronic physiological responses is unclear. We tested the hypothesis that glucocorticoid signaling is essential for the acclimation of aerobic exercise capacity to chronic HH, in part by mediating adaptive changes in skeletal muscle metabolism. Male F344 rats were administered the glucocorticoid receptor antagonist RU486 (RU; 60 mg/kg/d in chow) or no drug for 5 days prior to 15 days of continued normoxia (Fort Collins, CO; elevation 5,003 feet) or HH (simulated 17,200 feet in a hypobaric chamber) with or without continuous RU treatment (N=4-8/group). Graded treadmill exercise tests (GXT) were conducted on a motorized treadmill in normoxia, during acute HH exposure, and in HH after 15 days of HH acclimation. As expected, acute HH reduced GXT performance compared to normoxia in all rats, which improved following 15 days of acclimation to HH. RU pretreatment did not impact hypoxic GXT performance, but continuous treatment abolished improvements in GXT performance following chronic HH. RU attenuated HH-induced increases in hematocrit and muscle fatty acid oxidation efficiency assessed by high-resolution respirometry *ex vivo*, suggesting that glucocorticoid signaling may improve muscle oxygen utilization in response to chronic HH. RU also prevented HH-induced decreases in pyruvate dehydrogenase expression and increases in Krüppel-like factor 15, proteolysis and branched-chain amino acid aminotransferase in glycolytic muscle, implicating glucocorticoid signaling in a rewiring of glucose and protein catabolism to rid the cell of excess nitrogen in HH. In conclusion, these results demonstrate that glucocorticoid receptor signaling is essential for the acclimation of aerobic exercise capacity to HH, perhaps by mediating improvements in the bioenergetic efficiency of skeletal muscle metabolism.

Graduate Student / Biomedical Sciences

O-8. Mechanical compression as a model of disc degeneration disease.

Andres Bonilla, Mitchell Page, Steve Dow, Brian Johnstone, Christian Puttlitz, Jeremiah Easley.

Disc degeneration disease (DDD) is the main cause of low back pain in people. Diverse animal models have been developed to understand the disease progression and validate potential treatments for this condition. To date, the majority of DDD models involve direct trauma to the disc components, which does not accurately mimic the human condition. Models that do not cause direct trauma to the disc components such as disc stabilization have been described in animal models. However, mechanical compression has not been previously reported in a large animal model. Additionally, materials used for these disc stabilization models are not often MRI compatible, limiting the use of in-life MRI to follow the initiation and progression of DDD over time. We aimed to create a large animal model of DDD using mechanical compression devices made of MRI compatible materials. A mechanical compression device consisting of two threaded titanium pedicle compression rods, a bronze compression spring, and a plastic rod connector were implanted at L2-L3 and L4-L5 disc levels through a posterolateral approach in three skeletally mature sheep (n=6 disc levels). Three naïve sheep served as control samples. Standard lumbar radiographs and 3T MRI sequences were performed pre-surgery, 6 weeks, and 12-weeks (post implantation), as well as kinematic analysis and histopathology. Lumbar radiographs confirmed dorsal disc compression and ventral disc distraction in all affected discs immediately following surgery. MRI allowed serial disc imaging throughout the study period with moderate metal artifact. No signs of disc degeneration were noted on radiographic, or MRI compared to control discs. No significant differences were noted in kinematic analysis between compressed discs and control discs. Histological results are pending. In this study, we offered a reliable and reproducible sheep model of DDD, in which controllable and trackable degeneration could be achieved using a MRI compatible compression device.

Graduate Student / Clinical Sciences

O-9. Virulence determinants and class I integron-encoded antimicrobial resistance among Salmonella enterica serovar Typhimurium of swine origin.

Cydney Jardine, Nora Jean Nealon, Elizabeth Kim, Sangeeta Rao

Antimicrobial resistance (AMR) is an escalating global public health concern in *S. enterica*, a zoonotic livestock pathogen. Animals shedding or meat contaminated with *Salmonella enterica* also put the animal workers or meat handlers or consumers at risk. Previous studies suggest that integron (jumping gene cassettes) presence is associated with carriage of AMR genes especially conferring multidrug resistance. However, the association of virulence factor genes with integron-containing *S. enterica* is still unknown. The current study aims to identify and characterize virulence factors and AMR genes from integron-encoded S. Typhimurium isolated from swine and their environments using Whole Genome Sequencing (WGS) methods. S. Typhimurium isolated from swine and its' environments (n=32) was sourced from veterinary diagnostic laboratories from two institutes. Broth microdilution was used to establish phenotypic AMR. PCR and gel electrophoresis were used to identify integrons. DNA was isolated for WGS on an Illumina MiSeq. Denovo assembly algorithms reconstructed each genome (chromosome and/or plasmids) in SPAdes through a Geneious Prime interface. MEGARES database was used to screen each genome for AMR and virulence factor genes. A Fisher's exact test was used to examine statistical associations with integron presence. Genome analyses revealed that 27 out of the 32 swine samples collected contained integrons: 19 had an integron size of 1200 basepairs, and 8 had 1000 bps. Integron-containing S. Typhimurium showed a higher frequency of resistance to drugs such as beta-lactams, sulfa, tetracycline, and multiple phenicol drugs. The results from this study can be applied to predict the pathogenicity of S. Typhimurium isolates based on the presence of integron-associated AMR genes and identify antimicrobial resistant isolates that may pose a disease risk to swine and people. Further research will analyze specific virulence factors among integron-containing *S. enterica*, including rck factor which has been shown to assist *S. enterica* in evading an immune response.

Graduate Student / Clinical Sciences

O-10. Veterinarian's choices and practices of antimicrobial drug treatments among golden retrievers within the United States.

Linzy Jauch, Julia Labadie, Sangeeta Rao

Antimicrobial resistance is a global issue which impacts both public and animal health. Veterinarians use their professional expertise when providing antimicrobial treatments to patients; however, in certain circumstances, antibiotics are unnecessarily or inappropriately prescribed. The purpose of our study was to identify the antibiotic classes that are commonly used for a variety of conditions among golden retrievers across the United States, therefore indicating the veterinarians' preferred choice of antimicrobials. The data was retrieved from the Morris Animal Foundation Golden Retriever Lifetime Study, following 3,044 golden retrievers spanning from 2012-2020 and was collected from veterinary questionnaires including information on patient demographics, medical diagnoses, and treatments provided. Data was sorted into five geographical regions: Midwest, Mountain, Northeast, Pacific and South. R studio was used to clean and analyze the data in which the most common diagnoses were evaluated along with most frequently used antimicrobials overall as well as per region. Our results showed that the most prevalent diagnoses reported and treated with antimicrobials were otitis externa, diarrhea/ gastritis, hot spots, and bladder infections. Otitis externa accounted for the highest (39.8%) of total reported medical diagnoses and 42.8% of the diagnoses reported to be treated with antimicrobials. The Midwest region accounted for 23.6% of total reported antimicrobials prescribed, followed by the South at 22.3% and the Northeast at 20.4%. Of the total reported antibiotics prescribed for an infectious disease, aminoglycosides were the most frequent (19.7%), followed by nitroimidazoles (18.0%), penicillins (14.1%), and first-generation cephalosporins (13.6%). Despite limitations, our conclusions of otitis externa being the most prevalent condition reported among golden retrievers demonstrates more efforts should be made towards preventing the condition. Focus on preventative protocols will also abide with AVMA's efforts on antimicrobial stewardship. The analyses on associations between conditions and antimicrobial usage and regional differences are still being performed.

DVM Student / Clinical Sciences

O-11. Exploring the effects of standard two dimensional anatomical models and virtual reality models on visuospatial skills.

Brandon L. Lowry, Katelyn E. Brown, Carolyn A. Meyer, Kenny R. Ivie Jr., Brian P. Kelly, Chad M. Eitel, and Tod R. Clapp

Many reports demonstrate virtual reality has applications in medical science education. This emerging technology provides unique experiences to be immersed in content within collaborative and engaging environments. However, there is little evidence to support VR's use case as an educational intervention. Here we describe and evaluate the use of VR to increase spatial ability in graduate students taking a human dissection course. Human anatomy is often presented using two dimensional representations of the data but requires mastery of three dimensional structural relationships. By sorting students into two groups, control and experimental, we measured changes in visuospatial skill ability using mental rotation tests. The interventions used standard two dimensional anatomical models to demonstrate anatomy while the experimental group used virtual reality models. Preliminary results suggest that while both control and experimental groups demonstrated improvement between pre and post-intervention quizzes, there was no significant difference between groups. However, when categorizing students by GPA, the lower GPA students in the experimental group performed significantly better between pre and post quizzes. This suggests that immersive virtual reality may be an effective intervention for some students.

Undergraduate Student / Biomedical Sciences

O-12. Establishing the impact of osteosarcoma-derived exosomes in promoting lung metastasis.

Eric P Palmer, Laurel A Haines, Daniel P Regan

Osteosarcoma (OS) is the most malignant form of bone cancer primarily affecting children and young adults. Of those diagnosed, roughly 40% of patients will develop lung metastasis resulting in poor prognosis. Increasing data suggest extrinsic relationships between cancer cells and the non-malignant stromal cells within the tumor microenvironment (TME) are in part responsible for the progression of lung metastasis. Soluble factors released by the primary tumor, called exosomes, contain biological cargo that can remotely prime stromal cells within the lung, and contribute to the formation of a pre-metastatic niche. The mechanisms by which tumor-derived exosomes promote lung metastasis are not yet understood. Therefore, we have evaluated the effects of OS-derived exosomes on donor human lung fibroblasts, a key stromal cell within the lung. Exosomes were purified from 6 human OS cell lines (n=6) and characterized via tunable pulse resistance sensing (TPRS), western blot, and transmission electron microscopy (TEM). Human donor-derived primary lung fibroblasts (LF's; n=4) were 'educated' with OS-derived exosomes and assessed for; OS exosome uptake via flow cytometry and confocal fluorescent microscopy, exosome-induced cellular response via MAPK and Akt/mTOR phospho-kinase array, and exosome-induced cytokine secretion via a 45-plex multiplex cytokine array analysis of LF supernatants. Lung fibroblasts efficiently took up OS exosomes *in vitro*, which was associated with induction of MAPK pathway activation and significantly enhanced secretion of IL-6, IL-8 and CCL2 cytokines compared to untreated LFs. This OS exosome-mediated induction of IL-6 secretion in LFs occurred in a dose-dependent manner. Together, these data suggest OS-derived exosomes can induce a pro-inflammatory phenotype in lung fibroblasts characterized by secretion of known pro-metastatic cytokines/chemokines. These preliminary studies provide a first step in identifying novel molecular targets for modulation of the lung microenvironment for future investigation as potential therapeutic strategies to slow or prevent OS metastasis.

Graduate Student / Microbiology, Immunology and Pathology

O-13. Plasma fatty acid quantification in adults with mild, moderate and severe COVID-19 disease.

Sophia Stromberg, Bridget A. Baxter, Gregory Dooley, Stephanie M. LaVergne, Emily Gallichotte, Madison Tipton, Kailey Berry, Marcela Henao-Tamayo, Sangeeta Rao, Julie Dunn, and Elizabeth P. Ryan

SARS-CoV-2 has spread across the world, infecting hundreds of millions in 24 months. Many of the individuals who survive are left with new or persisting symptoms, also called post-acute sequelae of COVID-19 (PASC). Obesity is cited as a risk factor for disease severity and the development of PASC, and this may be attributed to the hyperinflammatory state observed in obesity. Plasma fatty acids are key modulators of inflammation, and dyslipidemia may increase risk of unfavorable disease outcomes. Plasma fatty acid profiles of 52 COVID-19 patients were analyzed by gas chromatography- mass spectrometry to assess perturbations during the acute and convalescent stages of infection. Participants were grouped based on disease severity and the presence of PASC. Significant differences between the fatty acid profiles were identified. Palmitic acid levels in individuals with severe disease were 268.8 – 1582.5 ng/ml, which was 92% higher than the uninfected adults. The levels of g-linolenic acid in individuals with moderate disease were 4.9 – 17.7 ng/ml, which was 146% lower than the uninfected adults. Depletion in this fatty acid was also observed in those who developed PASC. Linoleic acid levels in those with moderate disease were 298.65 – 583.5 ng/ml, which was 73% lower than those with mild disease and 75% lower than the uninfected adults. Depletion of eicosapentaenoic acid was observed in individuals with PASC and was also significantly depressed in those with moderate disease compared to the uninfected and mild disease severity groups. These data suggest a potential role for fatty acids in COVID-19 disease severity and in the development of PASC. Further investigation in larger cohorts is warranted to determine the practicality of fatty acids as a possible intervention target for reducing risk for developing long hauler symptoms.

Undergraduate Student / Environmental and Radiological Health Sciences

O-14. Elucidating sex differences in the anterior cruciate ligament response to injury using a novel mechanical rupture model.

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

Anterior cruciate ligament (ACL) injuries account for over 50% of all knee injuries. Females are up to six times more likely to experience an ACL injury compared to males participating in the same sports. A variety of factors are likely responsible for the increased prevalence of female ACL injury such as greater pelvic width and hormone fluctuations, although the exact cause remains unknown. It is also poorly understood if sex differences influence the healing processes of a damaged ligament or contribute to the development of secondary conditions such as post traumatic osteoarthritis. The aim of this project was to examine the variable injury responses of males and females following ACL rupture using a novel mechanical (non-surgical) rupture model. Under IACUC approval, unilateral ACL rupture was achieved via mechanical compression in male and female mice at twelve-weeks-old. Weekly voluntary activity monitoring (Anymaze) was conducted for eight weeks post-injury. Statistical analyses were performed using an unpaired Student's t-test to compare individual parameters for males and females at each timepoint with significance set to $p<0.05$. At five weeks following injury, male mice showed a decrease in both distance traveled ($p=0.0086$) and mean speed ($p=0.0075$) relative to female mice. No significant differences between sex for these parameters were observed prior to injury or at eight weeks following injury. These changes suggest that male mice are selectively minimizing movement five weeks after ACL rupture and could indicate differences in the injury response between males and females. Future work will examine compulsory gait (Digigait), histologic, and proteomic changes in both sexes following ACL rupture as well as the healing responses of other stifle ligaments. The translational impact of this work is broad as a greater understanding of sex factors influencing ligamentous injury response could aid in prevention and treatment of injury for both males and females.

DVM/PhD Student / Clinical Sciences

O-15. Preclinical *in vivo* assessment of replacing linezolid for spectinamide-1599 in the Nix-TB regimen.

Zohaib Ali, Amanda Walz, Camron Pearce, Taru Dutt, and Mercedes Gonzalez-Juarrero

Tuberculosis (TB) patients with highly drug-resistant-TB have limited treatment options. Nix-TB trial is testing three-oral-drugs, namely bedaquiline (B), pretomanid (Pa) and linezolid (L) combined as "BPaL regimen" against drug-resistant-TB with excellent favorable outcomes however some toxic effects are observed. Linezolid is a protein inhibitor with known toxicities under prolonged therapy. Spectinamide-1599 (1599) is another potent protein inhibitor of *Mycobacterium tuberculosis* (*Mtb*) wihout known adverse effects and cross-resistance with other TB drugs and is currently in preclinical studies as TB inhalational therapy. We hypothesize that inhaled 1599 has similar or higher efficacy than linezolid if combined with BPa (BPaS regimen). BPaL and BPaS regimens were tested in Balb/c and C3HeB/FeJ mice infected with low-dose Mtb aerosol infection. Bacterial burdens were determined post-treatment. Compared to untreated control, bacterial burdens in lungs of BPaL and BPaS treated Balb/c mice were decreased by >1 and 5 log10 CFU after 2- and 4-weeks treatment respectively and no statistically significant differences were observed between treatment groups. In C3HeB/FeJ mice, 4-weeks of BPaL and BPaS treatment decreased bacterial burdens by >3 log 10CFU compared to untreated control with no statistically significant differences between treatment groups. Comparative analysis of the cytokine profile in bone marrow, plasma, and lung homogenate samples from C3HeB/FeJ treatment groups showed statistically significant differences at bone marrow level. BPaL treated animals had higher concentration of proinflammatory cytokines and chemokines compared to BPaS group. Further investigation of the cells in bone marrow, blood and spleen based on immunophenotyping was done using flow cytometry. We concluded that inhaled 1599 is a potential replacement for linezolid if combined as BPaS regimen and further studies are warranted.

Graduate Student / Microbiology, Immunology and Pathology

O-16. Vitamin D2/D3 levels in the Northern Colorado Coronavirus Biobank: A biorepository for acute and convalescent patients (NoC0-COBIO).

Bridget A Baxter, Michael Armstrong, Sophia Stromberg, Kailey Berry, Madison Tipton, Nicole Natter, Jared Haberman, Michaela G Ryan, Stephanie M LaVergne, Kim McFann, Julie Dunn, Nicole Reisdorph, Elizabeth P Ryan

SARS-Co-V-2 has rapidly spread to infecting hundreds of millions worldwide. Longitudinal biobanking with the Northern Colorado SARS-CoV-2 Biorepository (NoCo-COBIO) was a resource of clinically integrated and quality-controlled samples to assess molecular markers of disease severity and post-acute sequelae of COVID-19 (PASC). Vitamin D deficiency is common in the US and changes immune functions, including T cells and macrophage. Since July 2020, 141 adults with a history of a positive SARS-CoV-2 nasopharyngeal PCR were recruited from the community or hospital settings. Participants were categorized as having mild (no oxygen required), moderate (1-5L/min oxygen requirement), or severe (> 5L/min oxygen requirement). Plasma Vitamin D (D2 + D3) were classified as < 30 ng/mL deficient, 30-50 ng/mL insufficient, 51-71 ng/mL optimal. Quantification by LC-MS/MS (n= 141 infected adults and 18 uninfected adults) and Fisher's exact were conducted to assess associations between disease severity and PASC. Sixty-seven percent of adults were deficient, 24% insufficient, and 8% (N = 12) had optimal levels. Participants with severe disease (n=37) had lower total vitamin D (21.8 ng/mL) and D3 (20.7 ng/mL) when compared to uninfected (n = 18) adults (35.1 ng/mL). Lower vitamin D and D3 levels were detected in obese (22.3 ng/mL) and overweight (29.0 ng/mL) adults when compared to normal weight (31.2 ng/mL). Forty-four percent (N = 42 of 95) had PASC between 29-83 days post PCR diagnosis. Eighty-two percent of hospitalized participants developed PASC, while 35% of those not requiring hospitalization developed PASC. The most common symptoms reported after 29 days of infection were fatigue, exercise intolerance, forgetful or absent minded, difficulty concentrating, loss of smell or taste and dyspnea. No significant correlations were detected between Vit D levels and the development of PASC. Additional integrated research attention is needed for Vit D with specific cellular immune response markers in this cohort.

Staff / Environmental and Radiological Health Sciences

O-17. Innate Immune Interactions of Bat Cells Infected with Middle East Respiratory Syndrome Coronavirus.

Phillida A. Charley, Kira Douglas, Tony Schountz

Middle East respiratory syndrome-related coronavirus (MERS-CoV) first emerged in Saudi Arabia in 2012. In humans, MERS-CoV causes a viral respiratory illness and can lead to death. Dromedary camels were shown to be a reservoir host, but the virus is believed to have originated in bats. The NS4a and NS4b accessory proteins in MERS-COV antagonize innate immunity in a human airway-derived cell line. Other studies have shown that NS4a inhibits type I interferon and is a double-stranded RNA binding protein, which can suppress the activation of RIG-I and MDA5. NS4b has been shown to interfere with the NF-kB pathway. Little is known about MERS-CoV interacts with bat cell innate immunity. We are examining these genes to determine if they similarly affect Jamaican fruit bat (*Artibeus jamaicensis*) kidney cell lines (AJK4 and AJK6) that we have determined are susceptible to the virus. The innate immune gene expressions increase with the MERS-CoV wild-type virus after 24 hours post-infection in the AJK4 cells and 48 hours post-infection in the AJK6 cells, but not with the mutant MERS-CoV. AJK4 cells produce less infectious virus compared to infectious virus from AJK6 cells.

Post-doctoral Fellow/ Microbiology, Immunology and Pathology

O-18. NTM exposure via drinking water enhance protective efficacy of BCG: An important role of humoral immunity.

Taru S. Dutt, Amy Fox, Burton R. Karger, Nathan Youssef, Rhythm Dhadhwal, Sarah Cooper, Elisa Rampacci, Andres Obregon-Henao, Brendan Podell, Marcela Henao-Tamayo

Tuberculosis (TB) continues to increase worldwide despite vigorous attempts to control it. Bacillus Calmette-Guerin (BCG) is the only licensed vaccine currently available for protection against TB, however, its efficacy is highly variable between countries. It has been hypothesized that BCG's variable protection is due, amongst others, to immunological interference by environmental, non-tuberculous mycobacteria (NTM). However, a definitive mechanism has not been identified so far. Considering the foregoing, we developed a murine model closely resembling the natural history of human exposure to different mycobacterial species, including: 1) BCG vaccination at an early age; 2) exposure to viable NTMs (*Mycobacterium avium subsp. avium*) via the oral route and 3) maintaining continuous NTM exposure even after TB infection, as occurs in endemic places. Surprisingly, we found that a low dose of NTM via the oral route enhanced BCG-mediated protection for 120 days post infection, as determined by decreased *Mycobacterium tuberculosis* (*Mtb*) burden in lungs and spleens of infected mice. This reduction in *Mtb* is directly correlated to the presence of B220+ B2 B-cells and CXCR5+ follicular helper T cells in the lungs. We also noticed the increased influx of cytotoxic T-lymphocytes in those lungs with reduced pathology. Therefore, we hypothesized that B cells are activated by T-dependent B cell activation mechanism and produce antibodies that stimulate the killing of *Mtb*-infected cells by enhancing antibody-dependent cell-mediated cytotoxicity. These results suggest that NTM exposure via the oral route elicit humoral mucosal immunity against TB, and this will be further dissected to leverage mucosal NTMs as a strategy to boost BCG.

Post-doctoral Fellow / Microbiology, Immunology and Pathology

O-19. Probiotic puzzle: host-microbiome response to rotavirus vaccination using recombinant *Lactobacillus*.

Darby Gilfillan, Kayl Ecton, Nurul Islam, Bridget Fox, Alora LaVoy, John Belisle, Zaid Abdo, Gregg Dean, Allison Vilander

Rotavirus causes 250,000 annual deaths among children under five. Lower-middle income countries have reduced protective immune responses to the vaccines currently available. An alternative vaccine is necessary. We engineered the probiotic bacterium *Lactobacillus acidophilus* as a recombinant, orally delivered vaccine platform (rLA) expressing the viral capsid protein VP8 and two adjuvants (FimH/FliC). Oral probiotic platforms are enticing vaccine candidates for enteric diseases because of easy administration and ability to generate localized mucosal and systemic immune responses. Here we evaluate anti-rotavirus immune responses induced by rLA immunization, along with the interaction between our vaccine platform and the host microbiome/immune system. We hypothesize rLA vaccination against rotavirus will induce protective immune responses. Systems-level understandings of mechanisms regulating vaccine-induced protection will inform our vaccine strategies. We vaccinated mice with our rLA on two immunization schedules (three and five doses) with subsequent rotavirus challenge. We evaluated anti-VP8 immune responses generated by the rLA using ELISpot and ELISA, and protection via fecal shedding of the rotavirus antigen. Results indicate delayed viral antigen shedding and increased antigen-specific B-cells in the five-dose vaccine group compared to controls and the three-dose group. To assess host immune system/microbiome/vaccine interactions, we orally immunized mice with the same rLA strain and collected sections of ileum containing a Peyer's patch 24-hours after dosing. We aim to understand these interactions structurally (with metagenomics), functionally (metatranscriptomics), and metabolically (metabolomics) at a mucosal inductive site. Preliminary results indicate metabolites differ based on sex in the controls compared to vaccinated mice. Metagenomic/metatranscriptomic data is being generated. Bioinformatics and statistical analyses are in progress using computational pipelines and R. Our research indicates rLA can induce a protective immune response against rotavirus and captures some immediate changes within the host microbiome/immune system environment in response to rLA vaccination. Future studies will further examine evidenced metaomic changes during immunization.

Graduate Student / Microbiology, Immunology and Pathology

O-20. Impact of maternal omega-3 fatty acid intake on ovine placental and fetal tissue metabolism.

Asma K Omar, Lance C Li Puma, Briana D Risk, Aria C Witt, Cheyanne S Izon, Luke A Whitcomb, Dorcas D Ka, Quinton A Winger, Gerrit J Bouma, and Adam J Chicco

Objectives: Dietary supplementation of omega-3 fatty acids such as docosahexaenoic acid (DHA) during pregnancy is often recommended to support optimal fetal brain development and cognitive function of the offspring. DHA supplementation also influences cardiometabolic risk parameters in adults, but its effect on fetal metabolism and subsequent risk is poorly understood. The aim of this study was to determine the effects of maternal DHA supplementation (MDS) on placental and fetal nutrient handling during pregnancy. Methods: White-faced ewes were fed either a control diet (Show-rite NewCo Lamb Feed) or a DHA-supplemented diet (control diet + 3% w/w algae-derived DHA) from 2-3 weeks before pregnancy until mid-gestation (75 days), after which a C-section was performed to collect the placenta and fetal tissues for metabolic analyses. Results: MDS significantly increased serum DHA levels and decreased serum triglycerides in the uterine (maternal) circulation, but not umbilical (fetal) circulation. Nevertheless, MDS resulted in significant DHA enrichment of the placenta and all fetal tissues examined, and differentially affected the protein expression of the four major fatty acid transport proteins FATP1, FATP4, CD36 and FABP in placenta, muscle, liver and heart, but had no effect on kidney or brain. Consistent with these findings, MDS tended to increase the capacity for fat over pyruvate oxidation in fetal muscle and heart, but favored a greater capacity for glucose uptake and oxidation in fetal liver. Conclusions: This study is the first to validate use of an ovine model for investigating the impact of maternal DHA supplementation on fetal metabolism and development. Results demonstrate a complex tissue-specific effect of MDS on fetal tissue carbohydrate and fatty acid metabolism that favors a greater capacity for serum glucose disposal and fatty acid oxidation. Whether these changes ultimately impact nutrient metabolism and cardiometabolic risk in the offspring later in life merits further investigation.

Graduate Student / Biomedical Sciences

O-21. Mitochondrial respiration in human placental cell lines.

Agata M. Parsons and Adam J. Chicco

Proper placental development and function is critical for pregnancy maintenance and maternal, fetal, and postnatal well-being. The placenta is comprised of trophoblast cells that are rich in mitochondria, consuming ~40% of oxygen used by the developing fetus. Consequently, mitochondria play an important role in trophoblast and placental function. The human placenta is difficult to study during pregnancy for obvious ethical reasons, forcing researchers to utilize primary or immortalized trophoblast cell lines to model placental cell physiology *in vitro*. The goal of this study was to characterize mitochondrial function in three well-established trophoblast cell lines (ACH-3P, BeWo, and Swan-71) to provide a foundation for future investigations of mitochondrial responses to insults typically observed in pregnancy disorders. Mitochondrial function was determined using a high-resolution respirometer with an integrated fluorescence module (Oroboros Instruments, Innsbruck, Austria) for simultaneous monitoring of O2 consumption (JO2) and hydrogen peroxide release rates (JROS). Basal cellular JO2 and JROS varied across the three cells lines, with both being highest in ACH-3Ps. Following cell permeabilization with digitonin and provision of mitochondrial substrates, JROS increased in the ACH-3P and BeWo cell lines, but decreased in Swan-71 cells. In all three cell lines, JROS decreased and JO2 increased following the addition of ADP, demonstrating expected responses to the shift from maximal LEAK to OXPHOS states in healthy mitochondria. Ongoing studies are evaluating mitochondrial responses to high levels of androgens associated with pregnancy disorders *in vivo*, such as pre-eclampsia and gestational diabetes. In response to testosterone, JROS increased and JO2 decreased in ACH-3P and BeWo cells, consistent with previously reported evidence from placentas with these pathologies. In summary, our preliminary studies have established a simple and repeatable protocol for characterizing mitochondrial function in trophoblast cells lines that is useful for future *in vitro* studies of placental physiology and pathophysiology.

Graduate Student / Biomedical Sciences

O-22. Dentate granule cell birth date-specific upregulation of feedback inhibition in a mouse model of posttraumatic epilepsy.

Young-Jin Kang, Sang-Hun Lee, Jeffery A. Boychuk, Corwin R. Butler, J. Anna Juras, Ryan A. Cloyd, and Bret N. Smith

Posttraumatic epilepsy (PTE) and behavioral comorbidities frequently develop after traumatic brain injury (TBI). Aberrant neurogenesis of dentate granule cells (DGCs) after TBI may contribute to the synaptic reorganization that occurs in PTE, but how neurogenesis at different times relative to the injury contributes to feedback inhibition and recurrent excitation in the dentate gyrus is unknown. Thus, we examined whether DGCs born at different postnatal ages differentially participate in feedback inhibition and recurrent excitation in the dentate gyrus using the controlled cortical impact (CCI) model of TBI. Transgenic mice expressing channelrhodopsin2 (ChR2) in postnatally born DGCs were used for optogenetic activation of three different DGC cohorts: postnatally early-born DGCs, or those born just before or after CCI. We performed whole-cell patch-clamp recordings from ChR2-negative, mature DGCs and parvalbumin-expressing basket cells (PVBCs) in hippocampal slices to determine whether optogenetic activation of postnatally born DGCs increases feedback inhibition and/or recurrent excitation in mice 8–10 weeks after CCI and whether PVBCs are major targets of ChR2-positive DGCs. In the dentate gyrus ipsilateral to CCI, activation of ChR2-labeled DGCs born before CCI produced increased feedback inhibition in ChR2-negative DGCs and increased excitation in PVBCs compared to those from sham controls. In sharp contrast, this upregulated feedback inhibition was absent in DGCs born early in life or after CCI. Surprisingly, ChR2-positive DGC activation rarely evoked recurrent excitation in mature DGCs from any cohort. These results suggest that DGC birth date-specific increased feedback inhibition in the dentate gyrus may contribute to hyperexcitability after TBI.

Staff / Biomedical Sciences

O-23. Transient and Persistent BVDV Infections and a Unique Post-natal Phenotype.

Jessica N. Kincade, Hana Van Campen, Terry Engle, Jeanette Bishop, Hanah Georges, Carolina Gonzalez-Berrios, Jordan Eder, Erin McDonald, Alexander Bally and Thomas R. Hansen

Bovine viral diarrhea virus (BVDV) infections cause reproductive losses, decreased weight gain and milk production, and immunosuppression. The impact of fetal BVDV infections depends on gestational age at the time of infection. Persistent infection (PI) occurs prior to 125 days of gestation, when the fetal immune system is unable to recognize and immunoneutralize the virus. Transient infection (TI) occurs if the fetus is infected after 150 days of gestation when the virus can be cleared by a more developed immune response. While PIs serve as the primary reservoir for BVDV infection, they only account for a small proportion of an infected cohort, while TIs may be twenty times more common. Because TI and PI animals experience a higher incidence of illness, we hypothesize that PI and TI fetuses have impaired immune cell function as a result of in-utero BVDV infection. 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-match PI calves were identified at a cooperating ranch. At four months of postnatal age, complete blood count (CBC) and immune cell populations were compared in control, TI and PI calves. Control and TI CBC were similar; whereas, PI calves had decreased hematocrit, hemoglobin, mean corpuscular volume, mean platelet volume, increased platelet numbers and decreased platelet size ($p<0.05$). TI calves had elevated eosinophils ($p<0.05$), while PI calves had decreased basophils ($p<0.05$). PI calves had increased monocytes ($p<0.05$) comparatively. Flow cytometry revealed that TI calves had lower populations of CD4+, CD8+, and activated and non-activated γδ T cells compared to controls. In conclusion, fetal TI and PI BVDV infection may result in altered postnatal cellular populations that contribute to possible increased susceptibility to infections postnatally. USDA NIFA Grants: 2019-67015-29866 and 2021-38420-34040.

Graduate Student / Biomedical Sciences

O-24. Vulnerability of cholecystokinin-expressing GABAergic interneurons in the unilateral intrahippocampal kainate mouse model of temporal lobe epilepsy.

Sang-Hun Lee, Young-Jin Kang, and Bret N. Smith

Temporal lobe epilepsy (TLE) is characterized by recurrent spontaneous seizures and behavioral comorbidities. Reduced hippocampal theta oscillations and hyperexcitability that contribute to cognitive deficits and spontaneous seizures are present beyond the sclerotic hippocampus in TLE. However, the mechanisms underlying compromised network oscillations and hyperexcitability observed in circuits remote from the sclerotic hippocampus are largely unknown. Cholecystokinin (CCK)-expressing basket cells (CCKBCs) critically participate in hippocampal theta rhythmogenesis, and regulate neuronal excitability. Thus, we examined whether CCKBCs were vulnerable in nonsclerotic regions of the ventral hippocampus remote from dorsal sclerotic hippocampus using the intrahippocampal kainate (IHK) mouse model of TLE, targeting unilateral dorsal hippocampus. We found a decrease in the number of CCK+ interneurons in ipsilateral ventral CA1 regions from epileptic mice compared to those from sham controls. We also found that the number of boutons from CCK+ interneurons was reduced in the stratum pyramidale, but not in other CA1 layers, of ipsilateral hippocampus in epileptic mice, suggesting that CCKBCs are vulnerable. Electrical recordings showed that synaptic connectivity and strength from surviving CCKBCs to CA1 pyramidal cells (PCs) were similar between epileptic mice and sham controls. In agreement with reduced CCKBC number in TLE, electrical recordings revealed a significant reduction in amplitude and frequency of IPSCs in CA1 PCs evoked by carbachol (commonly used to excite CCK+ interneurons) in ventral CA1 regions from epileptic mice versus sham controls. These findings suggest that loss of CCKBCs beyond the hippocampal lesion may contribute to hyperexcitability and compromised network oscillations in TLE.

Staff / Biomedical Sciences

O-25. CD146 expression by adult equine chondrocytes is not a strict indicator of suppression of stimulated lymphocyte proliferation.

Bethany E Liebig, Laurie R Goodrich, John D Kisiday

Mesenchymal stromal cells (MSCs) possess therapeutic properties that may promote healing of diseased joints. Chondrocytes, the only cells in cartilage, are a potential cell source for treating joints as they dedifferentiate from their native phenotype and acquire MSC properties with isolation and expansion. However, the extent to which chondrocytes must be expanded to acquire MSC properties has not been determined. One MSC property of interest is CD146 expression, a marker that has been positively associated with the therapeutic potency of MSCs. Previously, we reported that CD146 expression for adult chondrocytes is minimal (<14%) through 12 population doublings (PDs), but then significantly increases to >38% by 19 PDs and remains ~40% through 65 PDs. Given the association of CD146 expression with the therapeutic potency of MSCs, we hypothesize that this increase in CD146 expression for culture-expanded chondrocytes (CECs) indicates the adoption of other MSC properties, such as immunomodulation. To test this hypothesis, we evaluated the ability of CECs to suppress proliferation of stimulated lymphocytes, which is a common method for characterizing the immunomodulatory properties of MSCs. Equine articular chondrocytes were expanded in monolayer over 5 passages, totaling 33 population doublings (PDs) in 22 days. Co-cultures of CECs and stimulated lymphocytes were analyzed for each passage. CECs reduced proliferation of stimulated lymphocytes by at least 50% at all timepoints. Proliferation was highest at 5 PDs (~46%) and 12 PDs (~39%), then significantly decreased to ~23% at 19 PDs and remained ~19% through 33 PDs. In conclusion, CECs possess the ability to suppress lymphocyte proliferation independent of CD146 expression, which does not strictly support the hypothesis that CD146 indicates the adoption of MSC properties. However, the decrease in lymphocyte proliferation concomitant with increased expression of CD146 at 19 PDs suggests that CD146 expression may indicate an increase in immunomodulatory potency of CECs.

DVM/PhD Student / Clinical Sciences

O-26. Retrospective review of causes of mortality for zoo giraffe and okapi (1991-2020).

Taylor Locklear, Liza Dadone, Miranda Sadar, Robbie Rush, Sara Ferguson, Eric Klaphake, Matthew Johnston

Identifying historical causes of mortality in captive giraffe and okapi could improve species management and survival in zoological institutions. This study leveraged the Zoological Information Management System (ZIMS), a software program utilized by zoos worldwide, to identify the most frequently reported causes of mortality in giraffe and okapi historically, and in the past five years. In a 25-year review of giraffe mortality (1991 to 2015), 3207 giraffe deaths were reported, with 845 of those cases identified by ZIMS to have usable mortality data. The most common causes of giraffe death were neonatal issues (26.6%), trauma (24.7%), and infectious diseases (24.1%). In the past five years (2016-2020), 679 giraffe deaths were reported with 179 deemed to have usable records; the three most common causes of giraffe death were non-infectious disease (42.5%), trauma (22.3%), and neonatal issues (15.1%). In a 25-year review (1991-2015) of okapi mortality, 238 okapi deaths were reported with 64 cases having usable records; the three most common causes of okapi mortality were neonatal issues (45.3%), infectious disease (20.3%), and trauma (17.2%). In the past five years (2016-2020), 62 deaths were reported with 31 usable records; the top three causes of okapi mortality were non-infectious diseases (54.8%), neonatal issues (25.8%), and infectious diseases (19.4%). This study suggests a need to characterize the risk factors for neonatal mortality to prevent significant deaths in both species. Additionally, trauma is a persistent cause of mortality for giraffe, therefore ongoing evolution of management practices and exhibit design are needed. Finally, infectious disease remains a significant cause of mortality for okapi and warrants further investigation.

Resident / Clinical Sciences

O-27. Two novel species of Arthroderma isolated from domestic cats with dermatophytosis in the United States.

Alex Moskaluk, Sue VandeWoude

Dermatophytosis is a superficial fungal infection of keratinized tissues that can occur in humans and other animals. In domestic cats, the majority of cases are caused by *Microsporum canis* and can spread to other animals and humans via arthrospores. Between 2019 and 2021, 164 cases of suspected dermatophytosis were recorded in animals from a high-volume shelter in California. Samples (hair, nail, and skin scraping) were collected for routine screening from these individuals. One hundred and twenty-six of these were diagnosed as *M. canis* by culture and internal transcriber spacer (ITS) sequence. In four suspected dermatophytosis cases occurring in kittens in 2019, cultures grown at 20°C yielded fungi with colony morphology more similar to *Arthroderma* species than *Microsporum*. Morphologic and microscopic examinations were conducted, and gene segments for the ITS, β-tubulin, and translation elongation factor 1-alpha (TEF1) regions were sequenced from DNA extracted from these cultures. Sequences were aligned to other dermatophytes using maximum likelihood and neighbor-joining trees and were compared to previously described fungal species to assess nucleotide homology. We identified two previously undescribed fungal species, herein proposed as *Arthroderma lilyanum* sp. nov. and *Arthroderma mcgillisianum* sp. nov. *M. canis* co-cultured in two of the four cases. These species have significance as potential pathogens and should be considered as rule-outs for dermatophytosis in cats. The potential for infection of other species, including humans, should be considered.

Post-doctoral Fellow / Microbiology, Immunology and Pathology

O-28. Myoblast Exosome Production, Function, and MiRNA Cargo is Altered by Mechanical Stimulation: Therapeutic Implications for Skeletal Muscle Regeneration.

Katherine Williams, Michael Mullen, Thomas LaRocca, Karyn Hamilton, Chelsea Bahney, Nicole Ehrhart

Exosomes offer a potential cell-free regenerative therapy which may replicate the benefits of stem cell therapy while minimizing the associated risks and regulatory challenges of cellular therapeutics. Our previous work demonstrated that mechanical strain improves the myogenic functions of exosomes derived from myoblasts, suggesting that exosomes derived from mechanically strained myoblasts hold potential for use as a therapeutic to promote skeletal muscle regeneration. The goal of the current study was to investigate changes in exosomal miRNA cargo following mechanical stimulation of myoblasts and to identify candidate miRNAs that may modulate the myogenic properties of these exosomes. We hypothesized that exosomes derived from mechanically strained myoblasts would have differential expression of miRNAs with molecular functions involving myogenesis and skeletal muscle regeneration. C2C12 myoblasts were cultured under cyclical tension using a FlexCell FX-5000TT bioreactor alongside unstrained, static control myoblasts. Exosomes were then isolated from conditioned media. RNA was extracted from the exosomes and RNA sequencing and gene ontology enrichment analysis was performed to identify differential miRNAs and their associated molecular functions. 35 miRNAs were significantly downregulated in strained exosomes compared to static. The gene ontology (GO) terms associated with the mRNA targets of these downregulated miRNAs involved developmental, neural, cell signaling, transcriptional regulation, metabolic, and inflammatory processes. This study demonstrates that mechanical stimulation alters the miRNA cargo of myoblast exosomes. These altered miRNAs may have biological functions impacting muscle adaptation to mechanical strain by affecting gene expression of developmental, neural, metabolic, and inflammatory molecular pathways. Mechanically strained myoblast exosomes therefore hold potential as an alternative therapeutic to mimic the benefits of physical exercise on skeletal muscle via intercellular signaling to possibly enhance myogenesis and skeletal muscle regeneration.

DVM/PhD Student / Clinical Sciences

O-29. Clinical outcome of canine apocrine gland anal sac adenocarcinoma patients with non-surgical metastatic lymph nodes treated with stereotactic radiation therapy.

Theodore R Chang and Susan M LaRue

Canine apocrine gland and anal sac adenocarcinoma (AGASACA) is a malignant tumor of the anal sac and gland. Locoregional tumor spread is high (26-89%) and the tumor may metastasize to distant organs as well. The purpose of this study is to determine safety and efficacy of treating canine AGASACA with stereotactic body radiation therapy (SBRT). Medical records from 2016-2021 from a single institution were reviewed.

Signalment, clinical signs, tumor profile, laboratory data, SBRT dosimetry profiles, clinical response, survival time, treatment toxicity, and reason for death were collected from the medical records and from follow-up phone calls to owners and RDVM. Kaplan Meier curves and log-rank tests were performed on the survival analysis, and categorical predictors were utilized for prognostic evaluation.

Eleven dogs met inclusion criteria for this study. All dogs had surgical removal of primary tumor before or immediately after SBRT. Six patients received 3 fraction SBRT and 5 patients received 5 fraction SBRT to their metastatic lymph nodes. The fractions were administered on consecutive workdays. Six patients received adjuvant chemotherapy (Carboplatin (4), Palladia (3), Mitoxantrone, Doxorubicin, Cytoxan). Distant metastatic disease (54.4%) was the most common reason for death or euthanasia. Median progression-free survival (PFS) was 302 days. The median survival time (MST) of dogs in this study was 451 days. No prognostic factors except sex (Male MST 614 days vs Female MST 200 days, p=0.0086). had statistically significant impact on MST. No acute radiation effects were observed. Three dogs had late hindlimb paresis and/or lameness. At that time the dogs had advanced tumor recurrence, however exacerbation by late side effects cannot be ruled out.

Based on the described clinical outcome, one can assert that SBRT treatment of the non-surgical metastatic lymph nodes in canine AGASACA patient can be an effective treatment modality with low risks of toxicities.

Resident / Environmental and Radiological Health Sciences

O-30. Antiretroviral therapy decreases feline immunodeficiency virus RNA is saliva of infected cats.

Megan Conry, Jeffrey Kim, Mary Nehring, Elisa Behzadi, Sue VandeWoude

Feline Immunodeficiency Virus (FIV) is the feline analogue of Human Immunodeficiency Virus and is associated with gingivostomatitis and chronic gingivitis in infected cats. We are testing the effect of a novel combination antiretroviral therapy (cART) on salivary viral load of cats infected with FIV subtype C. We hypothesized that cART would result in a marked decrease in salivary viral load which ultimately relates to severity of oral disease. Saliva was collected from cats with FIV that had been treated with cART (2.5 mg/kg Dolutegravir, 20 mg/kg Tenofovir, 40 mg/kg Emtricitabine) or a placebo control (n=6/group) starting 4 weeks post-infection for 24 weeks. DNA and RNA extractions were performed using Qiagen DNeasy and QIAamp RNA extraction kits, respectively. We optimized the DNA extraction protocol using an ethanol precipitation step. Viral load was assessed by using digital droplet PCR, with FIV-C specific primers and probes. We quantified levels of proviral DNA and salivary RNA along with housekeeping gene CCR5 via ddPCR at weeks 2, 6, 12, 18, and 33. Mean proviral loads were 45800 copies/mL and 26200 copies/mL for cART and placebo cats, respectively. Mean viral loads were 4260 cDNA copies/mL and 31300 RNA copies/mL for cART and placebo cats, respectively, (p=0.064) These results suggest that while there is no significant difference in proviral loads, the cART protocol was effective at reducing FIV RNA viral loads in saliva. This finding indicates cART may have an impact on FIV transmission in multi-cat households and may impact oral mucosal expression of FIV disease.

DVM Student / Microbiology, Immunology and Pathology

O-31. Behavioral and physiologic effects of a single dose of oral gabapentin in rabbits.

Rachel E. Conway, Mollie K. Burton, Khursheed Mama, Sangeeta Rao, Lon V. Kendall, Marion Desmarchelier, Miranda J. Sadar

Stress in rabbits may influence veterinarians' abilities to assess their health and can lead to complications such as gastrointestinal stasis and poor anesthetic outcomes. Gabapentin has been used as an anxiolytic in various species. Five female and three male New Zealand white rabbits, aged 8 months to 1 year, weighing 3-4.5 kg, were administered a single dose of 25 mg/kg gabapentin orally to evaluate its effects on individual behaviors and selected physiologic parameters. Rabbits were assessed by a blinded observer using a Human Intruder Test (HIT) and scored for tractability (summed total score of 0-8, most to least tractable). Heart rate, respiratory rate, and temperature were also recorded. Each rabbit was assessed at baseline the day prior to administration at 1, 2, and 4 hours and at the same intervals post-gabapentin administration. Data were analyzed as continuous, binary, and continuous non-parametric as appropriate with (significance $p \leq 0.05$). No significant differences in physiologic parameters were observed between baseline and the time points post-administration. For the HIT, pressing down with ears flat (2 and 4 hours; $p=0.05$ and $p=0.013$) and approaching human (2 hour; $p=0.0219$) behaviors were decreased compared to baseline. Tractability scores were lower at the 2-hour timepoint compared to baseline (Friedman $p=0.0461$; Wilcoxon $p=0.0413$). These results suggest gabapentin 25 mg/kg orally decreased rabbits' reactivity, without significant effects on observed physiologic parameters. Gabapentin use is promising to reduce stress and anxiety in the presence of humans, and to facilitate handling in rabbits, with a peak effect at two hours.

DVM Student / Clinical Sciences

O-32. The use of Trazodone as an Anxiolytic Prior to General Anesthesia in Dogs.

Matthew Denton, Janelle Scott, and Gregg Griffenhagen

Canine patients often show signs of stress and anxiety when hospitalized or traveling to the hospital and veterinarians have become more apt to treat these adverse clinical signs. Trazodone, a serotonin antagonist and reuptake inhibitor, has a long track record of safe use in humans, and has become a commonly administered medication in dogs for relief of anxiety. While trazodone appears to be safe, the effects of a serotonergic medication can vary widely when coupled with general anesthesia. The goal of this study was to evaluate changes in sedation, intraoperative parameters, and duration of recovery in naïve patients receiving trazodone prior to elective surgery. Forty-five dogs (deemed healthy based on history and physical exam) between 4 months and 6 years of age undergoing spay/neuter procedures were enrolled at the Colorado State University Veterinary Teaching Hospital. Dogs were randomized to one of 3 groups; *group 1* (control) did not receive trazodone, *group 2* received one dose of trazodone (5 mg/kg) one hour prior to premedication, and *group 3* received 2 doses of trazodone (5 mg/kg), the night before surgery and on the morning of surgery. Variables assessed included: sedation score 10 minutes after premedication, ataxia score 30 minutes after extubation, dose of propofol, average vaporizer setting, minutes to extubation, and number of episodes of intraoperative hypotension. Normally distributed data was analyzed using ANOVAs and/or t-tests as appropriate, and the estimated marginal means used for multiple comparison analysis. Interim analyses revealed no differences between groups for all recorded variables with the exception of an increase in time to extubation and ataxia score after recovery in group 2 ($p < 0.05$). Trazodone appears to be safe when administered prior to general anesthesia but an increase in recovery time and ataxia should be expected when more than one dose has been administered.

DVM Student / Clinical Sciences

O-33. Imaging the cellular highway: microcirculation and endothelial glycocalyx in canine cardiopulmonary bypass.

Devon Diaz, Christopher Orton, Julien Guillaumin

Purpose: To evaluate microcirculation variables and endothelial glycocalyx using side stream dark field video microscopy (Glycocheck™) in dogs undergoing cardiopulmonary bypass (CPB).

Materials/methods: Prospective, single academic center study. Inclusion criteria were dogs with cardiac disease requiring surgical correction under CPB. Data collected included demographics, cardiac disease diagnosed, the microcirculation variables red blood cell (RBC) flow and 4-25 µm vessel density, and endothelial glycocalyx width assessed using perfused boundary region (PBR). Normality was assessed using Shapiro-Will's test. Results are presented as mean (± standard deviation) or median (range) as appropriate. Impact of CPB on RBC flow, vessel density and PBR between pre and post-CPB was tested with an analysis of variance (ANOVA) for repeated measures with Bonferroni correction.

Results: Six dogs were included. Mean age was 1.7±0.6 years. Median body weight was 29.0 kg (12.4-54). Cardiovascular disease processes included tricuspid valve dysplasia (n=2), Tetralogy of Fallot (n=1), atrioventricular septal defect (n=2) and mitral valve dysplasia (n=1). Data were collected at 4 time points: baseline (BL), while on the CPB pump (Pump), after the aortic cross clamp was removed (Clamp) and at surgical closure (Closure) and are presented in that order. Total 4-25 µm vessel density (mm/mm^2) were 209.1±51.2, 229.8±56.3, 145.9±30.0 and 205.0±40.8 (p=0.58). Total RBC flow (µm/s) were 266.5±77.6, 261.0±82.5, 120.7±38.2 and 161.3±32.3 (p=0.28). PBR (mm/mm^2) were 2.0±0.2, 2.4±0.2, 1.9±0.2 and 2.1±0.2 (p=0.46).

Conclusion: Microvascular circulation and endothelial glycocalyx was not modified by CPB. These results should be replicated with larger sample size.

Resident / Clinical Sciences

O-34. Alpha-enolase as a biomarker for early diagnosis of acute kidney injury and chronic kidney disease in cats.

Amanda L Diaz, Jennifer R Hawley, and Michael R Lappin

Kidney diseases are extremely common in domestic cats and can be an acute kidney injury (AKI) that is fatal, an AKI that leads to chronic kidney disease (CKD), or CKD. For years, research has focused on diagnosing kidney disease earlier than with currently available tests like the serum creatinine concentration which rises as the glomerular filtration rate goes down. The primary problem with this test is that by the time abnormal results are detected, there is already a 75% decrease in kidney function. Tests that detect kidney abnormalities earlier would allow for earlier supportive care to attempt to slow down this progressive and irreversible disease. Alpha-enolase is a glycolytic enzyme with a wide distribution in tissues, including kidneys. Studies in our laboratory have shown that alpha-enolase may be a viable biomarker of kidney damage. For example, immunohistochemistry was used to demonstrate the distribution of alpha-enolase in feline kidneys in different life stages. Cats over 10 years of age and cats in different IRIS stages of CKD have a different distribution of alpha-enolase within the kidney tissues than young, healthy cats. The goal of this study is to evaluate alpha-enolase as an early biomarker for diagnosing kidney disease in cats. The hypothesis of this study is that as the kidneys are damaged, the degeneration of the renal tubules will lead to increased concentrations of alpha-enolase in the urine and serum. In our assay titration experiments, concentrated cat urine (USG 1.078) spiked with alpha-enolase inhibited the detection of the enzyme. Additional titration experiments showed that standardization of the USG to 1.010 resolved the apparent inhibition. In our current experiment, we will test the hypothesis using results from the alpha-enolase antigen assay performed on banked serum and urine samples

DVM Student / Clinical Sciences

O-35. Surgical interventions and outcome in a population of feline trauma patients.

Corey J Fisher, Amanda A Cavanagh, David Liss, Taylor Adams, Sarah J Marvel, Kelly E Hall

Objective – To determine signalment, injury type, trauma severity score, and outcome of feline trauma patients undergoing surgical (emergency and operating rooms), and non-surgical treatment in addition to time to surgery, specialty services involved, and cost in the operating room surgery population.

Design – Retrospective evaluation of registry data on feline trauma cases.

Setting – University teaching hospital.

Animals – 251 cats presenting for traumatic injury.

Interventions – None.

Measurements and Main Results – Data on feline cases presenting for trauma between May 2017 and July 2020 were obtained from the Veterinary Teaching Hospital's Veterinary Committee on Trauma registry. Demographics and outcome were compared for cats undergoing surgical intervention in an operating room (OR, 12%, 31/251) and/or an emergency room setting (23%, 58/251) and feline trauma patients without surgical intervention (65%, 162/251). Among the two surgical groups, 99% survived to discharge compared to 73.5% of the non-surgical group (P <0.0001). For the OR surgical cohort, electronic medical records were reviewed to determine the specialty surgery service involved, time to and duration of anesthesia and surgery, and visit cost. The most common surgery services involved were orthopedics (41%, 12/29) and dentistry (38%, 11/29) and the most common surgeries performed were mandibular fracture stabilization (8/29) and internal fixation for long bone fractures (8/29).

Conclusions – Surgical intervention in feline trauma patients appear to be associated with higher survival rates, but no difference in mortality was found across surgery services. OR surgical intervention, in particular orthopedic surgery, was associated with increased length of hospitalization, increased cost, and increased use of blood products. A consistent significant difference in injury severity score was not found across surgical and non-surgical groups.

Staff / Clinical Sciences

O-36. Quantitative analysis of esophageal transit times in normal cats using contrast enhanced videofluoroscopy.

Kathryn M Goodman, Megan A Stadler, Cindy K Sotelo, Elissa K Randall

Esophagrams are a valuable tool when diagnosing dysphagic animals. There are no studies that provide quantitative data on barium coated food or liquid barium esophageal transit times in healthy cats. The purpose of this study was to establish normal parameters for esophageal transit times in cats. 39 cats without a history of GI disease were enrolled. A physical exam and survey thoracic radiographs were performed on those participating cats to rule out esophageal dilation or aspiration pneumonia. No cats were disqualified based on physical exam or radiographic findings. 23 enrolled cats voluntarily ate while in the hospital. Cine loops that included the beginning of the swallow (closure of the cranial esophageal sphincter after the bolus passes through) at the pharyngeal region through the entrance of the bolus into the stomach (closure of the caudal esophageal sphincter after the bolus passes through) were included in the results. 59 soft food, 36 kibble, and 13 liquid transits were captured. Soft food transit averaged 15.3 seconds (range 2.5 – 30.2, St Dev 6.1). Dry food transit averaged 16.3 seconds (range 7.9 – 38.3, St Dev 6.7). Liquid transit averaged 22.1 seconds (range 12.7 – 56.6. St dev 11.3;). Esophageal transit times were found to be variable but relatively similar between bolus types. Sample size, varying bolus sizes, and patient compliance were the biggest limitations of this study. The results of this study will provide reference numbers for normal esophageal transit time in cats for veterinarians performing esophagrams in cats with suspect esophageal dysfunction.

DVM Student / Environmental and Radiological Health Sciences

O-37. An increase in canine infected corneal ulcerations during the 2020 northern-Colorado wildfire season.

Katrina EV Jones, Søren Saxmose Nielsen, Joshua B Daniels, Michael R. Lappin, and Michala de Linde Henriksen

In the fall of 2020, Colorado experienced the two largest wildfires in state history. The smoke blanketed northern-Colorado for approximately three months (August, September, and October). The comparative ophthalmology service at the Colorado State University Veterinary Teaching Hospital (CSU-VTH) noted an influx of canine patients with infected corneal ulcerations during this time. The hypothesis of this study was that there was an increase in infected corneal ulcerations in dogs during the three months of the wildfires compared to previous years. It was also hypothesized that poor air quality (elevated air quality index (AQI)) was associated with infected canine corneal ulcerations. The medical records from canine patients presented to the ophthalmology service at the CSU-VTH with infected corneal ulcerations in August, September, and October of 2018, 2019 and 2020 were evaluated. Only corneal ulcerations with growth on their microbiology cultures were included in this study. Fisher's Exact test was used to compare the prevalence of canine patients with 'infected corneal ulceration' among all canine patients presented to CSU-VTH in each of the three years. One-way ANOVA was used to evaluate for differences between mean AQI per year. All statistical analyses were carried out in the statistical software R and a p-value<0.05 was considered significant. The study revealed an increase in prevalence of infected corneal ulcerations during the three months for 2020 i.e. 3.5%, (9/255), when compared with the two previous years, 2019: 1.0% (4/383, p=0.04), and 2018: 0.9% (4/457, p=0.01). The AQI was significantly elevated for dogs presented with infected corneal ulcerations in 2020 (70.2±5.8) compared to 2019 (19.7±8.7) and 2018 (45.6±8.7) (p<0.001). This study highlights the risk of developing an infected corneal ulceration during the wildfire season. As the duration and frequency of wildfires continues to rise globally, the effect of wildfire smoke on animal health should be investigated further.

DVM Student / Clinical Sciences

O-38. Get savvy about cavies: comparison of two portable lactate analyzers in guinea pigs (Cavia porcellus).

Ivana Levy, Alexa Spittler, Kelly Santangelo, and Miranda Sadar

Lactate, the product of anaerobic glycolysis, is generated during conditions of either hypoperfusion or increased glucose consumption. L-lactate measurements have been utilized as both diagnostic and prognostic tools for a variety of veterinary species. Guinea pigs can present in states of decompensatory shock with subsequent hypothermia; however, reference values for lactate have not been validated in this species. The purpose of this study was to compare measured lactate data from two portable analyzers. Whole blood from 40 male and 8 female anesthetized guinea pigs was analyzed in triplicate using the Lactate Plus and i-STAT portable analyzers within five minutes of collection. The results obtained with each method of measurement were compared utilizing Spearman's rank, Wilcoxon signed rank, Mann Whitney, and Bland-Altman analyses. Mean ± standard deviation of the L-lactate values measured by the Lactate Plus and i-STAT analyzers were 1.30 ± 0.35mmol/L and 1.11 ± 0.33 mmol/L, respectively. There was strong correlation between the analyzers (r = 0.85). Lactate values also significantly increased with the time taken to analyze the samples ($p < 0.05$). Thus, the concentration of l-lactate measured in this study is in the range of other small animals but should be analyzed without delay. While this study found strong correlation between portable analyzers, future work will aim for comparisons with a "gold standard" laboratory analyzer, creating a standardized reference interval for lactate in guinea pigs, and assessing the utility of L-lactate as a prognostic indicator for survival.

Graduate Student / Clinical Sciences

O-39. The use of increasing enclosure complexity to improve African elephant welfare at the Cheyenne Mountain Zoo.

Hannah N Rice, Rick P Hester, Jason L Bredahl, Liza Dadone, and 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 captivity and are manifested in response to a variety of welfare issues, including lack of stimulation and limited ability to perform normal behavior patterns. Our study aimed to provide novel methods for improving African elephant welfare for any institution wanting to reduce stereotypic behavior. Our hypothesis was that stereotypic behavior would be reduced in each individual elephant when environmental enrichments were added to the African elephant enclosure at the Cheyenne Mountain Zoo. Our sampling method was partial interval recording with one minute intervals. A behavior was recorded at its onset and only marked once for each interval period. The behaviors documented were defined as interacting, foraging, self-maintenance, waiting, stereotypy, and interacting with treatment. Baseline data was established prior to each enrichment introduction, then their enclosure was altered with one of three enrichment types. Our materials were the SIT application for interval timing, 10′ tall, 20′ wide sand mounds and tires and 18′ tall logs buried 6′ underground. Our results were that stereotypic behaviors and behaviors used to indicate positive welfare were not influenced by enrichment introduction. Although stereotypic behavior was not reduced, our preliminary photo and video documentation suggest sand mounds, mud wallows and logs create opportunities for captive elephants to perform natural behaviors that were not observed during baseline data collection and mud wallows or sand mounds can possibly be used therapeutically. Our ethogram failed to capture these observed changes and adjustments are being made in preparation for continued research next summer. We now hypothesize that implementing these enrichments next summer will be beneficial in increasing range of motion, overall exercise, and natural behaviors for captive elephants.

DVM Student / Clinical Sciences

O-40. Retrospective Analysis of Morbidity and Mortality of Gas Anesthesia, Injectable Sedation, and Combined Protocols in Avian Species.

Tatiana B. Rogers, Sangeeta Rao and Miranda J. Sadar

In clinical practice, chemical restraint may be used in avian patients during minimally invasive diagnostic testing and treatments. Chemical restraint may be administered in the form of inhalant gas anesthesia, injectable sedation drugs, or a combination of both. With several chemical restraint methods available, an increased understanding of adverse events associated with these methods is necessary to minimize odds of complications and mortality. The goal of this study was to describe the occurrence of adverse effects when gas anesthesia, injectable sedation, or a combination of both were used in birds undergoing elective or minimally invasive procedures. This retrospective study included 94 birds from 8 orders that underwent 121 chemical restraint events between July 1, 2017 and July 1, 2019 for minimally invasive diagnostic testing and treatments (physical exam, radiographs, blood collection, ultrasound, bandage change, computed tomography scans, echocardiogram, deslorelin implant placement), or elective procedures (talon, wing, and beak trims). Events included 44 gas anesthesia, 28 injectable sedation, and 49 combination protocols. Of the 121 events, mortality occurred in 10.0% of cases and morbidity occurred in 23.9% of cases. There were no statistically significant differences in the probability of mortality between any of the restraint methods. There was a significantly higher probability of morbidity with combinations compared to gas anesthesia or injectable sedation alone. The odds of complications occurring was 8.1 times greater with combinations compared to gas anesthesia, and 6.8 times greater than injectable sedation. Pre-existing mild to moderate systemic disease was present in 94.2% of patients.

DVM Student / Clinical Sciences

O-41. Investigating virtual versus hands-on learning interventions in promoting increased performance, confidence, and engagement in a large general microbiology course.

Delaney Worthington, Amelia Hines, Katriana Popichak, and Jennifer McLean

Thoughtful and proper design of course components, like active learning, is crucial in the online environment due to barriers remote learners face, such as isolation, disengagement, accessibility issues, and technology problems. These potential challenges, therefore, should inform how we implement active learning methods in online courses. When we pivoted to online instruction due to the COVID-19 pandemic, we converted six hands-on activities into interactive PowerPoint activities students could perform virtually. Preliminary data from the first asynchronous online MIP 300 General Microbiology course suggested that virtual activities were beneficial to student performance. Anecdotal feedback from students, however, indicated these PowerPoint activities were cumbersome and frustrating to use. Thus, we hypothesize that high-quality, easy-to-use active learning interventions covering historically difficult topics will increase student performance, confidence, and engagement among students in an asynchronous online course. To investigate this hypothesis, we converted six learning activities into high-quality, user-friendly digital learning content (eLearning) and compared them to existing, corresponding PowerPoint activities. ANOVA analyses were utilized to compare the impact of eLearning and PowerPoint interventions on activity-related quizzes, relevant test questions, and overall impact. Likert Scale surveys and focus groups provided qualitative data to help determine students' satisfaction and engagement. For two out of the six activities, student performance did not significantly change when exposed to the eLearning intervention rather than the PowerPoint intervention. Students ranked their levels of satisfaction and engagement either the same or higher for the eLearning intervention than for the PowerPoint intervention. Preliminary conclusions demonstrate that eLearning appears to increase student satisfaction and engagement. Analysis of the remaining four activities will provide a more comprehensive picture of the overall effects that each intervention has on student performance, satisfaction, and engagement.

Staff / Microbiology, Immunology and Pathology

O-42. Association between feline hyperthyroidism and thoracic radiographic evaluation of cardiomegaly and pulmonary hyperinflation.

Victoria Young, Sangeeta Rao, Sarah Shropshire, Angela Marolf

Hyperthyroidism frequently affects middle to older aged cats who can present with cardiorespiratory signs. The effects of hyperthyroidism on cardiac size and function have been previously documented. Anecdotally, pulmonary hyperinflation identified on thoracic radiographs may also be associated with hyperthyroidism; however, there is no literature to support this claim. The goal of this retrospective case control study was to determine any association between hyperthyroidism, pulmonary hyperinflation, and cardiomegaly with the following hypotheses: (1) hyperthyroid cats would not have evidence of radiographic pulmonary hyperinflation more frequently than control cats and (2) hyperthyroid cats were more likely to have evidence of radiographic cardiomegaly than control cats. Thoracic radiographs of 52 hyperthyroid cats and 51 non-hyperthyroid cats were evaluated for subjective and objective measurements of pulmonary hyperinflation and cardiomegaly. There were no statistically significant differences between hyperthyroid and non-hyperthyroid cats for any variable indicative of pulmonary hyperinflation. The presence of a valentine-shaped heart was significantly higher (P 0.0019) in hyperthyroid cats than non-hyperthyroid cats. Among hyperthyroid cats, a more severe total T4 elevation was significantly associated with a greater likelihood of a valentine-shaped heart (P 0.0017) and larger vertebral heart score (P 0.04). This study suggests that hyperthyroidism in cats is associated with cardiomegaly, specifically a valentine-shaped heart, but is not associated with radiographic pulmonary hyperinflation.

Resident / Environmental and Radiological Health Sciences

O-43. Chiropractic treatment improves lameness and axial skeleton pain and stiffness in horses.

Mikaela D. Maldonado, Samantha D. Parkinson, Melinda R. Story, and Kevin K. Haussler

Chiropractic care is a common treatment modality used in equine practice to treat back pain and stiffness. Clinically important interactions occur between axial and appendicular regions with regards to poor performance and lameness. The objective of this study was to evaluate the effect of chiropractic treatment on limb lameness and concurrent axial skeleton pain and dysfunction. Our hypothesis was chiropractic care would reduce lameness, spinal pain and stiffness, and muscle tone. In year 1, twenty horses with grade 1-3/5 lameness (AAEP scale) within at least one limb and in year 2, eighteen horses with a grade 1-3/5 hindlimb lameness were randomly assigned to treatment and control groups. Subjective and objective lameness examinations, spinal mechanical nociceptive thresholds and measures of spinal stiffness and muscle hypertonicity were performed on days 0, 14, and 28 by a blinded examiner. Chiropractic treatment was applied on days 0, 7, 14, and 21. Data was analyzed by a mixed model fit separately for each response variable ($p<0.05$). In year 1, the number ($p = 0.006$) and severity of painful vertebral locations within the cervical ($p = 0.017$) and lumbar ($p = 0.047$) regions decreased in the treatment horses. No significant change was observed in objective measures of fore or hind limb lameness across years, but in the year 2 subjective lameness scores improved in the treatment group ($p = 0.027$). Spinal stiffness significantly decreased in all regions in year 2 ($p = 0.024$). Muscle tone increased in control horses but not treatment horses in year 2 ($p = 0.012$). Chiropractic care had significant effects on lameness, spinal pain, stiffness, and muscle tone in these populations of horses. Future studies are needed to further evaluate the effect of chiropractic care in horses with specific known sources of limb lameness.

DVM Student / Clinical Sciences

O-44. Phenotypic assessments of metabolic status and potential for dietary supplements to mitigate insulin resistance in the obese mare.

Jordan M Marsh, Giovana Di Donato Catandi, and Elaine M Carnevale

Equine obesity is prevalent and associated with insulin resistance, metabolic syndrome, and laminitis. Methods to rapidly assess a horse's metabolic status and to improve insulin sensitivity have clinical relevance. We hypothesized that phenotypic measurements of mare adiposity correlate with systemic biomarkers for insulin resistance and that dietary supplements can be used to improve insulin sensitivity in the obese mare. Light-horse mares, matched for age and type, were assigned to three groups: normal weight, fed limited hay (NW, n=6); obese fed free-choice hay and 2.7 kg corn/oats daily (OB, n=7); or obese diet, fed as OB with dietary supplements containing complex nutrients, antioxidants, and targeted metabolic support (OBD, n=7). At 5 and 12 weeks after start of the study, phenotypic measurements were performed, included body condition score (BCS), percentage body fat (BF), and neck score (NS), and were positively correlated ($P<0.05$). At 5 weeks, significant ($P\leq0.05$) differences were observed between NW and OB for systemic leptin and insulin and for insulin sensitivity, determined using a proxy of the reciprocal of the square root of insulin or RISQI; OBD were not different from NW or OB. BCS and BF were correlated ($P<0.05$) with insulin and leptin (positive) and RISQI (negative). At 12 weeks, insulin was significantly lower for NW and OBD than for OB, and BF was significantly correlated with insulin. Using an oral sugar test, 0/6 NW, 2/7 OBD and 6/7 OB mares were determined to be insulin resistant. Additional assays are currently being performed; however, our current findings suggest that dietary supplements can be used to improve systemic indicators of insulin resistance. Our findings demonstrate the utility of observational criteria in assessing metabolic status in the mare and support the therapeutic use of nutritional supplements for the obese mare.

DVM Student / Biomedical Sciences

O-45. Evaluating the effects of telmisartan in healthy dogs as a preclinical model for Shar-Pei fever.

Kara Maslyn, Jennifer Hawley, Craig Webb, Tracy Webb, Michael R Lappin

Background: Chinese Shar-Pei dogs commonly develop Shar-Pei fever, a pro-inflammatory disease characterized by fever of unknown origin, renal amyloidosis, peritonitis, synovitis, and proteinuria. While traditional therapies help alleviate clinical signs, optimal therapies remain unknown. Telmisartan is an angiotensin II receptor blocker used primarily to help control hypertension and proteinuria in dogs, but other effects have been described.

Hypothesis/Objectives: The aim of this study was to evaluate the immunomodulatory properties and antioxidant effects of telmisartan in healthy dogs.

Animals: Eight healthy, mixed sex, purpose-bred research beagles were used for this study.

Methods: Dogs were given telmisartan 10 mg once daily for 28 days. A complete blood count (CBC), serum cytokine/chemokine panel, and the following antioxidant assays were performed six times over the course of the study; antioxidant levels of total antioxidant capacity (TAC), CuZn-superoxide dismutase (SOD), catalase (CAT), plasma levels of glutathione reductase (GR), and glutathione peroxidase (GPx). Group mean values after starting telmisartan administration were compared to baseline values by ANOVA with significance defined as $P < 0.05$.

Results: While significant changes in CBC or cytokines/chemokines were not detected, telmisartan significantly upregulated the activity of serum TAC ($P < 0.007$), serum SOD ($P < 1.78E-5$), and plasma GPx ($P < 0.001$) over the 28 day trial.

Conclusions/Clinical Importance: Telmisartan has antioxidant effects in healthy dogs which suggests an additional indication to use this drug in the management of Shar Pei fever. A clinical trial will be designed to evaluate the therapeutic effects of telmisartan in dogs affected by Shar-Pei fever.

DVM Student / Clinical Sciences

O-46. Determinants of Antimicrobial Use in Poultry Sector in South Asia- A Systematic Literature Review.

Fouzia Sattar, Mo D Salman, Ariana Joyce Dickson, Sangeeta Rao

Antibiotics are the cornerstone of therapy for bacterial infectious diseases. The One Health Concept, that human, animal, and environmental health are all interconnected, reflects that antimicrobial usage in livestock is a contributor to resistant bacteria. Reducing antimicrobial use in livestock and poultry is an essential step towards limiting antimicrobial resistance. Antimicrobial misuse and subsequent development of antimicrobial resistance have been related to species raised in intensive production systems, such as poultry. In South Asia, there are different regulations in place to restrict antimicrobial use within food producing animals as well as within the poultry sector. In this systematic review, we sought to summarize the evidence on better understanding the patterns and determinants of antibiotic use within poultry sector in the South Asia region. We conducted a comprehensive search of electronic databases (CAB Abstracts, MEDLINE, PubMed, Web of Science) containing keywords (Antimicrobial OR Antibiotic OR anti-biotic OR anti-microbial) AND (Poultry OR chicken OR broiler OR layer OR Turkey OR Quail OR Fowl OR Duck OR Goose OR Geese) AND (Pakistan OR India OR Bangladesh OR Afghanistan OR Maldives OR Bhutan OR Nepal OR Sri Lanka OR South Asia). Inclusion criteria were original studies that reported on antibiotics use in Poultry in South Asia, published in English language and no restriction for the publication date. A total of 7205 articles were identified by the initial systematic literature search which filtered down to 4832 unique records after removal of duplicates. These remaining records will undergo an abstract-screening process. All the research articles which meet the inclusion criteria are currently evaluated for a detailed review process. The findings of this systematic review will be used as the background for understanding antimicrobial resistance in poultry sectors through the antimicrobial use in poultry sectors and recent practices in South Asia to build reliable antimicrobial use stewardship.

Graduate Student / Clinical Sciences

O-47. Evaluation for anti-erythrocyte and anti-platelet antibodies in healthy dogs administered lokivetmab.

Megan Slaughter, Michael Lappin, Nida Chornarm, Jennifer Schissler, Sarah Shropshire

Lokivetmab (Cytopoint®) is an anti-IL-31 monoclonal antibody indicated in the management of canine allergic pruritis. IL-31 is a cytokine shown to activate the neuronal signaling pathway associated with itch in dogs. Multiple publications confer the efficacy and safety of lokivetmab for allergic disease when given at the recommended dosages. However, there have been anecdotal concerns that this therapy could trigger an immune-mediated response leading to immune-mediated hemolytic anemia (IMHA) or immune thrombocytopenia (ITP). Anti-erythrocyte antibodies (AEA) or anti-platelet antibodies (APA) can form in both IMHA or ITP respectively. Therefore, the aim of this study was to determine if AEAs or APAs develop following a standard-dose therapy of lokivetmab in healthy dogs. Determining if such antibodies form during lokivetmab therapy would be clinically beneficial for veterinarians who may be evaluating an anemic or thrombocytopenic patient shortly after receiving a lokivetmab injection. Whole blood samples were taken from 8 healthy beagles at baseline (day 0) prior to lokivetmab administration and on days 14 and 28 following lokivetmab administration. A complete blood count and flow cytometry to detect AEA and APA were performed at all time points. No dogs exhibited anemia or thrombocytopenia and all were negative for AEA and APA at all measured time points. Future work includes a clinical trial for the evaluation of anemia, thrombocytopenia, AEAs, and APAs in client-owned allergic dogs receiving lokivetmab.

DVM Student / Clinical Sciences

O-48. Rice bran in ready-to-use therapeutic foods for microbiota-targeted treatment of childhood malnutrition.

Annika M. Weber AM, Frank Wieringa, Dama Soekarjo, Ilyatun Niswah, Silvia Barbazza, Elizabeth P. Ryan

Severe acute malnutrition (SAM) is the cause for nearly half of all child deaths under the age five. In Indonesia, SAM affects more than 2 million children and ~1% receive adequate treatment. SAM treatment includes ready-to-use therapeutic foods (RUTF) which have shown immediate benefit and reduction in mortality rates, however, recovered children have persistent dysbiotic gut microbiomes, leaving them susceptible to infection and malnutrition relapse. Focus has been placed on prebiotic foods in disease treatment and host health promotion. Rice bran is a prebiotic of growing significance, as it is also dense in bioactive phytochemicals, fatty acids, and amino acids. Strong evidence suggests that rice bran positively influences the gut microbiome and metabolome in the protection against diarrheal diseases from bacterial and viral pathogens. An investigation is therefore warranted to assess how rice bran in an RUTF may improve SAM treatment and efficacy and offer a local malnutrition treatment option. The central hypothesis of this proposal is that rice bran in RUTFs will improve SAM treatment by targeting the growth of native gut microbiota and provide metabolites for the host involved in decreased gut permeability. To explore this hypothesis, we will determine the effect of RUTF + 5% rice bran on gut microbial composition in the treatment of SAM compared to RUTF alone and examine and establish blood metabolite profiles associated with RUTF + 5% rice bran in the treatment of SAM compared to RUTF alone. This study will yield new information of direct importance and impact to public health nutrition and advance our knowledge and treatment of SAM. Understanding microbiota and metabolite changes associated with microbe directed malnutrition treatments will improve our approach and treatment of this fatal condition and provide key insights into an accessible long-term solution for the treatment of SAM.

Graduate Student / Environmental and Radiological Health Sciences

O-49. Evaluating the confidence and competency of leadership and teamwork communication skills gained for the RECOVER BLS and ALS simulation CPR training course.

Ilana R Weisberg, Claire Tucker, and Kelly Hall

In human emergency medicine, teamwork and communication amongst the medical team have been shown to improve patient outcomes. Current research in emergency nursing highlights that employees trained in effective communication have improved work environments and patient outcomes, and feel more empowered. Comparatively, research in veterinary medicine related to teamwork is lacking and there is a need for further education and research. The RECOVER Basic Life Support (BLS) and Advanced Life Support (ALS) online courses use cardiopulmonary resuscitation (CPR) to highlight important teamwork aspects in triage. The objective of this project is to evaluate confidence and competence in team communication of in person training participants (primarily veterinary students and technicians) during their Emergency and Critical Care rotation. We hypothesize that these characteristics will improve following their exposure to the RECOVER online modules, the in-person component of the RECOVER BLS course, and a series of author-developed ALS scenarios. A further intervention to expose learners to teamwork communication topics- such as the group pause and closed-loop communication- is placed between the first and second simulations. Participants' confidence in communicating is measured on a self-reported Likert scale (1-5) before and after the in-person simulations. Competence is evaluated via a trained observer with 1-5 rating of communication skills. This study will inform design of veterinary school curricula leveraging best practices in veterinary teamwork training to improve patient outcomes and increase confidence of new graduates to lead teams and communicate effectively, especially during emergencies.

DVM Student / Clinical Sciences

O-50. Comparison of Advanced Imaging Modalities to Assess Bone Mineral Density in the Sheep Model.

Katie Bisazza, Brad B Nelson, Holly L Stewart, Katie J Sikes, Jeremiah T Easley

Osteoporosis is the most common bone disease in humans. Clinical diagnosis of osteoporosis is determined based on the bone mineral density (BMD) of an individual compared with the average BMD of the healthy population. BMD is most often measured using dual-energy X-ray absorptiometry (DEXA) imaging. Quantitative computed tomography (QCT) has been proposed as a potential alternative to DEXA because of its high sensitivity to changes in bone structure by easily distinguishing between trabecular and cortical bone. The objective of this study was to compare QCT to DEXA in the ovine preclinical model. Ten (n=10) intact, naïve, female skeletally mature ewes between 4-6 years of age underwent *in vivo* CT and DEXA scanning procedures of the lumbar spine region under general anesthesia (IACUC protocol #2060). Biopsies were harvested from the iliac crest of all animals immediately following scanning procedures for micro-computed tomography (MicroCT) scanning. Average BMD for lumbar vertebrae L2-L5 was reported for each modality. Measurements were compared between modalities using Pearson correlation and a simple linear regression model. Comparison between modalities revealed a good correlation between mean DEXA BMD and mean QCT BMD values at each vertebrae ($r=0.6480$, $p<0.0001$). Unlike DEXA, QCT has the ability to measure BMD of cortical and trabecular bone separately. For each vertebrae, trabecular BMD was significantly lower than cortical bone BMD ($p<0.0001$), as expected. Our results demonstrate that when cortical and trabecular bone BMD are measured separately, subtle differences can be observed between regions of interest that would otherwise have gone undetected using DEXA. MicroCT results are pending and BMD measurements will be compared to DEXA and QCT using similar statistical methods. Our future work will further investigate DEXA and QCT imaging features in sheep models of osteoporotic bone to detect changes in BMD over time.

Graduate Student / Clinical Sciences

O-51. Pharmacokinetics of Gabapentin after Single, Oral Administration in Domestic Rabbits (Oryctolagus cuniculus).

Mollie K Burton, Rachel E Conway, Noah G Mishkin, Khursheed R Mama, Heather K Kynch, Lon V Kendall, Miranda J Sadar

Gabapentin is used to treat epilepsy and neuropathic pain and, more recently, it has been prescribed as an anxiolytic in both human and veterinary medicine. Gabapentin has gained favor for its use as an anxiolytic as it is not classified as a controlled substance and has a wide margin of safety. As rabbits are easily stressed by handling, transport, and medical procedures, we propose gabapentin could be utilized as an anxiolytic. Current doses recommended for rabbits are extrapolated from other species or from limited studies. In this study, three male and three female 7-month-old New Zealand white rabbits received a single dose of oral gabapentin at 25 or 50 mg/kg in a randomized crossover design. Plasma samples were obtained at 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 8, 12, 24, and 48hours post administration and concentrations determined using liquid chromatography-tandem mass spectrometry. Sedative effects were also scored at all time points. Pharmacokinetic analysis revealed mean peak plasma concentrations of 17,695 and 16,830 ng/mL and mean time to maximum plasma concentrations at 1.75 and 1.5 hours for the 25 and 50 mg/kg doses, respectively. These results suggest non-linear absorption may occur, as doubling the dose did not result in increased plasma concentrations. Rabbits were mildly to moderately sedated at 2-3 hours with 25 mg/kg and 1.5-3 hours with 50 mg/kg with none experiencing profound sedation. Based on these results, further evaluation of oral gabapentin at a dose of 25mg/kg as an anxiolytic for rabbits is recommended.

Resident / Clinical Sciences

O-52. Colorado Dairy Farmer Knowledge, Attitudes and Practices for Livestock and Human Infectious Disease Prevention.

Robert Fathke, Tyler Ward, Stephanie Rouse, Jorge Rivera-Gonzalez, Mo Salman, Sangeeta Rao

Biosecurity and biosafety can be applied on dairy farms to prevent infectious diseases in animals and people. This pilot study aims to construct and use a knowledge, attitudes, and practices (KAP) questionnaire for the Colorado dairy farm environment to understand producer KAP on prevention of animal diseases, zoonoses, and infectious diseases transmitted person-to-person. Improved understanding of KAP can help frame recommendations toward preventing infectious diseases in animals and farm personnel. A KAP questionnaire was constructed based on published farm biosecurity and biosafety literature and producer input. Topics included vaccinations, perceived risks of animal diseases, zoonoses, COVID-19, trusted sources of information for disease prevention, perceived efficacy of preventive practices including personal protective equipment (PPE), and attitudes toward obstacles of disease prevention. English and Spanish questionnaire versions were used on four conventional and two organic Colorado Front Range dairies to assess worker (n=35), manager (n=13), and owner (n=2) biosecurity and biosafety KAP. Questions were constructed in multiple choice, Likert scale, and short answer format. KAP questionnaire construction revealed common animal-human infection prevention principles and areas where efforts can be streamlined, which guided construction of an integrated animal-human infectious disease prevention framework. Results highlight biosecurity and biosafety KAP trends and differences between farm owners, managers, and workers in organic and conventional settings and clarify application and perceived efficacy of preventive measures. Descriptive data are presented with preliminary analysis of KAP trends. Constructing and using a comprehensive questionnaire exploring KAP on animal and human infectious disease prevention is an important initial step in incorporating an integrated disease prevention framework on dairy farms. Questionnaire results shed light on Colorado Front Range dairy farmer infectious disease prevention KAP. Findings help shape recommendations for dairy farms and guide development of a holistic assessment framework to better prevent animal and human infectious diseases in various production settings.

Graduate Student / Clinical Sciences

O-53. Prognostic indicators for feline craniofacial trauma: a retrospective study of 130 cases.

Jennifer L Kelley, Mai T Le, Naomi K Hoyer, Kelly Hall, Sangeeta Rao, Jennifer E Rawlinson

Craniofacial traumatic injuries contribute significantly to the morbidity and mortality of domestic felines. Previous studies focused on feline craniofacial injuries have investigated the origin of injury, injuries sustained, and effectiveness of diagnostic tools. The aim of the study is to identify prognostic indicators for feline craniofacial trauma patients and determine their association with negative and positive outcomes. The Veterinary Committee on Trauma (VetCOT) Trauma Registry and Dentistry and Oral Surgery Case Log will be utilized to identify feline craniofacial trauma cases that were presented to Colorado State University's Veterinary Teaching Hospital between 2014 and 2020. Prognostic indicators to be evaluated will be: type and etiology of injury, signalment (age and sex), initial presentation to CSU Urgent Care vs referral, the Modified Glascow Coma Scale, Animal Trauma Triage (ATT) scores, craniofacial examination findings, diagnostic imaging technique, and injuries identified via imaging. Outcomes evaluated will be treatment pursued and patient status upon discharge. This information will help clinicians determine the likelihood of a successful outcome for feline patients with craniofacial trauma. This project is funded by the Young Investigator Grant program.

Resident / Clinical Sciences

O-54. Estrus suppression in the mare through the use of altrenogest releasing intravaginal rings.

Jessica Lederman, Jeremiah Easley, Marc Baum, John Moss, Jennifer Hatzel

This study proposes an alternative method for delivering altrenogest, a synthetic progestin, to decrease adverse behavior in mares through hormonal manipulation. We hypothesize that an intravaginal ring designed for the mare will provide a novel and efficacious method for sustained release of altrenogest to suppress adverse behavior commonly associated with estrus. Importantly, it will allow the mare to resume normal cyclicity upon removal for pursuit of reproductive procedures. Mares often demonstrate undesirable behaviors during their estrus phase (5-7 days) of their 21-22 day estrus cycle, due to elevated estrogen levels produced by the dominant ovarian follicle. Following ovulation, progesterone levels increase, overcoming effects of estrogen for 11-14 days; often alleviating the undesirable behavior. Altrenogest, currently delivered orally or by injection, provides estrus suppression through similar biologic activity as native progesterone. Mares often resist daily oral administration or injections, leading to noncompliance. The safety and ease for the application of intravaginal rings between the diameters of 13cm and 18cm was determined through pilot trials of control rings in the summer of 2020. Ring size (14.2 cm) was effective for both mare retention and safety. Pharmacokinetic and pharmacodynamic values were then evaluated at the onset, steady-state and tail-end of varying levels of vaginal altrenogest delivery in 7-day trials. The optimal level of altrenogest was determined and used in in a 15-day trial completed in November of 2021 to evaluate pharmacokinetic and pharmacodynamic values. Outward estrus behavior and physiological changes were assessed through exposure to stallions, routine transrectal ultrasound examination and hormonal assessment. The overall goal of this study is ring retention, steady-state levels of altrenogest at or above 0.5 ng/mL and behavioral estrus suppression for a 15 day duration. Comparative data will be analyzed using the Shapiro-Wilcoxon signed-rank test. Statistical significance will be set at $P \leq 0.05$.

Graduate Student / Clinical Sciences

O-55. Serosurveillance for anthrax exposure in Texas feral swine: A potential biosurveillance tool for mapping risk.

Rachel M Maison, Courtney F Pierce, Izabela K Ragan, Vienna R Brown, Michael J Bodenchuk, Richard A Bowen, Angela M Bosco-Lauth

Anthrax is a disease of concern for many mammalian species, including humans. Management primarily consists of preventative vaccination and observations at the clinical level, as environmental isolation is laborious and bacterial distribution across large landscapes difficult to confirm. Concurrently, feral swine (Sus scrofa) are an invasive species that rarely succumb to anthrax and whose range is extensive in the Southern United States. We report feral swine may serve as a biosentinel for anthrax based on serology from experimentally infected animals as well as comparative seroprevalence in swine taken from historically defined endemic and non-endemic anthrax regions of Texas, USA. Experimental infection data confirmed that feral swine exhibit measurable antibody responses to B. anthracis after intranasal exposure, and that the strength of immune response correlates positively with both inoculum dose and number of exposures. Field serology from two regions of Texas revealed overall seropositivity was 43.7% (n=478), and logistic regression demonstrated county status, age-class, sex, latitude, and longitude were informative for predicting antibody status. However, of these covariates, only latitude was statistically significant (β=-0.153, P=0.047). This suggests anthrax exposure in swine could serve as a proxy for bacterial presence when paired with continuous location data. These findings ultimately have implications for the identification of landscapes contaminated with B. anthracis, and subsequently relative human and animal exposure risk(s). Funding for this research was provided by the United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services.

Graduate Student / Biomedical Sciences

O-56. Feverfew: Cheerful foliage or source of an anticancer compound?

Lisa J Schlein, Barbara J Rose, and Douglas H Thamm

Parthenolide (PTL) is a potent anti-cancer compound that was originally isolated from the feverfew plant. In our research, we have identified several types of canine cancer that are sensitive to PTL. Some of these cancer types, like histiocytic sarcoma and mast cell neoplasia, are more common in some dog breeds than in humans, providing a natural, translational study population for rare and deadly human diseases.

Growth inhibition assays were performed with canine cell lines and primary lymphoma cells isolated from canine patients, using PTL alone or in combination with redox-perturbing standard-of-care therapeutics. Cell death was assessed using flow cytometry. Immunofluorescence was used to assess NFκB localization, and canine cells were transfected with a reporter gene cassette containing the NFκB consensus sequence followed by firefly luciferase gene. PTL's effects on glutathione and reactive oxygen species generation were assessed with a colorimetric assay and a fluorescent H2DCFDA assay, respectively. Over 200 paraffin-embedded biopsy and necropsy specimens from dogs with naturally occurring tumors were assessed with immunohistochemical (IHC) staining for active nuclear NFκB (canonical and alternative pathways).

Canine cell lines and primary cells are sensitive to PTL and undergo dose-dependent apoptosis following exposure to drug. PTL exposure also leads to glutathione depletion, reactive oxygen species generation, and NFκB inhibition in canine cells. Standard-of-care therapeutics broadly synergize with PTL. Many spontaneous canine tumor samples have nuclear p65 IHC staining that is of greater magnitude than observed in comparable, normal cell populations, indicating PTL's promise in canine cancer treatment. Preliminary data show that immunodeficient mice can model disseminated histiocytic sarcoma with intraperitoneal xenografts of canine cells, but there is a long latency period to tumor development. Additional studies are underway to continue development of a mouse model of this disease.

Graduate Student / Clinical Sciences

1. Investigation of the canine immune landscape with single cell transcriptomics.

Dylan Ammons, Jade Kurihara, Lyndah Chow, and Steven Dow

Dogs make up a population of animals that are constantly around humans. They live in the same environment, often sleep in the same bed, and not surprisingly develop similar diseases. In many instances disease progression closely resembles that of their human counterparts, and thus they have the same need for therapeutics. The need for novel treatment is especially prominent in the context of cancer, in particular osteosarcoma. This highly malignant cancer of the bone typically arises in the limbs of canine patients and is generally treated by amputation of the affected limb. Although this intervention prolongs the life expectancy of the patient, it is likely that they will develop metastasis to distant sites. The process of metastasis is mediated by a variety of factors, but it is hypothesized that circulating immune cells may play a part in the movement of metastatic tumor cells and potentially its prevention. As such we used single cell RNA sequencing to investigate how circulating white blood cells are altered in canine osteosarcoma patients. In total, we plan to study cells from 5 healthy and 5 osteosarcoma patients to determine how cancer influences immune cell abundance and the transcriptome of each cell population. Analysis to date has allowed for classification of major immune cell populations (T cells, B cell, monocytes, neutrophils, etc.) with the identification of various subpopulations within each major immune cell type. These data have the potential to lead to the discovery new therapeutic targets that could be targeted for intervention and will ultimately provide an unbiased classification of canine immune cells in health and disease.

DVM/PhD Student / Clinical Science

2. Testing the validity of using a biochemical analytical assay for diagnosing canine cognitive dysfunction, as a translational model for Alzheimer's disease.

Madi Barton, Brittany MacQuiddy, Julie Moreno, and Stephanie McGrath

Alzheimer's disease and other dementias are some of the most prevalent neurodegenerative disorders and are predicted by the World Health Organization to be the second leading cause of death in the United States within the next decade. Neurodegenerative disorders are associated with the accumulation of misfolded proteins in the brain followed by irreversible loss of neurons. Canine cognitive dysfunction (CCD) is a commonly recognized neurodegenerative disease in older dogs and is comparable to Alzheimer's disease in humans. Currently, there are no widely used treatment options that are effective for treating dogs with CCD due to delays in diagnosis and inferior animal models used in past research. The aim of this study is to develop an antemortem analytical assay for diagnosing CCD to be used as a translational model for Alzheimer's disease. This will be accomplished by analyzing blood and cerebrospinal fluid from client owned dogs with naturally occurring CCD and assessing the degree of inflammation and aggregate protein accumulation to accurately diagnose CCD. The goal after development of the assay is to perform clinical trials evaluating possible treatments for both animals and humans, using the biochemical assay to confirm the diagnosis of neurodegenerative disease and evaluate the efficacy of treatments.

DVM Student / Biomedical Science

3. SARS-CoV-2 in felids.

Laura Bashor, Erick Gagne, Angela Bosco-Lauth, Mark Stenglein, and Sue VandeWoude

SARS-CoV-2 (SARS2) virus populations evolve rapidly to changing environments within their hosts through the selection of viral variants. Experimental and observational data suggest that felids are highly susceptible to SARS2 infection. However, it remains to be determined if SARS2 undergoes species-specific adaption following human-to-felid transmission. To evaluate SARS2 evolution in felids, we employed an experimental model system to observe variant emergence and selection in domestic cats. Three cohorts of cats (N=23 cats total) were directly inoculated with SARS2 or infected through cat-to-cat contact transmission. RNA recovered one-to-three days post-infection was used to sequence full SARS2 genomes at a high depth of coverage prior to analysis of within-host viral variants. We detected 129 single nucleotide variants (SNVs) and structural variants (SVs), mainly nonsynonymous SNVs that result in an amino acid change. Notably, we observed genomic signatures of positive selection and the emergence of nine within-host variants located at the same genomic positions as mutations characteristic of SARS2 Variants of Concern (VOC). These experimental results underscore the rapidity of SARS2 adaptation in felid hosts, and a theoretical potential origin for VOC in human populations. However, an analysis of publicly available SARS2 genomes recovered from naturally infected domestic and wild felids suggests human-to-felid transmission, while frequent, is typically unilateral, and felid-specific SARS2 evolution is not evident at this scale.

Graduate Student / Microbiology, Immunology and Pathology

4. Estrogen Receptor Beta in oxytocin neurons has an implicated role in HPA axis regulation.

Anna I Bautista, Julietta A Sheng, Sarah M Tan, Renata M Daniels, Alex M Miller, Robert J Handa

Estrogen receptor beta (ERβ) is hypothesized to be an important component in modulating the stress response of the hypothalamic-pituitary-adrenal (HPA) axis by decreasing anxiety and depressive-like behaviors in mice. ERβ is shown to be 75% colocalized with oxytocin (OT) neurons in the paraventricular nucleus (PVN). Reducing ERβ in OT neurons is expected to lead to increases in anxiolytic behaviors along with increases in neuronal activation in the PVN, the central regulator of the HPA axis. The relative amount of activation is expected to be higher in females as compared to males due to the pre-existing notion that females are more sensitive to the effects of stress. To understand these effects, ERβ-OTcre knockout mice were used for this study. Mice were restrained for 20 minutes in 50 mL conical tubes followed by euthanasia and aldehyde perfusion. Brains were extracted, postfixed in 4% paraformaldehyde, and cryoprotected in a 30% sucrose phosphate buffer. Brains were then sectioned at 35 µm and immunohistochemistry was performed with c-FOS antibody (1:1000). Sections were imaged with confocal microscopy (Zeiss LSM880). c-FOS expression was then quantified using IMARIS. Analysis is ongoing, but results are expected to demonstrate increased c-FOS expression in the mice that underwent restraint knockout condition compared to the control. Mutant female mice of the knockout condition under restraint are also hypothesized to display elevated c-FOS expression in comparison to male. If greater levels of neuronal activation as indicated by the number of c-FOS+ cells are observed in females compared to males, it will align with the hypothesis that ERB in OT neurons may be important for the regulation of stress response in adult mice. The data will also contribute to the body of literature that females are more sensitive to stressors in adulthood.

Undergraduate Student / Biomedical Sciences

5. Endogenous opioid signaling in the retina modulates sleep/wake behavior.

Casey-Tyler Berezin, Nikolas Bergum, and Jozsef Vigh

Opioid drugs, like morphine, are powerful analgesics, but long-term use leads to sleep disturbances and subsequent negative outcomes such as increased risk of addiction and overdose. The mechanism underlying opioid-induced sleep disturbances is unclear. Our overall hypothesis is that opioids accumulate in the eye and disrupt an endogenous opioid system in the retina that regulates sleep/wake cycles.

In the human and mouse retina, intrinsically photosensitive retinal ganglion cells (ipRGCs) help set sleep/wake cycles to environmental light/dark cycles by firing action potentials in response to light. Importantly, light-evoked firing by ipRGCs is inhibited by the activation of their μ-opioid receptors (MORs). In this study, we test the hypothesis that activation of these MORs by the endogenous opioid peptide β-endorphin modulates sleep/wake cycles.

Our immunohistochemical experiments reveal that there is a statistically significant increase in β-endorphin protein expression in the retina in nighttime versus daytime, as well as in dark versus light conditions. In addition, telemetric recordings of sleep/wake behavior in transgenic mice which lack functional MORs on ipRGCs show reduced wakefulness during the subjective night compared to wild-type mice.

Therefore, we conclude that β-endorphin expression in the retina depends on both circadian timing and light exposure, which is consistent with previous studies showing circadian regulation of β-endorphin expression in the brain. In addition, our data support the hypothesis that endogenous opioid signaling in the retina is critical to healthy sleep/wake cycles. Furthermore, these results suggest that chronic opioid use may cause aberrant signaling through MORs on ipRGCs that could contribute to opioid-induced sleep disturbances.

Graduate Student / Biomedical Sciences

6. A novel target for opioid-induced sleep disorders.

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-induce 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 Science

7. Using the Ovariectomized Ewe to Model Postmenopausal Osteoporosis Disease Progression.

Katie Bisazza, Brad B Nelson, Katie J Sikes, Russell Anthony, Laurie Goodrich, Kirk McGilvray, Holly L Stewart, Jeremiah T Easley

Osteoporotic fractures constitute the majority of reported fractures in the U.S. and are becoming an increasing burden on the medical industry. Ovariectomized (OVX) sheep are commonly used in osteoporosis research because they are comparable to humans in both bone size and microarchitecture. However, the cellular pathways involved in bone turnover in ovine models of osteoporosis have not been fully elucidated. The objective of our study is to further investigate the disease progression of osteoporosis using a previously established OVX-corticosteroid combination ovine model. Sixteen (N=16) intact, naïve, skeletally mature ewes were enrolled in the study, where ten (N=10) animals were assigned to the experimental group (E) and the remaining six (N=6) animals were assigned to the control group (C) (IACUC protocol #2060). Sheep in group E underwent OVX and were administered a high-dosage regimen of corticosteroids for four months following surgery, whereas sheep in group C remained intact throughout the study and were not administered corticosteroids. All animals will undergo dual-energy x-ray absorptiometry (DEXA) imaging at baseline and every three months to monitor bone density changes. Group E animals will additionally undergo computed tomography (CT) scanning at the same time points to evaluate in-life trabecular and cortical bone density fluctuations following OVX. Iliac crest biopsies will be obtained from all animals at baseline and every three months throughout the study. Biopsies will be divided for micro-computed tomography (microCT) scanning to evaluate bone microarchitecture and untargeted proteomic analysis to evaluate protein production and/or degradation in the bone. All animals will be survived until twelve (12) months following baseline sampling. The study is currently in progress. Understanding the cellular pathways involved in osteoporosis disease progression could aid in future cellular therapy research and lead to novel pathway targets for disease treatment in humans.

Graduate Student / Clinical Sciences

8. Reported neurocognitive symptoms post SARS-CoV-2 infection in adults and the relationship with IL-6, IL-8, and D-dimer inflammatory markers.

Kailey Berry, Madison Tipton, Bridget A Baxter, Nicole Natter, Taru S. Dutt, Luke Whitcomb, Stephanie M. LaVergne, Tracy L Webb, Sophia Stromberg, Kim McFann, Greg Ebel, Julie Dunn, Adam Chicco, Marcela Henao-Tamayo and Elizabeth P Ryan

SARS-CoV-2 infection that results in COVID-19 disease impacts nervous system function in some adults. The role for the strong inflammatory response to clear the virus and persistent inflammation for developing post-acute sequelae of COVID-19 (PASC) is unknown. This project involves adults (64 male, 75 female) that enrolled in the Northern Colorado SARS-CoV-2 Biorepository (NoCo-COBIO) starting in July 2020 (NCT04603677). Participants provided blood at multiple times within 6 months and with connection to the RAND SF-36 quality of life (QoL) survey. Symptom surveillance reports were used to identify neurocognitive alterations. Blood plasma was separated for longitudinal analysis of cytokines in 139 adults, and 96 adults had one or more follow-up collections. Participants reporting headache, dizziness, loss of consciousness, deafness, numbness in extremities, tingling in extremities, loss of taste, loss of smell, change in taste, forgetful or absent minded, confusion and difficulty concentrating at 1-6 months after the initial study visit were classified with neurologic symptoms associated with COVID-19. Neurocognitive symptoms are hypothesized to be associated with persistently elevated plasma levels of IL-6, IL-8, and D-dimer. At visit 2, 49% (n = 47) were affected with a neurocognitive symptom from COVID-19, whereas 51% (n = 41) and 32% (n = 25 individuals) in visits 3 and 4. Changes over time in these 3 analytes and between disease severity groups (mild, moderate, severe) will be shown and compared to participants that displayed no neurocognitive symptoms at visit two. A range of D-dimer levels was reported for visits 1 (69 – 178,272.1 µg/µL), 2 (59.8 – 286,883 µg/µL), 3 (76.4 – 301328.9 µg/µL), and 4 (93.2 – 375,816.6 µg/µL). The data obtained from this study emphasizes the importance of further neurocognitive research related to PASC from SARS-CoV-2 infection.

Undergraduate Student / Environmental and Radiological Health Sciences

9. Indirectly targeting the intervertebral disc nucleus pulposus tissue for a model of degenerative disc disease.

Andres Bonilla, Katie Bisazza, Jaiden Oropallo, Zach Cartwright, Steve Dow, Brian Johnstone, Christian Puttlitz, Jeremiah Easley.

Lower back pain affects 80% of the human population with over 40% of those cases been attributed to degenerative disc disease (DDD). Many therapeutic strategies for DDD are under investigation worldwide, however, their translation to a clinical setting is hampered by lack of an appropriate animal model. Current animal models of DDD require direct trauma to the disc tissue, which poorly recapitulates the human condition of DDD. Extracorporeal shock wave therapy (ESWT) is a non-invasive medical technology that delivers acoustic waves to specific tissues within the body to reduce pain and promote healing. When used in excess, ESWT can result in tissue damage. We hypothesize that an ovine preclinical model of DDD can be created by targeting shock waves to the disc without using direct trauma. For this purpose, we used focused ESWT which was delivered to the intervertebral disc to develop a model of DDD. Six (N=6) sheep were equally randomized into two groups. Group A (N=3) sheep received ESWT at L2-3 and L4-5 discs (n=6 disc levels per group), through a lateral retroperitoneal approach. Group B (N=3, n=6 disc levels) served as untreated controls. Evaluation was performed with lumbar radiographs, magnetic resonance imaging (MRI), as well as post-sacrifice gross evaluation, kinematic analysis, and histopathology. Preliminary results reveal no significant changes in radiographical or MRI assessment. However, gross evaluation showed moderate morphological changes around the disc levels treated with ESWT. Kinematic analysis exhibited differences between the control levels and the levels treated with ESWT. At gross evaluation, morphological changes were found, which suggests a reaction of the disc tissue to ESWT. To our knowledge, this is the first time that adverse changes in the intervertebral disc are demonstrated after application of ESWT. Our preliminary results suggest the potential use of ESWT to develop a preclinical model of DDD.

Graduate Student / Clinical Sciences

10. Verifying correlation between equine clinical examination parameters and inertial measurement unit data to optimize wearable inertial sensor development.

Cassidy R Bouse-Eaton, Wes Anderson, Steven Simske, Erin Contino, Melissa King, Christopher Kawcak

The purpose of the study was to verify mathematical correlation between clinical examination and inertial measurement unit (IMU) data in order to optimize inertial sensor development for gathering limb-based data for analysis. The intent is to validate expanding the study to a larger cohort. A single horse had four IMU sensors placed on specified locations on the forelimbs. The horse was trotted for a distance of 15.5 meters at least 6 times to gather IMU data and then was evaluated for lameness, carpal joint effusion, range of motion, and flexion test scoring. Following the initial data collection, lameness was induced in the left forelimb via a surgically-induced carpal osteochondral fragment. Data collection was repeated at day 10 and 14 post-operation, and then every 7 days through 70 days total. 3D acceleration data was collected via the IMU sensors and analyzed via machine learning techniques. Analysis was performed using 516 time-series features, with feature standardization followed by appropriate coefficient of variance (CoV), repeatability, and correlation measurements. Data analysis included separating and using the movement data to calculate features that are statistically likely to assist identifying differences over time when lameness is surgically induced in a horse. Using four traditional classification methods, the 516 calculated features were, combined, able to effectively distinguish between the different scores assigned with each metric, with the strongest predictive algorithm being the k-Nearest Neighbors (kNN). Although these results are inconclusive statistically, the methodology used in this pilot study supports further examination with a larger sample size.

DVM Student / Clinical Sciences

11. Immunophenotyping immune cells of the Jamaican fruit bat.

Bradly E. Burke, Savannah Rocha, Tony Schountz

There are over 1,400 species of bats, each providing important ecological roles, including pollination and seed dispersal, and consummation of insects – such as crop pests and mosquitos which spread arthropod-borne diseases. Bats represent major reservoir hosts for viruses that are transmissible to humans and livestock, including, henipaviruses, filoviruses, coronaviruses, and lyssaviruses. Moreover, bats are susceptible to other pathogens such as Pseudogymnoascus destructans – the causative agent of white nose syndrome – that has killed over 6 million North American bats. As such, bats interconnect ecological and agricultural importance, and human health. Human and rodent immunology provide a foundation to understand bat immune systems and responses; however, clear differences exist between well understood species and bats. Jamaican fruit bats (Artibeus jamaicensis) represent an underdeveloped animal model for immunology research. This gives rise the inherent roadblock of a paucity of bat specific reagents–such as antibodies. Limited reagents constrain the use of high throughput technologies such as flow cytometry, cell sorting, and highly qualitative and anatomical data from fluorescent microscopy. In this study, 12 commercially available antibody clones were identified to cross-react with Jamaican fruit bat epitopes: CD3e, CD8a, CD19, CD34, CD44, CD45RA, CD80, CD104, CD161, major histocompatibility complex-II (MHC-II), and asialo-GM1. These were identified, by pre-screening protein homology in silico for cross-reactivity potential, tested via flow cytometry and florescent microscopy, validated by cell sorting and RT-qPCR, and have demonstrated cross-reactivity with Jamaican fruit bat epitopes. Furthermore, we have produced hybridoma cells lines against three Jamaican fruit bat epitopes: CD3g, CD4, and IgG. Characterization of bat immune responses will provide valuable insight into selection pressures placed on bat-borne viruses driving critical spill-over events. Furthermore, these reagents will demonstrate how Jamaican fruit bat, and likely other bat species, respond to infections, providing aid to bolster conservation efforts we currently, and will continually face.

Graduate Student / Microbiology, Immunology and Pathology

12. Point of care ultrasound measurement of caudal vena cava diameter and collapsibility index for predicting hypovolemic states in dogs.

Jenna H. Cardillo, Kristin Zersen, Brianna Potter, Amanda Cavanagh

Point of care ultrasound is a promising tool for reliable, accessible, and non-invasive volume assessment in ill or injured animals. The ability to evaluate volume status is critical in optimizing fluid resuscitation and avoiding detrimental fluid overload. However, a reliable, practical method of measuring intravascular volume status has not been identified and traditional point of care diagnostic tests lack precision. The purpose of our study is to evaluate the relationship of caudal vena cava (CVC) diameter and CVC collapsibility index (maximum diameter – minimum diameter/maximum diameter x 100) evaluated using point of care ultrasound with cardiac output in normovolemic and hypovolemic dogs. No information exists about the relationship between caudal vena cava imaging and cardiac output in dogs. Anesthetized spontaneously breathing research beagles were instrumented with pulmonary arterial, peripheral arterial, and jugular venous catheters. Cardiac output was monitored using thermodilution. Dogs were exsanguinated to a mean arterial pressure of 40mmHg, or a maximum 60% blood volume lost, then auto-transfused. CVC diameter and CVC collapsibility index were measured from the subxiphoid ultrasound view at baseline, after exsanguination, at 50% auto-transfused volume, and then again after full auto-transfusion. Abdominal CVC: abdominal aortic diameter was also measured at these time points from a paralumbar view. Preliminary results indicate CVC diameter markedly decreases and CVC collapsibility index increases in hypovolemic states. Statistical analysis is pending. Initial conclusions from this study indicate caudal vena cava assessment in dogs using point of care ultrasound is a useful volume assessment tool. This study was funded by the principal investigator's faculty start up fund and the ultrasound machine was donated by Universal Imaging.

Resident / Clinical Sciences

13. Evaluating Bluetongue Virus Reassortment in *Culicoides sonorensis*.

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

Infection of susceptible ruminants with bluetongue virus (BTV) can result in devastating disease with significant economic losses. Geographical expansion of BTV and increased incursions of novel serotypes, evident by recent outbreaks, demonstrate the aptitude of the virus and the *Culicoides* midge vector to infiltrate new regions. However, factors driving BTV's expansion are not clearly defined. As a segmented double stranded RNA virus, reassortment between BTV strains may result in genetic variation that enhances BTV transmission dynamics and effectuates epizootic events. The objective of this current study was to evaluate if reassortment occurs in *Culicoides sonorensis* when coinfected with BTV strains BTV-10 ATCC and BTV-17 California. To establish single and co-infections, C. sonorenesis were fed a blood meal containing BTV-10 ATCC, BTV-17 California, or both BTV strains at equal titers. *C. sonorensis* specimens were collected every other day and processed for BTV qRT-PCR to track virogenesis over time. Co-infected *C. sonorensis* were collected on day ten for plaque isolation and propagation. The complete genotypes of isolated plaques were identified using Next Generation Sequencing. Plaque isolation and propagation from pools of coinfected *C.sonorensis* from day 10 post-infection yielded viable BTV with subsequent genotyping of plaques aligning with the BTV-10 ATCC parental strain. While reassortment was not detected in the processed plaques in this study, it is a phenomenon found in field isolates. Thus, further studies on determinants of BTV reassortment such as genetic relatedness between strains and temperature are warranted. Future findings will add an important dimension to the modeling of viral evolution

Graduate Student / Microbiology, Immunology and Pathology

14. Transcutaneous Vagal Nerve Stimulation of the Auricular Branch in the Mouse.

Emily A Castellanos, Julietta A Sheng, Sarah ML Tan, Bret N Smith, and Stuart A Tobet

Metabolic syndrome and obesity are prevalent major health issues that can coincide with affective mood disorders such as depression. Transcutaneous vagal nerve stimulation (tVNS) is a therapy that has been implicated and increasingly investigated in the treatment for both major depressive disorder (MDD) and obesity. Recently, a non-invasive method of stimulating the vagus nerve through the auricular branch in the ear has been of high interest in developing therapies for neuropsychiatric and metabolic diseases. However, which neurons are activated by tVNS in the central nervous system (CNS) and how stimulation of the vagus nerve overlaps anatomically with the hypothalamic-pituitary-adrenal (HPA) stress axis is not well described. The objective of this study is to map c-FOS expression in the CNS after tVNS in male and female mice to determine the brain regions activated by tVNS and the degree of overlap with the HPA axis. Corticotropin Releasing Hormone (CRH)-cre and Arginine Vasopressin (AVP)-cre mice are crossed with Ai14 reporter mice to induce robust tdTomato expression in cre-expressing neurons that project to the brainstem. Adult wild-type C57 and the reporter mice underwent acute tVNS for 30 minutes while anesthetized, followed immediately by transcardial perfusion. c-FOS expression was quantified through immunohistochemistry to determine areas of high neuronal activity and co-expression of the c-FOS positive neurons with the various cre-expressing cell types. Preliminary data show stimulated neurons in the brainstem nucleus of the solitary tract (NTS), demonstrating relay of neuronal signaling of vagal stimulation in the CNS. Ongoing studies will determine downstream target sites within the CNS that are activated by tVNS, which may also be involved in HPA axis activity or satiety signaling related to mood disorders and metabolic syndromes.

Graduate Student / Biomedical Sciences

15. A review of biodosimetry methods for wildlife mammals experienced radiation exposure.

Yuiko Chino and Thomas E Johnson

Wildlife can be exposed to a significant amount of radiation from accidents at nuclear facilities as well as detonations of improvised nuclear devices. Radiation can impact mammalian wildlife for a significant time, potentially causing injury to exposed individuals and the overall population Therefore, it is important to properly estimate radiation dose to exposed animals. Biodosimetric methods such as dicentric chromosomal analysis, micronucleus assay, and proteomic assay have been studied for humans to estimate radiation dose from accidental radiation exposure. These methods may also be useful to estimate the dose to wildlife exposed to radiation and estimate the impact. While biodosimetric methods for humans have been established and reviewed, those for wildlife are limited and requires further investigation. In this study, we reviewed the literature for mammalian wildlife *in vivo* and vitro biodosimetric methods and provide a table of information for each species. A collection of available biodosimetric methods, the dose range, and significance of the dose response is provided. Difficulties in implementation of the methods described is included, as well as biological and ecological characteristics.

Graduate Student / Environmental and Radiological Health Sciences

16. Anti-erythrocyte antibodies detected by flow cytometry assay in 235 anemic client-owned dogs.

Nida Chornarm, Melissa Brewer, Sarah B Shropshire, Christine S Olver, Steve Dow, and Michael R Lappin

There are multiple causes of anemia in domestic dogs, some of which are life-threatening. Of those, immune-mediated hemolytic anemia (IMHA) is a potentially fatal disease in dogs resulting in red blood cell rupture(hemolysis). IMHA can be primary (idiopathic) or secondary from conditions such as infectious disease or cancer. Anti-erythrocyte antibodies bound to the red blood cell surface play a major role in the hemolytic process. Use of a flow cytometry assay to detect anti-erythrocyte antibodies on the surface of erythrocytes can be quantitative (percent IgG binding) and is an alternative diagnostic method to the Coombs test (direct agglutination test or DAT).The objective of this study was to compare the percent IgG binding among 4 groups of anemic dogs; idiopathic IMHA, neoplasia, blood-borne pathogen infection, and other etiologies. From previous studies in healthy dogs, a cut-off of < 5% was defined as negative in the flow cytometry assay. Results from a total of 235 samples from client-owned dogs with anemia (hematocrit ≤ 35%) evaluated at the Veterinary Teaching Hospital were included. The mean percentage of anti-erythrocyte antibodies in idiopathic IMHA dogs (n=58) was 29.6%, range 0.24% – 99.8%. Subgroups included IMHA in crisis (mean = 34.02%; n=39), IMHA on treatment (mean = 12.75%; n=7), Precursor-targeted immune-mediated anemia(PIMA) (mean = 29.87%; n=8) and other characteristics of dogs in IMHA group (mean = 15.4%; n=4). The mean percentages for anti-erythrocyte antibodies in dogs due to neoplasia (n=84), blood-borne pathogen infection (n=5), and other etiologies (n=88) were 1.82% (0.08% – 29.23%), 3.25% (0.71% – 5.18%) and 2.87% (0.12 – 95.1%), respectively. In conclusion, the results support that dogs with idiopathic IMHA have numerically higher levels of anti-erythrocyte antibodies compared to dogs with other causes of anemia. The study also suggests that dogs with PIMA are also likely to have some degree of peripheral hemolysis.

Graduate Student / Clinical Sciences

17. Spatial patterns of immune protection reveal granuloma heterogeneity among BCG vaccinated, *Mycobacterium tuberculosis*-infected mice.

Sarah K Cooper, Hadley E Gary, Macallister C Harris, James E DiLisio, Amy Fox, Burton Karger, Marcela Henao-Tamayo, Joshua J Vasquez, Brendan K Podell

During *Mycobacterium tuberculosis* (*Mtb*) infection, granuloma formation in the lung plays a critical role in orchestrating complex host-pathogen interactions. As discrete and multifocal immune microenvironments, each individual granuloma may evolve independently within the same lung. This may lead to a spectrum of response including successful containment, potential elimination, or failed control of *Mtb*. Our understanding of the granuloma has been limited by a lack of experimental tools to simultaneously evaluate both the host and pathogen without disrupting the granuloma microenvironment. To address this, we have developed methods to interrogate immune cell and their spatial location through multiplexed immunohistochemistry (IHC) as they relate to location and replicative state of the *Mtb* bacilli, measured by *in situ* hybridization (ISH). Because adaptive immunity plays a critical role in controlling bacterial growth and TB disease progression, we applied these techniques to *Mtb*-infected, BCG-vaccinated and unvaccinated mice to determine influential spatial relationships between immune populations and *Mtb* bacilli. Data from this study highlight the striking heterogeneity in response to *Mtb* infection occurring within and across mice in both vaccinated and unvaccinated groups. This heterogeneity, present even among an inbred mouse strain, highlights the potential for a granuloma-targeted approach to better understanding immune protection. This more granular approach to identify successful and unsuccessful granulomas may offer new insight into correlates of immune protection.

Graduate Student / Microbiology, Immunology and Pathology

18. A Radioanalytical Method For The Separation Of Radium From Hydraulic Fracturing Waste Water Using Ion Exchange Resin.

Maelle Coupannec, Steffen Happel, Ralf Sudowe.

A major concern arising from hydraulic fracturing is the generation of a large volume of flowback water potentially containing various amounts of dissolved naturally occurring radioactive material. Up to four million gallons of water-based fluid is injected per well, of which 10–70% is subsequently recovered as flowback. Improving the measurement and separation of "Technologically Enhanced Naturally-Occurring Radioactive Material" (TENORM) is therefore a priority. Radium has gained attention as a source of potential environmental contamination in process waters from hydraulic fracturing sites and bodies of water affected by the mining industry. Providing accurate radium measurements is an essential step for appropriate disposal of the brine water as regulated or non-regulated NORM-containing wastes. The oil and gas industry produces water with high dissolved solid material content leading to a significant scientific challenge in achieving separations of radium due to the high concentration of chemical analogs such as barium, strontium, and calcium. Multiple extraction chromatographic resins developed by TrisKem Chemical were investigated at different nitric acid concentrations to quantify and enhance the separation of radium from the chemical analogs. Identifying a resin capable of extracting radium from the fracking water would also allow the reduction of costs associated with handling, treatment, and disposal of the wastewater.

Graduate Student / Environmental and Radiological Health Sciences

19. Evaluation of factors associated with surgical site infection in equine proximal interphalangeal joint arthrodesis: 54 cases (2010-2019).

Alyssa M Daniels, Lynn M Pezzanite, Gregg M Griffenhagen, Dean A Hendrickson

Frequency of surgical site infection (SSI) following orthopedic implant placement in horses has been reported but not compared with respect to antibiotic protocols administered. The objective was to determine factors associated with SSI in horses undergoing proximal interphalangeal joint (PIPJ) arthrodesis. Records from CSU VTH were evaluated (2010-2019) and horses undergoing PIPJ. arthrodesis identified. Patient signalment, supervising surgeon, reason for surgery, limb, implants placed, anesthetic time, duration casting/coaptation postoperatively, antibiotic regimens, incidence/onset SSI, and adjunct treatments were recorded. Logistic regression was used to estimate the contributions of covariates to the occurrence of infection postoperatively. During the study period, 54 PIPJ arthrodeses were performed. Surgical site infection was reported in 2/54 (3.7%) at d15,30 postoperatively, which resolved with medical management. Arthrodesis was performed due to osteoarthritis (33/54, 61.1%), fracture (11/54, 20.4%), subluxation (5/54, 9.3%), osseous cyst-like lesion (2/54, 3.7%), osteoarthritis with cyst-like lesion (2/54, 3.7%), and severed lateral collateral ligament (1/54, 1.9%). Perioperative systemic antibiotics were administered in all cases, in 15/54 (27.8%) horses for 1-3 days, and 39/54 (72.2%) for >3 days. Most frequently used protocols were cefazolin/gentamicin (20/54, 37%), cefazolin/gentamicin/doxycycline (14/54, 25.9%) and potassium penicillin/gentamicin (10/54, 18.5%). Regional limb perfusion was performed in 31/54 (57.4%) preoperatively and 7/54 (13%) postoperatively. Intraoperative lavage with antibiotic-infused fluids was used in 52/54 (96.3%). Survival to dismissal was 98.1% (53/54 horses); one horse was euthanized for support limb laminitis. No association was identified between antibiotic selection or duration (1-3 versus >3 days), regional antibiotic perfusion, intraoperative antibiotic lavage, or anesthetic time (< or >3hours) and SSI, however modeling was complicated by quasi-complete or complete separation of the data. Limitations include retrospective nature and low infection rate overall. In summary, short duration antibiotic therapy (<3 days postoperatively) did not result in a higher risk of development of SSI following equine pastern arthrodesis.

DVM Student / Clinical Sciences

20. Use of a commercially available electrolyte supplement in the management of acute diarrhea in shelter dogs.

Hailey Davis, Patricia Franco, Rachael Isdale, Katrina EV Jones, John Marsella, Michael R Lappin

Acute diarrhea in dogs is common in stressful situations and there are a number of specific bacterial, parasitic, and viral causes for both small and large bowel diarrhea. The commercially available electrolyte supplement be assessed in this study was previously shown to lessen diarrhea in calves in stressful situations. In a previous study in research beagles in our laboratory, the supplement was shown to be safe with no negative effects on the fecal microbiome. The primary hypothesis to be tested in this study is that dogs with acute diarrhea that are administered the supplement will have improved stool character over time and higher diarrhea resolution rates than dogs supplemented with the placebo. All dogs approved for entry to the study by the shelter staff and tested for common fecal parasites. All dogs were fed a standardized diet and administered fenbendazole for nematodes and Giardia spp. infection. The dogs were randomized to the electrolyte solution or placebo groups and both products were administered on Day 0 and Day 1. Dogs were monitored for vomiting, dehydration, and appetite daily and fecal scores were recorded through Day 5. To date, 12 dogs were randomized to be administered the electrolyte solution and 8 dogs were administered the placebo group. Side effects were not noted in either group. The median day to first normal stool was 2 for both groups. The proportion of days with diarrhea after starting treatment was 47.5% for the electrolyte solution dogs and 57.7% for the placebo dogs but this result was not significantly different. The results of the study suggest that electrolyte solution is safe for use in dogs but additional cases will need to be evaluated to determine the extent of any positive effects.

DVM Student / Clinical Sciences

21. Dengue Virus Reprograms Macrophage Gene Expression and Metabolism.

Jasmine Donkoh, Forrest Ackart, Randy Basaraba, Sandra Quackenbush, Joel Rovnak

Dengue virus (DENV) is the most prevalent arthropod borne flavivirus in the world, causing dengue hemorrhagic fever and dengue shock syndrome Macrophages are a site of DENV replication and the imbalance between macrophage pro-inflammatory and anti-inflammatory phenotypes during DENV infection leads to severe disease outcome. To investigate the effect of macrophage phenotype on DENV serotype 2 (DENV2) replication, we treated cells with either interferon gamma (IFNγ) or interleukin 4 (IL-4) to polarize cells towards a proinflammatory or anti-inflammatory phenotype and infected cells with DENV2. DENV2 shows preferential replication in cells treated with IL-4 compared to cells treated with IFNγ or non-treated cells. DENV2 infection upregulates expression of the anti-inflammatory cytokine, interleukin 10 (IL-10), and the inflammatory chemokine, CXCL10 in all phenotypes. DENV serotype 2 (DENV2) infection of macrophages leads to induced expression of the host mediator complex protein, cyclin dependent kinase 8 (CDK8). CDK8 is a transcriptional co-factor that regulates expression of interferon gamma-stimulated genes and certain cytokines/chemokines. Expression of CDK8 increases coincident with DENV2 replication throughout a 36-hour time course. Treatment with Senexin B, a CDK8 inhibitor, decreases DENV2 mRNA and infectious particles in THP-1 macrophages. We also found that inhibition of CDK8 activity with Senexin B increases IL-10, but decreases CXCL10 gene expression, regardless of macrophage phenotype. This suggests dependence upon CDK8 activity for virus induction of CXCL10 and IL-10. We hypothesize that CDK8 plays a key role in regulating a macrophage's ability to transcribe genes that control polarization and anti-viral immunity.

Graduate Student / Microbiology, Immunology and Pathology

22. Prevalence of Upper Respiratory Pathogens in Colorado Front Range Rescue Horses.

Conner Dugan, Elsbeth O'Fallon, Carson Zweck-Bronner, and Gabriele Landolt

The Colorado Front Range unwanted horse population is an important group of equids that present a risk to national horse populations. The stressful and transitional environments with commingling of mixed origin equids play host to various contagious pathogens that may or may not produce clinical signs. Infections of Equine Influenza Virus (EIV), strangles (*Streptococcus equi ssp. equi*), *Streptococcus equi* ssp. *zooepidemicus*, and equine herpesviruses 1 and 4 (EHV-1 and 4) may present with systemic signs of illness. However, subclinical infections are possible and are often undiagnosed. This includes the gammaherpesviruses, equine herpesviruses 2 and 5 (EHV-2 and 5). We hypothesized that equids rescued from feedlots and auctions with clinical and subclinical infections of respiratory pathogens present a contagious disease risk to the general equine population as these horses are rehomed. Existing data from polymerase chain reaction (PCR) analysis of nasal swabs of clinical and subclinical horses from various rescue organizations across the front range was analyzed to characterize the prevalence of upper respiratory infections in this target population. These data represent a diverse population of equids by including ages ranging from newborn foals to 28 years, and various breeds including ponies, donkeys, Quarter Horses, and Draft breeds. The prevalence of respiratory infections in this group is consistent with those found for a non-rescue population with exceptions being increased occurrence of strangles, EIV, and EHV-4. The quantified prevalence of infections in this population provides useful information about the risk level these horses present to other horse populations upon dispersal. This information will guide veterinarians, rescue operations, and horse owners in risk assessment and implementation of biosecurity and quarantine considerations.

DVM/MPH Student / Clinical Sciences

23. Intrapulmonary host-directed therapy using bioactive vitamin A for Mycobacterium tuberculosis infection in guinea pigs.

Hadley E. Gary, Macallister C. Harris, Sarah K. Cooper, Connor R. King, James E. DiLisio, Brendan K. Podell.

Previous studies have demonstrated that subclinical vitamin A deficiency (<200ug/L) in humans increases the risk of tuberculosis (TB) disease progression. Our lab has shown that oral supplementation of vitamin A, in the form of retinyl palmitate, to deficient animals over the course of TB infection reinstates the protection afforded by vitamin A sufficient status, suggesting that vitamin A may function as a host-directed therapy. The current work involves exploring the potential use of the bioactive form of vitamin A, all-trans retinoic acid (ATRA), as a host directed therapy during infection delivered via an intrapulmonary route in a liposome encapsulated formulation. Guinea pigs were fed diets either deficient in vitamin A (VAD) or sufficient in vitamin A (VAS) and exposed to low-dose aerosol of *Mtb* after vitamin A deficient status was achieved. 10 days after infection, animals were treated four times at 72-intervals with 25 µg of ATRA. On day 30, tissues were collected. Histopathology analysis showed minimal difference in total lesion burden between treated and untreated groups, but relative absence of lesion necrosis in VAD guinea pigs, as we have previously reported. Bacterial burden was higher in the VAD groups as expected; however, it was not decreased within the ATRA treated groups indicating that it could have an immunosuppressant effect on the host capacity to control bacterial growth. We have thus concluded that the bioactive form does elicit the same response in the host as the oral supplementation during active TB infection.

Staff / Microbiology, Immunology and Pathology

24. Cryopreservation of stallion sperm results in a loss of Phospholipase C zeta.

Raul A Gonzalez-Castro and Elaine M Carnevale

Phospholipase C zeta (PLCZ1) is a membrane-bound protein in sperm that triggers oocyte activation. Frozen or refrozen stallion sperm are often used for clinical ICSI. However, sperm are exposed to cryoinjuries and membrane protein modifications during cryopreservation which could cause loss of PLCZ1 and fertilization failure. We hypothesized that freezing and refreezing reduces sperm-associated PLCZ1; however, thawing frozen sperm at a lower temperature would avoid multiple membrane phase changes and reduce cryodamage. Semen from 12 stallions was frozen and thawed at 22°C (RT) or 5°C (LT) prior to refreezing. Sperm viability, acrosome integrity, and PLCZ1expression were analyzed by flow cytometry. Presence of PLCZ1 in lysates of fresh, frozen, and refrozen sperm and freezing extenders were determined by western blot. Data were analyzed using a mixed model for repeated measures and Tukey pairwise comparisons. As main effects, freezing and refreezing of sperm did not affect ($P>0.05$) acrosome integrity; but sperm viability decreased ($P<0.05$) after each freezing cycle. However, sperm refrozen at LT had similar viability ($P>0.05$) to frozen sperm. Also, cryopreservation as main effect reduced ($P<0.05$) PLCZ1 positive sperm and mean fluorescence intensity (MFI), although no differences ($P>0.05$) were observed between frozen and refrozen sperm. Within the live sperm subpopulation, freezing as main effect negatively affected ($P<0.05$) PLCZ1-MFI; but similar MFI was observed among fresh, frozen, and RT-refrozen sperm. Sperm having intact acrosomes had higher ($P<0.05$) PLCZ1-MFI than acrosome-reacted sperm. Viability and acrosome integrity were positively correlated ($P<0.05$) with PLCZ1 positive sperm and MFI. Congruently, PLCZ1 band density was reduced in sperm lysates after each freezing cycle, with a concomitant increase of PLCZ1 released into the freezing extenders. We concluded that cryopreservation results in a loss of PLCZ1 from the sperm membrane but thawing at a low temperature (5°C) versus room temperature prior to refreezing improves sperm survival.

Post-doctoral Fellow / Biomedical Sciences

25. Influence of sex and BMI on changes to quality of life scores in adults from 3 months to one year post COVID-19 disease.

Jared M Haberman, Bridget A Baxter, Stephanie M LaVergne, Sophia Stromberg, Kailey Berry, Madison Tipton, Tracy Webb, Kim McFann, Julie Dunn, and Elizabeth P Ryan.

Between July 2020 and October 2021, 139 adults were enrolled into the Northern Colorado SARS-CoV-2 Biorepository (NoCo-COBIO). Of those enrolled, 64% (n=89) completed an SF-36 survey at one timepoint while 55% (n=77) completed two. A snapshot assessment of Quality of Life (QoL) in Northern Colorado adults with COVID-19 and that develop post-acute sequelae of COVID-19 (PASC) was completed using the RAND SF-36 health survey. Moving forward, this cohort had a second QoL survey completed 3-6 months after the initial survey. The RAND SF-36 consists of 36 questions sorted into 8 scaled categories: Physical Functioning, Role Limitations due to Physical Health, Role Limitations due to Emotional Problems, Energy/Fatigue, Emotional Well-being, Social Functioning, Pain, and General Health. Each question and scale score were evaluated on a scale of 0-100; lower scores are indicative of lower QoL. By obtaining SF-36 data at multiple timepoints, changes in scores across categories can be tracked. An ANCOVA test will be performed on SF-36 scores with age, sex, BMI, disease severity, hospitalization, PASC, and IL-6 levels as covariants. Further, independent sample t tests will allow for determination of significance for each metric. It is hypothesized that participants with persistent IL-6 levels, older age, and increased BMI will present with greater magnitude of change in QoL scores when compared to younger, normal weight adults. Conversely, participants with reductions in IL-6 after disease onset and without PASC, will have scores with an improved QoL. Assessing changes to QoL for these participants will yield insightful information regarding targeted interventions to improve QoL alongside the role for IL-6 as a marker for correlative use by the growing number of COVID-19 survivors.

Undergraduate Student / Environmental and Radiological Health Sciences

26. Osteosarcoma exosomes selectively home to the lung and elicit pro-metastatic changes in resident alveolar macrophages.

Laurel A Haines, Eric P Palmer, Sophi J Schofield, Chris Andretsos, Katie E Cronise, Daniel P Regan

Osteosarcoma (OS), the most common primary malignant tumor of bone, often progresses to a highly fatal metastatic disease with limited treatment options. Following resection of the primary tumor, one-third of OS patients relapse with metastases, almost exclusively in the lung. Metastasis is preceded by the formation of a pre-metastatic niche, a process by which distant sites in the body are "primed" for tumor cell seeding by factors secreted by the primary tumor. Of these secreted factors, nano-sized extracellular vesicles, also known as exosomes, have been shown to mediate pre-metastatic changes in tissue-resident cells, and display highly specific organotropism in certain metastatic cancers. Little is known about the role of OS exosomes in modulating the pulmonary microenvironment during OS metastasis. We hypothesize that OS exosomes selectively home to the lung and instruct resident alveolar macrophages to construct a pro-metastatic microenvironment characterized by inflammatory and structural changes. To investigate this, we evaluated human OS exosome biodistribution and cellular uptake in mice using intravital imaging, flow cytometry, and immunofluorescence. We also investigated the immunological effects of OS exosomes *in vivo* in mice and in primary human donor-derived lung alveolar macrophages. We show that OS exosomes selectively track to the lung, can be taken up by alveolar macrophages, and elicit distinct changes in tumor-promoting cytokines. Our findings demonstrate that OS exosomes can alter the lung microenvironment through their interactions with alveolar macrophages prior to circulating tumor cell arrival. These pro-tumorigenic changes may promote metastasis during OS and could serve as early indicators and potential therapeutic targets for patients with metastatic disease.

DVM/PhD Student / Microbiology, Immunology, and Pathology

27. Kinetics and effector function of lipid antigen CD1-restricted immunity in *M. tuberculosis* infected guinea pigs.

Macallister C. Harris, Hadley E. Gary, David F. Ackart, Sarah K. Cooper, Ildiko Van Rhijn, D. Branch Moody, Brendan K. Podell

CD1 is a group of glycoproteins on antigen-presenting cells (APCs) that present lipid antigens to T cells. A number of CD1-restricted lipid antigens have been identified from *Mycobacterium tuberculosis* (*Mtb*). However, the evolution and effector functions of CD1-restricted immunity have not been evaluated in response to specific lipid antigens during *in vivo Mtb* infections, owing to challenges in animal models reflecting the repertoire of group 1 CD1 complexes present in humans. Guinea pigs represent the only rodent model with human CD1 orthologues. The objective of this work was to determine the kinetic expression of CD1 complexes in tissue, and effector function of CD1 restricted T cells during *Mtb* infection in the guinea pig at innate, early adaptive and late adaptive immune stages of TB disease. Limited CD1 expression was present early in disease, but expression increased substantially at 30 days after infection, during the early adaptive response and was followed by a decline at day 60 infection. Correspondingly, CD1-restricted immunity elicited a strong cytotoxicity effector function at day 30, aligning with the presence of high CD1 expression. Cytotoxic activity was identified at the sites of infection, in the lung and spleen, and was detected against synthetic antigens, glucose monomycolate, and mycolic acid, both of which are CD1b restricted antigens. Importantly, we also identified that CD1 is expressed not only on myeloid antigen presenting cells but is also highly expressed on B cells. Now with demonstrated effector function in a tissue-dependent and antigen-restricted manner, this lays the groundwork for better understanding how CD1-restricted responses contribute to immunity against *Mtb* infection and the development of TB disease.

Resident / Microbiology, Immunology and Pathology

28. Outcomes of horses undergoing ventral midline celiotomy for colic with dorsal mesenteric attachment abnormalities of the colon and cecum.

Sara M Wist, Sloane Hoblick and Diana M Hassel

Congenital elongation of dorsal mesenteric attachments of the colon and cecum is a newly recognized condition identified during surgical exploration of colic patients that has unknowns with respect to history, presenting signs and survival. These cases are characterized by an unusual ability to exteriorize larger portions of the cecum and colon, including the cecocolic ligament, through a ventral midline celiotomy. The purpose of this study was to compare surgical colics with abnormal elongated attachments (cases) with control surgical colic cases with similar surgical lesions and normal anatomy. Survival rates, presenting signs and prevalence of pre-operative recurrent colic episodes were evaluated. Data from 94 surgical colic patients at Colorado State University's Veterinary Teaching Hospital presenting from 2018-2021 were included. Of the 92 included patients, 18 (19.6%) were found to have abnormally loose dorsal mesenteric attachments and 74 (80.4%) had normal attachments. Survival to hospital discharge for the 18 cases was 78% versus 64% for controls. When excluding the 13 horses euthanized intraoperatively, the survival rate for cases was 82% versus 76% for controls. Age, presenting PCV, total plasma protein and blood lactate were not significantly different in cases versus controls. Surgical procedure, defined as resection or no resection performed, was also compared. Excluding horses euthanized intraoperatively, 5 (29.4%) cases and 5 (8.1%) controls had a large colon resection performed. Out of the 92 patients, 11 (61.1%) abnormal cases and 19 (25.7%) control cases had a history of pre-operative recurrent colic episodes. Identification of abnormal dorsal mesenteric attachments of the colon and cecum paired with large colon resection provides comparable short-term survival of equine surgical colic patients and is associated with a higher prevalence of pre-operative colic episodes. Long-term outcomes and prevalence of post-operative colic is currently being investigated.

DVM Student / Clinical Sciences

29. Prevalence of FeLV proviral DNA in samples from client owned cats in a university blood bank.

Rachael Isdale, Kristine Kofron, Melissa Brewer, Chirs Luttenegger, Michael Lappin

Feline leukemia virus (FeLV) is a retrovirus that can cause many health issues in cats including anemia, other cytopenias, lymphoma, leukemias, and immunodeficiency. FeLV does not infect people but can be passed among cats by saliva, feces, milk, and blood transfusion. The Colorado State University Veterinary Teaching Hospital maintains a blood bank to provide blood products to patients in need. The feline blood donor program is populated by cats owned by clients, veterinary students, and staff. Owners are requested to have cats used as a blood donor to live indoors to lessen exposure to infectious disease agents. If the blood donor cat lives with other cats, those cats are also requested to be housed indoors. With 87 feline units drawn from 32 active donor cats in 2021, it important to know that the blood being transfused is free of any potential harmful pathogens that could be transferred to the recipient. The American College of Veterinary Internal Medicine maintains blood donor screening guidelines for blood borne pathogens and in general, the feline blood bank follows the optimal testing protocols which includes screening all cats for FeLV antigen and feline immunodeficiency virus antibodies. There have been a few reports of FeLV antigen negative cats occasionally having enough FeLV proviral DNA in blood to infect other cats. To date, the feline blood donor program has not included this PCR assay in our general screening program. The purpose of this study is to apply a newly titrated FeLV proviral DNA PCR assay (Antech Diagnostics) to banked DNA from past and present feline donors. All samples tested were negative for both FeLV P27 antigen and for proviral DNA by PCR assay. The results suggest that use of indoor cats in blood donor programs lessens the risk of FeLV carriage and transmission to recipients.

Staff / Clinical Sciences

30. Spatial transcriptomics illuminates pathways correlated to immune control of *Mycobacterium tuberculosis* infection.

Connor R King, Sarah K Cooper, Hadley E Gary, Burton Karger, Amy Fox, Taru S Dutt, Marcela Henao-Tamayo, and Brendan K Podell

Infection with *Mycobacterium tuberculosis* (*Mtb*) generates a chronic inflammatory response leading to formation of organized structures referred to as a granuloma. While immune cell composition and overall inflammatory profiles of granulomas have been studied extensively, our ability to identify cellular responses that successfully constrain the *Mtb* organism have been limited by a lack of experimental tools to evaluate both host and pathogen parameters without disrupting the granuloma microenvironment. Spatial transcriptomics is a novel technique that allows for the evaluation of the organization within the granuloma. To investigate these structures in mice, gene expression matrices were obtained from a BCG vaccinated and an unvaccinated mouse infected with *Mtb*. The granulomas were then assembled into matrix subsets for analysis. The gene expression in these granulomas was compared using Gene Set Enrichment Analysis (GSEA) to discern large scale pathways that are upregulated and downregulated in the presence of BCG vaccination. Patterns of gene expression were subsequently analyzed with the R package CoSTA to illustrate spatial relationships of genes. Finally, the R package SPOTlight was used to deconvolute smaller areas within the granuloma to discern the immune cell composition. All of this information unveiled phenotypic differences in granulomas that are correlated to an improved immune response to *Mtb*. To date, GSEA has revealed that the IL-2 signaling pathway is upregulated in the BCG vaccinated mouse while scavenger receptor mediated uptake of ligands, an innate immune system process, is downregulated. These pathways suggest that the improved outcome of infection in BCG vaccinated animals may be due to an improved ability to sustain a lymphocyte-based immune response.

Undergraduate Student / Microbiology, Immunology and Pathology

31. Differences in CD146 expression between neonatal and adult chondrocytes are not reflected in microRNA expression.

Caroline E Kuldell, Bethany E Liebig, Gerrit J Bouma, and John D Kisiday

Neonatal and adult connective tissues differ in their propensity for rapid, scar-free healing. To investigate this phenomenon, we evaluated the propensity of neonatal and adult equine chondrocytes to adopt mesenchymal stromal cell (MSC) properties *in vitro*, given that MSCs possess anti-inflammatory and immunomodulatory functions that promote connective tissue repair. Because chondrocytes have been shown to acquire MSC properties with ex vivo expansion, we followed markers for MSC properties as a function of population doublings (PDs). Previous work from our lab indicted that chondrocytes can express CD146, a cell-surface protein that has been positively associated with the therapeutic potency of MSCs. For adult chondrocytes, flow cytometry indicated that CD146 expression is low (<12% positive) through 19 PDs, and then increases to ~40% positive with subsequent expansion. In the current study, neonatal chondrocytes were ~90% positive for CD146 with as few as 5 PDs. These data suggest the potential that neonatal chondrocytes can rapidly and robustly adopt an MSC-like phenotype. Next, using PCR we investigated expression of microRNAs (miRs) as an indicator of phenotype, with bone marrow MSCs used as a reference. We focused on 10 miRs that are highly expressed by MSCs, although 9 of the selected miRs were generally similar between adult chondrocytes and MSCs, independent of the extent of chondrocyte proliferation. However, one candidate, miR-145, was approximately 400-fold lower in adult primary chondrocytes compared to bone marrow MSCs, and increased with time in culture to ~20-fold lower than MSCs by 33 PDs. In neonatal chondrocytes, miR-145 expression was ~100-fold lower than MSCs through 5 PDs but increased in the same manner as adult chondrocytes with additional PDs. Therefore, the miR analysis does not support the trend indicated by CD146 expression that neonatal chondrocytes adopt MSC properties more rapidly than adult chondrocytes.

DVM Student / Clinical Sciences

32. Closing the Nuclear Fuel Cycle: Minor Actinide Separations.

Samantha A Labb and Ralf Sudowe

To meet the increasing demand for clean and reliable energy, the production of electricity through nuclear energy is an integral element to meet the baseload needs for the future. As a side effect, there will be an increase in the nuclear waste inventory and, with no long-term storage options, waste management solutions need to be developed. Partitioning and Transmutation (P&T) of spent nuclear fuel is a rational approach to the challenge of reducing the volume and radiotoxicity of high-level waste. A major technological challenge for this option is the ability to separate the minor actinides, americium and curium, from one another. Thus, finding ways to efficiently separate these radionuclides is crucial in helping overcome resistance to nuclear power. The significant scientific challenge in achieving these separations is due to the nearly identical chemical behavior of americium and curium. These minor actinides have predominant trivalent oxidation states, similar ionic radii, and similar ionic bonding in complexes. However, while curium appears only in the trivalent oxidation state in solution, americium can exist in higher oxidation states provided a strong oxidizing agent is present. Thus, this project focuses on the exploitation of this difference in redox chemistry to achieve an efficient separation of americium from curium. The oxidizing agent, sodium bismuthate, has been shown to efficiently oxidize americium in extraction chromatographic systems giving high separation factors. This method, however, suffers from poor adsorption capacity and flow rate properties, gradual dissolution of the material during the separations, and gas production in nitric acid. As a result of our previous findings, the use of a novel extraction chromatographic resin developed in collaboration with TrisKem Inc. that incorporates sodium bismuthate into polyacrylonitrile as in the commercially available MnO2-PAN resin will be evaluated and characterized for its ability to achieve these separations.

PhD Student / Environmental and Radiological Health Sciences

33. Safety and analgesia of liposomal bupivacaine administered intra-abdominally and peri-incisionally for laparoscopic ovariectomy in mares.

Blaine Larson, Dean A Hendrickson, Gregg M Griffenhagen, Luke Bass, Mana Okudaira, Lynn M Pezzanite

Abdominal surgery is commonly performed in horses, either for emergency exploratory celiotomy or elective procedures such as ovariectomy. Current options for postoperative pain management (e.g. nonsteroidal anti-inflammatories, opioids) can be associated with potentially life-threatening side effects. Liposomal local anesthetic solutions may provide extended duration analgesia postoperatively but have not been assessed for safety or efficacy following intra-abdominal administration in any species. The objectives of this study were to evaluate two doses of 1.33% liposomal bupivacaine (20 or 40mL) compared to bupivacaine HCL (BHCl) for analgesia following laparoscopic ovariectomy in mares. Fifteen healthy mares were enrolled. Horses were restrained in stocks and administered α-2 agonist sedation for bilateral paralumbar fossa ovariectomies as previously described. All horses received 30mL 0.75% bupivacaine to pre-operatively block the port sites. Horses then were administered either 5 or 10mL liposomal bupivacaine volume expanded to 20ml (LB20 and 40 respectively, n=6/group) or 20mL 0.75% BHCl (n = 3) in each mesovarium prior to ovary removal. Skin incisions were anesthetized with either 10 or 20mL LB diluted to 40mL total (LB20 and 40, n=6/group) in the treatment groups. Horses were monitored by physical examination and pain scoring (Bussieres Composite Pain Scale, Horse Grimace Scale) for 72h. Abdominocentesis for fluid analysis was performed at 72h. Analgesia achieved with all treatment protocols allowed completion of procedures. Transient postoperative complications occurred in 3 mares (skin suture dehiscence (n=2), mild colic (n=1)). No complications resulting from LB administration were appreciated. Peritoneal fluid analyses didn't differ from those previously reported following ovariectomy procedures. Study limitations included small patient sample size and lack of follow-up past 72h or histopathology. In summary, bilateral standing laparoscopic ovariectomies were successfully completed and analgesia considered adequate for 72h with both LB doses administered. Further evaluation of extended duration local anesthetics intra-abdominally for improved postoperative pain management is warranted.

DVM Student / Clinical Sciences

34. Co-activation of nicotinic acetylcholine receptors leads to improvement of brain rhythms and memory in AD.

Rahmi Lee and Seonil Kim

Different subtypes of GABAergic inhibitory interneurons produce hippocampal oscillations where reduced activity in these interneurons is linked to lower oscillatory activity and memory loss in AD. In the early stages of AD, beta-amyloid peptide (Aβ) is linked to decreased hippocampal oscillations due to decreased GABAergic inhibition, resulting in cognitive impairment, the mechanism however is unknown. A prominent AD pathology in the human brain is the loss of cholinergic neurons and nicotinic acetylcholine receptor (nAChR) expression. Our findings show that co-activating the subtypes α7- and α4β2-nAChRs *in vivo* improves memory in an AD mouse model. We hypothesize that Aβ reduces hippocampal GABAergic activity by selectively inhibiting α7- and α4β2-nAChRs, resulting in hippocampal oscillation disruption and memory loss in AD, and that selective co-activation reverses the Aβ-induced pathological effects. The AD mouse model, 5XFAD transgenic mice, with wild type (WT) littermates treated intraperitoneally with α7- and α4β2-nAChR agonists 1 μM PNU-282987 and 2 μM RJR-2403 Oxalate respectively at concentrations of 5mg/ml for 7 days. We compare both mice models with same volume of 0.9% saline as control. We performed stereotaxic surgery to insert electrodes into CA1 of hippocampus to measure local field potential of theta and gamma oscillations. At control conditions we found that 5XFAD and WT mice had similar power spectrum density (PSD). After consolidation there was definite decrease in PSD for 5XFAD mice. Then we found that with co-stimulation of nAChRs we can rescue the PSD. We also performed fear conditioning to see if memory consolidation increases with dual injection of the agonists. We observed that with control conditions 5XFAD has clear deficit in contextual memory which is then successfully rescued by co-activation.

Graduate Student / Biomedical Sciences

35. Ocular penetration of oral acetaminophen in the horse.

Sera Lee, Jacky Peraza, Hannah M Terhaar, Rachel C Hector, and Kathryn L Wotman

Acetaminophen has been increasingly used as an oral analgesic and anti-inflammatory in horses and may represent a safer drug choice than traditional non-steroidal anti-inflammatory drugs due to its lack of renal and gastrointestinal side effects. This study aimed to determine the ocular penetration of orally administered acetaminophen in the healthy equine eye. Six clinically healthy horses as determined by physical examination, complete blood count and serum chemistry, and full ophthalmic examination were used: three mares and three geldings, aged 11 ± 4 years, weighing 568 ± 65 kg (mean ± standard deviation[s.d.]). Horses were administered 20 mg/kg acetaminophen orally every 12 hours for a total of six doses. Commercial acetaminophen tablets were crushed and mixed with sweet feed and corn syrup. Physical examinations (heart rate, respiratory rate, rectal temperature, capillary refill time, mucous membrane color, and gastrointestinal motility by auscultation of borborygmi) were performed before each dosing. One hour after the last dose of acetaminophen, horses were sedated and simultaneous serum and aqueous humor samples (from a randomly selected eye) were collected. Samples were analyzed for acetaminophen concentration. Acetaminophen was detected in both serum and aqueous humor; the aqueous humor:serum acetaminophen ratio was 44.9 ± 15.9% (mean ± s.d.). Physical exam parameters were unchanged from baseline throughout the dosing period. Acetaminophen readily penetrates the aqueous humor of horses with normal ophthalmic exams, and is well tolerated over multiple doses. As such, it may be a safe and effective drug to treat inflammatory ocular conditions in horses.

DVM Student / Clinical Sciences

36. Altering the mycobacterial cell envelope structure differentially affects cell surface properties in *Mycobacterium abscessus* – implications for host adaptation.

Elena Lian, Juan M Belardinelli, Kavita De, Shiva K Angala, and Mary Jackson

Mycobacterium abscessus is an environmental bacterium recently recognized as an emerging pathogen. With dominant clones of the bacterium circulating worldwide in individuals with cystic fibrosis (CF), the bacterium's transition to an emerging pathogen highlights the adaptation of *M. abscessus* to a human host environment. One such adaptation relates to the mycobacterial cell envelope, where the *ubiA* gene is mutated at a higher rate than by chance in *M. abscessus* isolates from chronically infected CF patients. The UbiA enzyme initiates the synthesis of the only known arabinose donor required for synthesizing two major mycobacterial cell envelope polysaccharides integral in envelope integrity and permeability, arabinogalactan and lipoarabinomannan. Since bacteria interface with their surrounding environment through the cell envelope, we hypothesize the *ubiA* mutations affect the structure and surface properties of the *M. abscessus* cell envelope, consequently altering infection dynamics and outcomes in the bacterium's favor. Four isogenic strains expressing the clinically relevant *ubiA* mutations were generated using *M. abscessus* subspecies abscessus ATCC 19977, a strain genetically similar to one of the dominant clones circulating in the CF population worldwide. The functional consequences of these *ubiA* mutations were evaluated by identifying any differences in abundance and/or structure of the two major cell envelope polysaccharides, growth profile, or cell surface properties. Current results indicate the *ubiA* mutations affect the abundances of arabinogalactan and lipoarabinomannan, but that these differences differentially impact phenotypic outcome. Furthermore, the extent of biochemical and phenotypic differences depends on the location of the mutation in UbiA. These results demonstrate the clinically relevant *ubiA* mutations exert a functional consequence, and *ubiA* is likely under evolutionary pressure during infection to adapt and be more fit for a human host environment.

Graduate Student / Microbiology, Immunology and Pathology

37. Development of a novel vaccine for Feline Enteric Coronavirus using recombinant *Lactobacillus acidophilus*.

Mikaela E. Linch, Benjamin E. Curtis, and Gregg A. Dean

Coronaviruses are present throughout the world and affect a wide variety of species. In cats, feline infectious peritonitis virus (FIP) has a nearly 100% mortality rate and to date no effective vaccine has been produced. Because FIP emerges when the common and innocuous enteric virus, feline enteric coronavirus (FECV), mutates within an individual, we hypothesize that vaccination against FECV will protect cats from FIP emergence. This a novel approach which we believe will be integral in combating this devastating disease. FECV enters the body through the mucosa of the intestines. Because of this, an orally delivered vaccine which can stimulate the mucosal immune system will be more effective at directly neutralizing the virus. The probiotic gram-positive lactic acid bacteria, *Lactobacillus acidophilus*, is perfectly suited for traversing the harsh environment of the gastrointestinal tract and delivering vaccine epitopes directly to the mucosal immune system. Using common molecular techniques, we have engineered *Lactobacillus acidophilus* to display FECV epitopes which we have identified as essential to viral biding and entry with host cells as well as replication and construction of the virion. Polymerase chain reaction (PCR) was used to construct plasmids carrying *Lactobacillus acidophilus* genes, which were modified with our target epitope sequences and confirmed by PCR and Sanger sequencing. The sequences were integrated into *Lactobacillus acidophilus* using double crossover homologous recombination. Peptide expression was confirmed by flow cytometry for surface expressed epitopes, and cytoplasmic epitopes were confirmed by mass spectrometry and western blotting. We have successfully integrated the desired viral epitopes into *Lactobacillus acidophilus* and have begun a vaccine trial in young, weaned kittens. The Morris Animal Foundation is funding this study.

DVM Student / Microbiology, Immunology and Pathology

38. Molecular Characterization of Giardia intestinalis isolates in Dogs from a Rescue Shelter in Northern Colorado.

Patricia Franco, Valeria Scorza, Hailey Davis, Rachel Isdale, Katie Jones, Michael R. Lappin

Giardia intestinalis is a common gastrointestinal parasite in humans and animals worldwide. Dogs can harbor strains of *G. intestinalis* that are dog-specific (assemblages C and D), and other strains that are shared with other animals and humans and are considered potentially zoonotic (assemblages A and B). *G. intestinalis* comprises eight subtypes or assemblages which have been categorized based on genetic differences. We aimed to genotype *G. intestinalis* isolates on fecal samples from dogs housed in a rescue shelter in northern Colorado experiencing acute diarrhea. Fecal samples were classified as normal or diarrheal based on a standardized fecal scoring system. Samples that classified as diarrheal were tested by a commercial ELISA test, centrifugal flotation, and a commercial immunofluorescence assay (FA). DNA from *G. intestinalis* positive samples was extracted (2-3 g), and published PCR assays and the sequencing of the amplified genes beta-giardin (bg), glutamate dehydrogenase (*gdh*), and triose phosphate isomerase (*tpi*) were performed. The assemblage of each sample was determined based on the genotyping results of one or more genes. Of the 42 fecal samples, 17 (40.5%) were positive for *G. intestinalis* by at least one of the tests used. Genotyping results showed that only dog-specific *G. intestinalis* assemblages C and D were detected in the samples, with assemblage C being the most prevalent. The bg primers showed the highest amplification rate, followed by *tpi* and *gdh* primers. Dogs in this study harbored dog-specific assemblages C and D. Therefore, our findings, suggest a low risk of zoonotic transmission of *G. intestinalis* from dogs to people, since assemblages A and B were detected.

This study was funded by the Center for Companion Animal Studies.

Staff / Clinical Sciences

39. Enrichment media improves detection of *Streptococcus equi* subsp. *equi* in environmental screening samples.

Blaire C MacNeill and Joshua B Daniels

Strangles is a global, highly infectious, and economically significant equine upper respiratory tract disease caused by *Streptococcus equi* subsp. *equi* (*S. equi*). At Colorado State University (CSU), environmental samples are routinely collected and tested for the presence of *S. equi*, amongst other pathogenic bacteria, for the purposes of both reducing incidences of infectious diseases and improving biosecurity. Currently, definitive diagnosis of the organism is made by culture or polymerase chain reaction (PCR) testing; however, environmental surveillance of the organism has proven challenging, as environmental bacterial species out compete the *S. equi* and reduce the sensitivity of both culture and PCR. To overcome this challenge, samples were placed into an enrichment media, Todd Hewitt Broth with 8 µg/ml amikacin sulfate, and incubated for 24 hours before being plated to Columbia CNA (colistin, nalidixic acid) agar and incubated at 35°C for an additional 24 hours. Additionally, aliquots of the samples in the enrichment media were submitted for PCR analysis. Using Todd Hewitt Broth with 8 µg/ml amikacin sulfate increased the detection limit of *S. equi* in culture by three orders of magnitude. Using this method may also increase the sensitivity of PCR testing, however further validation is needed. Culture is a low cost, widely accessible method for the isolation and identification of pathogenic bacteria. Use of this selective enrichment media will help to increase sensitivity of tests, leading to improved detection of *S. equi* in environmental samples tested at CSU.

DVM Student / Microbiology, Immunology and Pathology

40. Defining specialized M1 phenotypes in *Mycobacterium tuberculosis* infection.

Pablo Maldonado and Marcela Henao-Tamayo

Mycobacterium tuberculosis (*Mtb*) is the infectious bacterium that causes pulmonary Tuberculosis (TB) which is still of major concern globally, killing approximately 2 million people per year. TB progression is largely combated by macrophages, innate immune cells, during initial infection. Macrophage phenotypes exist on a spectrum of pro-inflammatory and anti-inflammatory responses, M1 or M2 respectively. This nomenclature also describes the macrophage's ability to kill *Mtb* and other pathogens (M1). The extent of M1 specialization remains largely unexplored as subcategorization has focused on M2 subtypes which support specialized functions in homeostasis. Our current understanding of M1 does not assign it distinct properties as we do with M2, rather M1 falls into a black box of antimicrobial activity. Indeed, we have yet to determine if macrophages can phenotypically differentiate into specialized M1 subtypes in response to specific pathogens. Lack of M1 characterization is due, in part, to the methodologies available to evaluate M1 polarization which traditionally focus on evaluating M1 cell surface markers, gene expression, or metabolomics, but fail to comprehensively connect these findings to macrophage specialization against pathogens such as *Mycobacterium tuberculosis*. A comprehensive M1 profile specialized for pathogen neutralization is therefore lacking. In this study, we begin to understand phenotypic differences in the M1 phenotype of tuberculosis TB resistant and TB susceptible mice.

Graduate Student / Microbiology, Immunology and Pathology

41. FIV quick assay: a novel technique for diagnosis of feline immunodeficiency virus.

Clara CP Mankowski, Molly West, Jennifer L Malmberg, Mary Nehring, and Sue VandeWoude

Feline immunodeficiency virus (FIV) infects between 1-10% of domestic cats in North America. While sensitive diagnostic tests for FIV exist, these analyses cannot distinguish vaccination from infection, leading to up to 20% false positive diagnosis, and do not determine magnitude of FIV replication. Due to high nucleotide variation between FIV strains, which may differ as much as 20%, standard PCR tests have false negative results ranging from 10-100%. Few full-length domestic cat FIV (FIV_{fca}) genomes have been analyzed, leading to a lack of understanding genomic variation related to disease severity and geographic distribution. We used PrimalScheme to design a multiplex PCR assay using six reference genomes from three subtypes. This resulted in identification of 10 primer pairs across all major FIV genes that targeted the most highly conserved genomic regions. Multiplex PCR using these primers was optimized using DNA samples from cats experimentally infected with three molecularly cloned FIV strains. We tested an optimized protocol in a blinded fashion on 21 field isolate samples (7 negative and 14 positive) previously diagnosed with traditional PCR . Twelve FIV-positive seven FIV-negative were in agreement with prior testing. One sample was identified as negative that had previously tested positive, and one sample tested positive that had previously been diagnosed negative. All samples were processed on a MinION sequencer to verify FIV diagnosis, which revealed this assay detected FIV in a sample previously diagnosed negative by traditional PCR. The use of this multiplex PCR promises superior sensitivity to current diagnostic methods for FIV and will ultimately allow characterization of isolate strain identity. This PCR could also prime full-genome sequencing of additional FIV_{fca} genomes. Future work will develop a pipeline to analyze positive or negative sequences, align sequenced isolates with known genomes, and develop strategies to fully sequence individual genomes.

Staff / Microbiology, Immunology and Pathology

42. Rabacfosadine (TANOVEA) for the Treatment of Relapsed Multicentric Canine Lymphoma.

Elise Martens, Craig A Clifford, Kristen M Weishaar, Gerald S Post, Corey F Saba, David M Vail, Mary K Klein, Brenda S Phillips, Robert B Rebhun, Mona S Rosenberg, Kaitlin M Curran, Timothy M Fan, Philip J Bergman, Douglas H Thamm

Introduction: The aim of this study was to evaluate the efficacy and adverse event (AE) profile of rabacfosadine for the treatment of relapsed multicentric canine lymphoma. Previous studies evaluating rabacfosadine for relapsed lymphoma have assessed selected patient populations, various doses or with additional treatment. Therefore, there is a need for data evaluating a larger number of dogs receiving standard doses of single-agent rabacfosadine in the relapse setting. Methods: This was a single arm, open-label, multi-institutional prospective trial. Dogs received rabacfosadine at 1.0 mg/kg IV every 21 days for up to 5 treatments. Response was assessed via VCOG-CTCAE criteria, and the progression free interval (PFI) was calculated by the Kaplan-Meier method. The effect of variables on PFI and overall response rate (ORR) was evaluated. Adverse events (AEs) were summarized. Results: 187 dogs were evaluated. Most dogs were heavily pre-treated (66% received ≥2 previous protocols). The ORR was 48% (20% CR, 28% PR). Immunophenotype, substage and degree of pre-treatment were significantly associated with PFI. Responders (CR or PR) had a significantly longer median PFI than non-responders (126 days and 63 days for CR and PR, respectively, versus 21 days for NR). Immunophenotype and degree of pre-treatment were significantly associated with response (ORR=65% for dogs with B cell lymphoma at first relapse). AEs were similar to those reported in other studies. Conclusions: These data confirm the efficacy of rabacfosadine in the relapse setting, and affirm improved efficacy in less heavily pre-treated patients with B cell disease.

Resident / Clinical Sciences

43. WHO Let The Dogs Out: How Virtual Animal Anatomy Facilitated a Successful Transition to Online Instruction and Supported Student Learning During the Coronavirus Pandemic.

Jason F Martin, Olivia R Arnold, Andrea Linton, Jay D Jones, Andrew C Garrett, Damon W Mango, Katie A Juarez, Gene Gloeckner, and Christianne Magee

Anatomy faculty with cadaver-based laboratory courses were presented with a significant challenge in March 2020 to create equivalent learning experiences without cadaveric access. The undergraduate domestic animal anatomy course at Colorado State University was halfway into a 16-week semester when COVID-19 lockdown orders and the transition to remote instruction began. The new course curriculum was critically evaluated using student surveys and course outcome data. Most students (92.5%) agreed that the transition to online learning was a success; however, students who valued face-to-face lectures prior to March were less likely to perceive the transition as a success. Qualitative and quantitative analyses of survey results suggest that the resources perceived as most helpful for the transition to online learning were not the same as those that helped facilitate animal anatomy learning. Most students (92.5%) agreed that the Virtual Animal Anatomy (VAA) helped them learn anatomy, and 82.2% indicated that the VAA was a valuable resource following the transition to online learning. Additional resources associated with transition success included course instructors, weekly quizzes, written descriptions of anatomical structures, and open laboratory sessions. In contrast, those resources associated with facilitating learning included guided quizzes and asynchronous lecture recordings. These findings suggest that the VAA can support online anatomy learning when used in conjunction with other best practices for online teaching.

Graduate Student / Biomedical Sciences

44. Fecal bacterial microbiota and insights into antibiotic-induced perturbation of the microbiome in domestic ferrets (*Mustela putorius furo*).

Tiera S McAdam, Kaela K Amundson, Mike J Wilkins, and Barb A Wolfe

Research shows that the gastrointestinal (GI) microbiome plays an important role in the immune response to pathogens. Bacteria-host relationships have been shown to take part in cell-mediated immunity and promote differentiation of T-helper cells (Th17) which produce IL-17, a potent pro-inflammatory cytokine. Domestic ferrets have been shown to harbor high numbers of *Clostridia*-related species in their gut microbiome which is known to play a large role in cell-mediated immunity. Antibiotics are used extensively to treat a wide variety of GI bacterial infections. These antibiotics are typically broad-spectrum antibiotics which have been shown to alter the commensal microbiome in animals. Based on previous studies which show that use of broad-spectrum antibiotics can alter the gut microbial community in minks, we hypothesize that antibiotic use in domestic ferrets (*Mustela putorius furo*) will significantly alter the gut microbiota, leading to changes in immune response to pathogens. To address this hypothesis, we performed metagenomic analyses to determine microbial richness and functional profiling of microbial genes. Microbial diversity in all samples showed a high relative abundance of *Firmicutes* with *Clostridia*-like species making nearly 50% of the diversity. The individuals with no history of antibiotic use show a relatively higher level of microbial diversity than those treated with antibiotics. With the knowledge of how certain commensal bacterial species contribute to the immune response towards pathogens, this will give us insight into how the immune response may be affected due to perturbations of the gut microbiome.

DVM Student / Clinical Sciences

45. Ambient temperatures experienced by free-ranging giraffe across sub-Saharan Africa.

Malea R McGimsey, Matthew S Johnston, Michael B Brown, and Liza Dadone

As the health and wellbeing of animals living under human care continues to be scrutinized, the care standards implemented must be intentional and informed by current knowledge. Giraffe are an iconic staple at many zoos throughout North America and Europe, covering a broad range of ambient temperatures. However, thorough investigation into their natural lives is limited, and no studies have been done outlining the actual temperatures within which wild giraffe are living. Thus, it is unclear which temperature ranges should be considered acceptable. In this study, data transmitted by GPS units affixed to over 200 free-ranging giraffes in sub-Saharan Africa was analyzed to characterize the ambient temperature ranges they experienced. It was found that the lowest temperature experienced for all giraffes was -0.5ºC, and the highest was 50ºC. Grouped by species, the temperature ranges experienced within two standard deviations from the mean were 25.3-33.9ºC, 19.4-33.5ºC, 23.1-32.8ºC, and 23.0-30.0ºC, for the species *Giraffa camelopardalis, G. giraffa, G. reticulata*, and *G. tippelskirchi*, respectively. This data can now be used to inform the management of captive giraffe. In knowing what temperatures are experienced by giraffe in their natural lives, we can make informed decisions about best practices and care standards for facilities with giraffe under human care.

DVM Student / Clinical Sciences

46. Assessing northern sea otter (Enhydra lutris kenyoni) immune function in Alaska.

Malea R McGimsey, Tuula E Hollmen, Katrina L Counihan

Southern sea otters (Enhydra lutris nereis) as well as the southwest stock of northern sea otters (E. l. kenyoni) are listed as threatened under the Endangered Species Act. These animals are susceptible to a number of infectious diseases and harmful toxicants which could have damaging effects on their populations, yet current knowledge is limited regarding the immune system that defends them from adverse effects. While some immune system analyses have been done in southern sea otters, to our knowledge no studies have yet been done investigating immune health and function in northern sea otters. The primary goal of this study is to assess lymphocyte function in northern sea otters from Prince William Sound, Alaska. Lymphocytes were isolated from blood collected from 60 free-ranging otters. Function will be evaluated through mitogen induced proliferation of T and B cells, using concanavalin A and lipopolysaccharide, respectively. Dependent on available field data, individual and group comparisons can be made such as age and gender differences in immune function. Prince William Sound was the site of the 1989 Exxon Valdez oil spill, which impacted the sea otter population and the ecosystem as a whole. Investigating sea otter lymphocyte function will give insight as to the health status of this population and the potential risk posed by pathogens and toxicants

DVM Student / University of Alaska, Fairbanks

47. Autism-associated δ-catenin G34S mutation promotes GSK3β-mediated premature degradation and social deficits.

Hadassah Mendez-Vazquez, Kaila Nip, Madeleine C Moseley, Matheus Sathler, Rosaline A Danzman, Jessica P Roberts, Libby Koch, Seonil Kim

Social impairment is core symptom in several mental disorders, including autism spectrum disorder (ASD), depression, and schizophrenia. However, the physiological, cellular, and molecular factors underlying social behavior are poorly understood. In humans, mutations in the δ-catenin gene are linked to severely impacted ASD patients from multiple families. Mice with disruptions in δ-catenin gene show social deficits, indicating δ-catenin is an important gene for social behavior. δ-catenin is a post synaptic scaffolding protein and is important for AMPA GluA2 subunit localization through the δ-catenin-n-cadherin-GRIP/APB complex. A glycine to serine loss-of-function mutation at residue 34 (G34S) in the n-terminal region of δ-catenin has been identified in humans as an ASD-associated missense mutation. The G34S mutation, according to the Group-based Prediction System, is an added target for GSK3-mediated phosphorylation. Transfected human neuroblastoma cells expressing WT and mutant δ-catenin were found to have significantly lower G34S δ-catenin levels. Chemical and genetic inhibition of GSK3β resulted in a significant increase in G34S δ-catenin levels. A δ-catenin G34S knock-in mouse model was used to investigate the effects of G34S δ-catenin loss-of-function mutation on cortical and hippocampal levels of δ-catenin, GluA1 and GluA2, and we found a significant decrease in all three proteins in the cortex, but not the hippocampus. Additionally, we used the three-chamber sociability test in 3-month-old male and female δ-catenin G34S KI mice to investigate disruptions in sociability and social novelty. Males showed normal sociability, but females were found to have impaired sociability as compared to the WT. Female G34S mice had normal social novelty preference while male G34S mice showed atypical social novelty preference as compared to the WT. Thus, we believe the G34S δ-catenin mutation induces a loss-of-function via GSK3β-mediated δ-catenin degradation, and the G34S δ-catenin mutation causes decreased cortical δ-catenin levels, and impaired sociability and social novelty with sex specific deficits.

Graduate Student / Biomedical Sciences

48. Pharmacokinetics of oral ondansetron in dogs admitted to a tertiary referral hospital, a pilot study.

Angela Molli, Brooke Gallagher, Daniel Gustafson, Kristin Zersen, and Sarah Shropshire

Gastrointestinal upset, including vomiting, is a very common presenting complaint in canine patients. Consequently, anti-nausea and anti-emetic drugs are frequently prescribed by veterinarians. Ondansetron, a 5-HT3 receptor antagonist, is often prescribed to treat nausea and vomiting, however there are no studies investigating the optimal oral dose and frequency of administration in clinically nauseous dogs. The current recommended dose range is 0.5–1 mg/kg by mouth (PO) once to three times daily (TID). In addition to establishing an optimal dose and frequency, studies investigating ondansetron's anti-nausea clinical efficacy are needed. Using a previously published nausea scoring system, seven dogs with a nausea score of 2 or greater were enrolled in this pilot study. Each dog was randomly assigned to one of four different groups (0.5 mg/kg PO BID, 0.5 mg/kg PO TID, 1 mg/kg PO BID, 1 mg/kg PO TID) and ondansetron was administered as assigned. A baseline nausea score and blood were collected from each patient upon enrollment and at multiple time points over a 24-hour period. Analysis of serum drug levels in this limited sampling data set, while comparing to a previous pharmacokinetic study of intravenously administered ondansetron, showed a bioavailability of orally administered ondansetron to be approximately 10%. The low oral bioavailability at currently recommended doses requires further research into the pharmacokinetics of oral ondansetron in dogs, as well as the clinical efficacy of the anti-emetic and anti-nausea properties of the drug at these dosages in the clinically nauseous patient. This pilot study has guided a larger oral ondansetron pharmacokinetic and pharmacodynamic study with the hopes of providing answers to these very clinically relevant questions.

DVM Student / Clinical Sciences

49. Longitudinal SARS CoV-2 identification in human stool and associated gut microbiota.

Nicole Natter, Bridget A. Baxter, Maddie Tipton, Kailey Berry, Kristen Otto, Abby Veath, Jason Corwin, Pankaj Trivedi , Emily N. Gallichotte, Emily Fitzmeyer, Michael C. Young, Gregory D, Ebel, August Luc, Jim Huang, Carol Wilusz, Stephanie M LaVergne, Ki

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) has caused the deaths of millions and infected over 200 million since its emergence in December of 2019. Little is known regarding the persistence of SARS-CoV-2 positive detection following acute infection in human stool. A cohort of adults with confirmed SARS-CoV-2 infection were asked to submit stool samples over the course of six months to one year as part of the Northern Colorado COVID-19 Biorepository. Of 139 participants enrolled, 109 adults provided at least one stool sample. There were 63 females and 46 males whereby, 39.4% tested positive at least once in the stool and 14% had persistent positive stool detection by either real-time PCR or ddPCR. At baseline 16 people tested positive with stool by both PCR and ddPCR, 2 people tested positive in PCR only, and 19 people tested positive in ddPCR only. During longitudinal follow up over six months we had 2 people test positive by both PCR and ddPCR, 6 people test positive in PCR only, and 13 people test positive in ddPCR only. We conclude that the intestinal colonization of virus merits further investigations with respect to changes to microbiota and persistent immune activation, as well as development of post-acute sequelae, namely long-COVID symptoms. A relationship between BMI and microbiota composition was identified by 16S sequencing that will be further interrogated for differences with persistent virus detection in stool. In this study, we found that adults who are overweight and obese had a higher PCR detection rate than those who were normal weight. Additionally, females were more likely to have PCR persistence in their stool. Future studies are needed to evaluate differences between variants with respect to virus detection and intestinal involvement during disease and in the months following initial infection.

Undergraduate Student / Environmental and Radiological Health Sciences

50. Intramedullary stents as a device to prevent post-radiation fractures in canine patients with osteosarcoma.

Vivian Ojeda, Ben Gadomski, Erin Estrada, Chloe Brehus, Michael Poland, Quinn Smith, Bernard Séguin

Appendicular osteosarcoma presents in thousands of primarily large breed dogs in the United States with a common treatment being limb amputation. As an alternative, stereotactic radiation therapy (SRT) has become an appealing treatment for a subset of patients. However, estimates from studies show that over 50% of dogs fracture their bone at 11 months post-SRT. Leading to these fractures still resulting in amputation or even euthanasia. In this study, three groups are tested with 6 pairs of left and right humeri in each. The first group served as the control, containing a "non-defective" and "defective" right and left pair. The second tested the effect of cementoplasty alone by testing a "defective" and "defective with cementoplasty" right and left pair. The third group will allow for testing of the stent with cementoplasty by containing a "defective with cementoplasty" and a "defective with cementoplasty and stent" right and left pair. Because torsional forces acting on the afflicted bone are suspected to be a significant cause of these fractures, all humeri specimen were placed within a Mechanical Testing System (MTS) to observe their ability to withstand torsional forces. The model developed where a defect in the diaphysis was created proved to significantly weaken the bone ($p=0.016$), requiring on average 31% of the force to cause a fracture. It is predicted that the placement of the stent with cementoplasty will provide the bone models with the greatest resistance to fracture as compared to the other two groups when tested in torsion by way of the stent transferring the forces from the weakened bone to the cement. The aim of achieving these results will be to provide a basis for implanting these intramedullary stents in live patients with osteosarcoma treated with SRT to prevent bone fracture and promote better quality of life.

DVM Student / Clinical Sciences

51. Temporal comparison of plasma total mercury in Steller sea lions (*Eumetopias jubatus*) of the Aleutian Islands.

Julia Orluk, J. Margaret Castellini, Stephanie Crawford, and Lorrie Rea

Populations of Steller sea lions (*Eumetopias jubatus*) in Alaska experienced a sharp decline in the late 1970's to early 2000's. Hair samples in pups from the Western Aleutian Island populations (WAI) have shown increased total mercury ([THg]) from 2011-2019, which may relate to the poor reproductive success of these areas. No hair samples were available prior to 2011 to study temporal changes, however the capability of new instruments to detect lower limits of mercury have made it possible to use archived plasma samples to assess Steller sea lions sampled prior to 2011. This study incorporates new data from 1991-2007, expanding the temporal analysis of [THg] to further assess the increasing trend in the WAI populations. A Nippon MA3000 analyzer was used to measure [THg] in archived plasma samples. Agattu Island (WAI) has had increasing [THg] in hair samples since 2011, however no significant difference among individual years of plasma samples [THg] was found (Kruskal-Wallis ANOVA H=10.58, p=0.10). When Agattu samples were binned by time periods of 1997-2007, 2011-2013, and 2015-2017, the 1997-2007 time period was found to be significantly lower than the other bins (H=10.28, p=0.0059). Similar increases in plasma [THg] were not found at Ugamak or Bogoslof Islands (Eastern Aleutian Islands) during these same time periods (H=2.57, p=0.46 and H=1.71, p=0.43 respectively). These results suggest changes occurring sometime between 2007 and 2011 have resulted in an increase in the bioaccumulation of mercury in the WAI Steller sea lions, prompting further inquiry as to the nature of such changes and the need for continued monitoring in this region.

DVM Student / University of Alaska, Fairbanks

52. Early Failure of CHOP Protocol Indicates Poor Response to Rescue Protocol in Dogs with Lymphoma.

Ashley Parker, Jenna H Burton, Caitlin M Curran, Amber Wolf-Ringwall, Douglas H Thamm

Introduction: Dogs with lymphoma that fail CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy before completion of their protocol are commonly thought to have a poor long-term outcome; however, no previous studies have evaluated the effect of early CHOP relapse on progression free interval (PFI) or overall survival time (OST) for patients undergoing rescue chemotherapy. The aim of this study was to determine if dogs with multicentric lymphoma that fail CHOP prior to completion have a worse outcome with rescue therapy than dogs that complete CHOP. Methods: Data were collected from 6 previous retrospective or prospective studies in 193 dogs with multicentric lymphoma that received CHOP-based chemotherapy then received either lomustine (CCNU), L-asparaginase and prednisone or rabacfosadine (Tanovea-CA1), with or without prednisone or L-asparaginase. Results: Length of CHOP PFI (progression during versus after completion of CHOP) was significantly associated with PFI and post-relapse OST for both rescue protocols. Patients achieving a complete response to CHOP had a significantly longer PFI for both rescue protocols. Immunophenotype (B vs. T cell) was not significantly associated with response, PFI or OST for L-asparaginase/lomustine but was significantly associated with PFI for rabacfosadine. Conclusion: Dogs with multicentric lymphoma that fail a CHOP protocol prior to completion are likely to have shorter PFI and OST with rescue therapy. Immunophenotype does not significantly affect outcome with L-asparaginase/lomustine but is associated with PFI for rabacfosadine.

Resident / Clinical Sciences

53. Characterization of neuronal fibers, immune cells, and coronavirus in a murine organotypic lung slice.

Brielle H Patlin, Suad Elmegerhi, Hadley Gary, Brendan Podell, Rushika Perera, Stuart A. Tobet

Organotypic lung slice models can be used to examine infection processes ex vivo relative to changes in neuronal fibers and immune responses. Neuronal fibers and cell bodies were compared in lung sections/slices that were fixed immediately versus lungs that were fixed at regular intervals between one- and six-days ex vivo. Neuronal elements were examined using immunofluorescence (IF) for peripherin, vasoactive intestinal peptide, calcitonin gene related peptide, and choline acetyltransferase. These molecules are related to neuronal intermediate filaments, neuropeptides, and neurotransmitters. Beyond characterizing neuronal elements, we also examined cellular immune components that may interact with neurons when infection is triggered ex vivo (e.g., SARS CoV2 in a humanized ACE2 mouse). For this purpose, lung slices were characterized by IF using antibodies directed against Surfactant protein C, Z01, CD3, CD4, CD8, and CD19. These elements were maintained ex vivo for 6 days ex vivo setting the stage for future perturbation studies. Presence of CD19 immunoreactive B cells in lung slices changed upon the addition of the SARS CoV2 virus. While located relatively sparsely in germinal center-like clusters in healthy tissue, these cells changed in location and increased in quantity in infected tissue. This change in immune response was matched by a significant change in surfactant protein C immunoreactivity in the tissue indicating a decline in tissue health. Changes in slice health, immune cell location, and innervation as assessed in an organotypic model can be used to model SARS CoV2 infection.

Undergraduate Student / Biomedical Sciences

54. Use of computed tomography and nuclear scintigraphy for diagnosis and staging of primary anterior uveal osteosarcoma in a client-owned rabbit.

Hannah Patterson, Michala de Linde Henriksen, Hannah M Terhaar, Zachary Dvornicky-Raymond, Christopher Olmo, Douglas H Thamm, Leandro Teixeira, and Miranda Sadar

The purpose of this study was to evaluate if a computed tomography (CT) scan paired with nuclear scintigraphy could be used to determine whether a uveal osteosarcoma (OSA) diagnosed in a rabbit on histopathology was primary or secondary (metastatic). A 4-year-old, female spayed, angora mix rabbit was referred to the Colorado State University Veterinary Teaching Hospital (CSU-VTH) Avian, Exotic, and Zoo Medicine Service for progressive drainage and discomfort of the left eye (OS). An examination performed by CSU-VTH Ophthalmology service revealed a yellow and white vascularized mass taking up 95% of the anterior chamber causing blindness OS. The globe was enucleated and submitted to the Comparative Ocular Pathology Laboratory of Wisconsin for histopathology. Histopathology revealed an anterior uveal OSA and chronic secondary glaucoma, but it was not able to discern between primary intraocular (extraskeletal) and secondary (metastatic) OSA. The rabbit underwent a pre- and post-contrast whole body CT scan paired with nuclear scintigraphy to evaluate for any skeletal or soft tissue abnormalities. The radioactive isotope used was technetium (Tc-99m) oxidronate. Results from the CT scan and nuclear scintigraphy showed no evidence of metastasis or tumor regrowth, suggesting the anterior uveal mass was a primary, intraocular (extraskeletal) OSA. Two hundred and fifty-eight-days following enucleation, the rabbit was comfortable in the right eye and had no appreciable signs of systemic abnormalities according to the owner. This case report describes primary uveal OSA in a rabbit. Computed tomography paired with nuclear scintigraphy has not previously been reported in a client-owned rabbit but was effective in staging uveal OSA in this individual.

DVM Student / Clinical Sciences

55. Assessing the host immune response to *Mycobacterium abscessus* infection in cystic fibrosis.

Camron Pearce, Amanda Walz, Zohaib Ali, Charlotte Avanzi, Mercedes Gonzalez-Juarrero

Mycobacterium abscessus (*Mab*) can cause severe and chronic pulmonary infection in immune susceptible patients. *Mab* has become increasingly prevalent since its first isolation in 1953, and in the last decade, has become a major health concern for patients with cystic fibrosis. The focus of this study is to look at the differences between an immune competent and deficient host during *Mab* infection to uncover new targets for antibiotic therapy. Results have provided an improved *in vitro* cell infection assay that will be used for preparing a single cell suspension from *Mab* infected mice and subsequent imaging and transcript analysis. We will utilize single cell RNA sequencing, flow cytometry, and imaging technologies to help paint a clear picture of the host response to *Mab* pulmonary infection for improving patient outcome.

Graduate Student / Microbiology, Immunology and Pathology

56. Short-term Treatment of Keratoconjunctivitis Sicca (KCS – dry eye) in Three Dogs With Equine Interleukin-1 Receptor Antagonist Protein (IRAP) and its Effect on Clinical Parameters.

Jacky Peraza, Katie Jones, Kathryn Wotman, Jeff Christensen, David Frisbie, and Michala de Linde Henriksen

Interleukin-1 receptor antagonist (IL-1ra) has been shown to have anti-inflammatory effects on keratoconjunctivitis sicca (KCS, dry eye) in humans. This study investigated the short-term clinical effect of topical equine IL-1ra protein (IRAP) in dogs diagnosed with keratoconjunctivitis sicca (KCS). Three systemically healthy research dogs that were diagnosed with KCS and treated with topical hyaluronic acid eye lubrication bilaterally (OptixCare) were included in the study. The study was conducted daily over seven days. Each dog received a complete ophthalmic examination that included tear production measurement (Schirmer tear test (STT), mm/min), slit lamp biomicroscopy and indirect ophthalmoscopy on Day 0, Day 3, and Day 6 at 7am and 7pm. Day 0=topical OptixCare TID OU. Day 1-3=topical equine autologous serum TID OU, Day 4-6=topical equine IRAP TID OU. The clinical parameters that were evaluated in the study were epiphora, blepharospasm, chemosis, and hyperemia. The subjective scoring degree for the clinical parameters were 0=normal-, 1=mild-, 2=moderate-, 3=severe findings. Each dog had an eye with decreased STT ('KCS eyes', mean STT 8.3mm/min) and an eye with normal STT ('normal eyes', mean STT 16.7mm/min) at the initial ophthalmic examination. A one-way ANOVA test did not show statistical differences between STT for the KCS eyes between Day 0, Day 3, Day 6 (total mean=11.6mm/min, p=0.694), or for normal eyes (total mean=16.3mm/min, p=0.269). Subjectively, no clinical differences were found between eyes with KCS and their clinical parameters at 7am and 7pm for Day 0, Day 3, and Day 6. Treatment with short-term topical equine IRAP does not seem to have influence on STT or clinical parameters for KCS in dogs when compared to topical OptixCare or equine autologous serum. Specific binding to canine IL-1 receptors could be involved in the lack of effect of equine IRAP in this study and a future study will involve topical canine IRAP.

Staff / Clinical Sciences

57. Evaluating B7-H3 as a tumor antigen target in canine osteosarcoma for CAR T cell therapy.

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

Chimeric antigen receptor (CAR) T cells target surface antigens of tumor cells to mediate an adaptive immune response. CAR T cell therapy targeting CD19 has shown remarkable clinical success against B cell malignancies. However, human clinical trials of CAR T cell therapy with solid tumors has not shown the same efficacy as shown in preclinical mouse models. One of the challenges in applying CAR T cell therapy to solid tumors is the immune suppressive tumor microenvironment (TME). Canine osteosarcoma (OS) has been well established as a spontaneously occurring immune competent translational model for pediatric OS and would serve has a robust model for CAR T cell therapy that would recapitulate the TME and account for individual tumor antigen expression variability. The costimulatory marker B7-H3 has been showed to be overexpressed in OS and correlated with increased metastasis, poor prognosis, and decreased tumor immune infiltration. Our aim is to develop a protocol to evaluate the prevalence of B7-H3 within formalin fixed paraffin embedded FFPE canine OS and glioma tissue by immunohistochemistry (IHC). This IHC protocol would allow for a reliable high through put screen for B7-H3 on clinical biopsies of canine OS and gliomas, allowing for proper evaluation of the viability of B7-H3 as a tumor antigen target for canine CAR T cell therapy. We established cross reactivity of a human B7-H3 antibody to canine B7-H3 by western blot. Antigen preservation of B7-H3 was evaluated using canine OS cell lines fixed in either 2% paraformaldehyde or formalin and stained by IHC and imaged by confocal microscopy.

DVM Student / Clinical Sciences

58. SFTSV: Characterizing a novel bunyavirus in North American Wildlife.

Maggie Priore, Rachel Maison, Stephanie Porter, Jeff Root, Nicole Nemeth, Angela Bosco-Lauth

Severe Fever with Thrombocytopenia Syndrome Virus (SFTSV) of the order *Bunyavirales*, family *Phenuiviridae*, was isolated from an acutely febrile patient in 2009 in Henan Province, China. Positive cases have since been confirmed in Japan and South Korea. This newly emergent virus is of major human health concern as it is classified as a viral hemorrhagic fever hallmarked by thrombocytopenia, leukocytopenia, and occasional neurological manifestation in humans. Its primary vector, the Asian long-horned tick (*Haemaphysalis longicornis*) has become established in much of the Eastern U.S. and has spread as far west as Arkansas. With establishment of the long-horned tick underway, it is crucial to determine the reservoir host potential and disease pathogenesis of wildlife in the U.S. that may play a role in the viral ecology of SFTSV in order to further our understanding of potential human exposures. Thus far, feral hogs (*Sus scrofa*), striped skunks (*Mephitis mephitis*), raccoons (*Procyon lotor*), and desert cottontail rabbits (*Sylvilagus blaire*) were experimentally inoculated with SFTSV and evaluated for clinical disease and evidence of host competence as measured by virus isolation and seroconversion. Daily sampling (blood, oral and rectal swabs) occurred for eight days post inoculation (DPI). Animals were euthanized and necropsied on either 7 or 28 DPI for serological, virological and histopathological evaluation. With much of the analysis pending, preliminary data indicates that raccoons develop viremia (virus in blood) for several days with evidence of viral dissemination to select lymphoid tissues. This suggests that in the event of an incursion of SFTSV to the U.S., it is possible that a sufficient reservoir host exists to establish an endemic vector-host cycle in wildlife. Future directions include pathogenesis studies based on past completed animal models, vector competency studies, and cross-neutralization studies to determine the serologic diagnostic specificity between SFTSV and similar bunyaviruses.

Graduate Student / Microbiology, Immunology and Pathology

59. Clinical Outcome in Dogs with Appendicular Osteosarcoma Treated with Palliative Radiation Therapy With and Without Bisphosphonates.

Beck Ringdahl-Mayland, Douglas H Thamm, and Tiffany Wormhoudt Martin

Introduction
Hypofractionated radiation therapy (RT) and bisphosphonates are commonly utilized for palliative intent treatment of canine osteosarcoma (OSA). Consensus has not been reached as to whether these treatments should be administered concurrently. The primary objective of this study was to evaluate outcome in dogs treated with hypofractionated RT, with and without the addition of bisphosphonates. A secondary objective was to identify prognostic factors in this population.
Methods
Dogs with presumed and confirmed OSA of the appendicular limb treated with daily hypofractionated RT (8 Gy x 2 fractions) at the Flint Animal Cancer Center between 2010 and 2019 were evaluated retrospectively. Clinical data were abstracted from the medical records, and adjuvant therapies were noted. Outcome was assessed using medical records and electronic follow up.
Results
165 dogs were included. 68 dogs received bisphosphonates as part of their palliative intent treatment. Median survival time from the first RT treatment to death was not significantly different between groups (p=0.758). Only age (>/= 9 years) was found to be prognostic in this population (p=0.031). Factors not associated with survival time included bisphosphonate drug type, timing of bisphosphonate administration, tumor location, weight, breed, sex, time from diagnosis to treatment, concurrent administration of chemotherapy, and amputation.
Conclusions
This study suggests no difference in outcome for dogs treated with and without bisphosphonates in addition to hypofractionated RT. Prospective studies are needed to determine if the addition of bisphosphonates to hypofractionated RT leads to an improved quality of life in dogs undergoing palliative intent treatment for OSA.

Resident / Environmental and Radiological Health Sciences

60. Repurposing β-blockers as anti-cancer immunotherapeutics to target myeloid derived suppressor cells.

Cody Rinker, Dylan Ammons, and Steven Dow

Myeloid derived suppressor cells (MDSCs) are a heterogenous population of immature myeloid cells which arise in response to chronic inflammation, such as that associated with cancer. There are two distinct subsets of MDSCs, polymorphonuclear (PMN) and monocytic (M), both of which correlate with poor outcomes in cancer patients. In human and murine models, β-adrenergic stress has been shown to promote the differentiation and immunosuppressive functions of MDSCs through interactions between catecholamines and β-adrenergic receptors. This study aims to evaluate the potential of β-adrenergic antagonistic drugs (β-blockers) to decrease the differentiation and immunosuppression caused by MDSCs. We hypothesize that the quantity and function of circulating MDSCs in canine cancer patients will correlate with degree of β-adrenergic stress and that treatment with β-blockers will decrease their immunosuppressive capabilities and abundance. To this end, we quantified the MDSC burden in the blood of healthy dogs and dogs with cancer to determine if there was a detectable increase in MDSC populations. We confirmed that canine PBMCs express β1 and β2 adrenergic receptors via flow-cytometry and western blotting, then investigated if β-adrenergic activation enhances MDSC immunosuppressive functions and differentiation. Finally, we assessed whether non-selective or β1-specific β-blockers could reverse the effects of β-adrenergic activation. These studies provide insights into the relationship between β-adrenergic stress, MDSC burden, and cancer immune suppression. Ultimately, the findings from these studies can inform how the β-adrenergic signaling axis could be targeted in the clinic.

DVM Student / Microbiology, Immunology and Pathology

61. The impact of TP53 missense and truncating mutations on protein function and tumor progression in canine osteosarcoma.

Brittney Sanfacon, Rupa Idate, and Dawn L. Duval

The transcription factor P53 is well known for its role in cancer development across many species. P53 mutations have been identified in approximately 80% of canine osteosarcomas (OSA), but recent studies suggest that not all p53 mutations have equal effects on protein function and response to chemotherapy. An exon-6 truncating mutation found in a subset of canine OSAs, known as P53-psi may be unable enter the nucleus and regulate DNA damage responses. Instead, this truncated P53 is directed to the mitochondria where it may alter membrane pore permeability. The goal of this study was to generate expression constructs of the canine P53-psi protein using Wild type (WT)-P53 template DNA and PCR technology, and assess its function when stably expressed in the DH82 P53-null cell line. Stably pools of these cells have been generated and are being evaluated for P53 expression prior to further evaluation of drug sensitivity and mitochondrial function. Another goal of this project was to determine sensitivity in canine OSA cell lines with either WT P53 or various missense P53 mutations to the standard of care treatments, Doxorubicin and Carboplatin. It has been suggested that cancers with P53 mutations have greater sensitivity to DNA damaging chemotherapy and longer disease-free intervals after treatment. Canine OSA cell lines Gracie (WT), HMPOS (R226H) and OS2.4 (I284T) were tested for their sensitivity to both drugs using a resazurin-based bioreductive fluorescent assay to assess cell viability. Initial results indicate that the HMPOS cell line exhibits the most sensitivity to doxorubicin. Future studies will explore the mechanisms that drive this increased sensitivity to DNA damaging chemotherapy.

DVM Student / Clinical Sciences

62. One Health, One Cat at a Time.

Leah K Sauerwein, Danielle M Frey, Michael R Lappin, Gilbert Kersh, Tom Gelatt, Colleen G Duncan

Colorado State University has a rich history of forging bonds with unique communities that are oftentimes underserved areas in the realm of animal healthcare. Following a model similar to the CSU/UAF led Hub Outpost Project, which provides veterinary services to the Yukon-Kuskokwin Delta of southwest Alaska, this pilot study aims to assess the needs and feasibility for a similar clinic on St. Paul Island in the Pribilof Islands of Alaska. This island is part of the Alaska Maritime Wildlife Refuge and to protect this marine ecosystem, dogs are not permitted on the island, so domestic cats are the most common household companion animal. The aims of this project are to 1) review the literature for feline diseases in Alaska that could be relevant to Pribilof feline companions, local wildlife or the public and 2) test archived feline serum samples provided by the Alaska Native Rural Veterinary Organization for evidence of exposure to top candidate diseases identified and 3) seek financial and logistical support for a mobile veterinary clinic to St. Paul Island. Preliminary work suggests there is exposure of cats on St. Paul Island to common feline pathogens, some of which (ex. Toxoplasma gondii and Coxiella burnetti) could impact both people and marine wildlife. This pilot study demonstrates there are likely several ways that a mobile veterinary clinic event could benefit the health of animals, people and the environment of St. Paul Island. Future work involves community discussions, funding and logistical planning.

DVM Student / Microbiology, Immunology and Pathology

63. A retrospective analysis of traumatic brain injury (TBI) in canine trauma patients.

Leah K Sauerwein, Claire D Tucker, Sangeeta Rao, Kelly E Hall

Objective –Describe the canine traumatic brain injury (TBI) population in order to make comparisons to injury patterns in humans and help inform knowledge gaps for future clinical trials.

Design – Retrospective analysis of canine patient data collected from the Colorado State University Veterinary Teaching Hospital and stored in the Veterinary Committee on Trauma (VetCOT) registry between April 2017 and October 2021.

Setting – Veterinary teaching hospital

Animals – Two thousand forty dogs presenting to CSU VTH assessed for trauma within 24 hours and with a recorded Modified Glasgow Coma Scale (MGCS) score. Measurements and Main Results – Primary outcome of the study was presence of head injury and with a secondary outcome as the presence of TBI as assessed by MGCS. 346 dogs had moderate to severe TBI (MGCS <18.) 296 dogs had head injury and a reported MGCS score. The mean MGCS score (rank 3-18) in cases of head trauma is 15.6.Smaller breed dogs were more likely to have head injury than larger breed dogs. When adjusting for weight, toy and small breed dogs have a higher likelihood of developing moderate to severe TBI (MGCS <18) than medium, large, and giant breed dogs. The likelihood of moderate to severe head injury increases with each increase in age. The most common presentation of traumatic injury for patients with head injury was to have both blunt and penetrating injuries (37.1%). Median blood lactate and blood glucose values were significantly higher in subjects with head injuries. The likelihood of death or euthanasia was significantly higher in patients with head injury. 97% of dogs without head injury survived to hospital discharge compared to 85% of dogs with head injury.

Conclusions – Head trauma and associated TBI worsen prognosis for traumatic injury in dogs. Future prospective studies are needed to inform clinical management.

DVM Student / Clinical Sciences

64. Maternal stress alters hypothalamic development.

Julietta A. Sheng, Robert J. Handa, Stuart A. Tobet

Maternal stress increases fetal exposure to glucocorticoids (GC), altering development of the hypothalamus, an important regulator of homeostasis, stress responses, and behavior. Gonadal hormones exert organizational effects during critical periods of development that result in sexually dimorphic development of hypothalamic structure and function. In the current study we found that late gestation exposure to synthetic GC, dexamethasone (DEX) led to long-term reductions in neuropeptide, arginine vasopressin (AVP), expression in the paraventricular nucleus of the hypothalamus (PVN) in a female-biased fashion. We detected this first in neonates and followed it into adulthood. However, prenatal DEX treatment is a partially confounded model of fetal stress. In humans, DEX is given therapeutically to promote lung maturation in preterm fetuses. Ongoing studies are determining the role of DEX in the context of a prenatal environmental stress, e.g., a restricted nutritional stress. These studies will test the interaction between exposure to endogenous GCs due to an environmental stress and excess exogenous GC (i.e., DEX). The central hypothesis is that in utero stressors alter development of PVN structure and function (e.g., neuropeptide roles). Oxytocin (OT), related to AVP by amino acid sequence and co-located chromosomally, will be examined to identify coordinated actions of stressors. Studies will utilize transgenic mice, AVPcre and OTcre to determine the influence of fetal stress on projections of PVN AVP and OT neurons in adulthood using stereotaxic placements of anterograde recombinant adeno-associated viral particles. By examining alterations in PVN neuropeptide neuron development, we will be able to better understand the relation between maternal stressors and adult risk for neuropsychiatric disorders. Funded by ORWH U54-MH118919

Graduate Student / Biomedical Sciences

65. Measuring Systemic Genomic Instability in Yeast.

Joseph A Stewart, Lucas Argueso

In the field of evolution, gradualism is described as the slow and random accumulation of mutations that over time lead to genetic diversification. Although this Darwinian model is well supported and widely accepted, it cannot explain the rapid changes seen in some instances, such as in tumors. Recent reports in various organisms, including from our group using budding yeast, provide evidence for an alternative mode of rapid and non-independent accumulation of chromosomal rearrangements known as punctuated equilibrium caused by systemic genomic instability (SGI). Although SGI can offer an additional explanation to how we obtain mutations over time, we know very little about its causes and duration. We hypothesize several conditions that have the potential to cause SGI, but one we favor the most is noise in gene expression. Given the rapid nature of how these mutations occur, we also hypothesize that SGI happens over a very short timeframe, as short as a single cell cycle. Using a variety of unique and customized techniques for measuring a specific mutation type (loss of heterozygous) in the yeast genome, we have developed a system to measure the rates and timing of SGI as well as the potential influence of noise in gene expression. Investigation surrounding SGI will help us better understand how complex genomes arise over time and more insight into cancer progression.

Graduate Student / Environmental and Radiological Health Sciences

66. Markers of endothelial injury in canine trauma patients: a pilot study.

Jordan Tarbutton, Claire Tucker, Tracy Webb, and Kelly Hall

The endothelial glycocalyx is a complex network of glycoproteins and proteoglycans on the surface of cells lining blood vessels throughout the body. When injury occurs, this glycocalyx surface sheds, releasing molecules such as syndecan-1, heparan sulfate, hyaluronan, and ve-cadherin. The extent of endothelial glycocalyx damage may play a role in the development of systemic inflammatory processes and multiple organ failure, causing long term complications and a worsening prognosis in trauma patients. Additionally, studies suggest the initial fluid resuscitation methods we commonly use today - such as large volume, rapid crystalloid boluses - may cause an increase in glycocalyx shedding and, ultimately, a poorer outcome. The purpose of this pilot study is to determine whether these molecules can be measured in canine trauma patients and utilized as biomarkers of glycocalyx shedding. We hypothesize that in measuring these molecules, we can utilize them as biomarkers of glycocalyx degradation in canine trauma patients. Canine patients presenting to the Colorado State University Urgent Care service for a recent traumatic injury will have these molecules measured at various timepoints after injury. They will be classified into mild, moderate or severe injury based on a validated trauma injury score. This information will inform the design of future studies to compare various resuscitation methods and their effects on the glycocalyx leveraging translational aspects of trauma patient care.

DVM Student / Clinical Sciences

67. Intestinal Model for Parkinson's Disease Development.

Hayley N Templeton, Luke A Schwerdtfeger, Casey P McDermott, Savannah M Rocha, Ronald B Tjalkens, Julie A Moreno, and Stuart A Tobet

The pathogenesis of Parkinson's disease (PD) is incompletely understood, but there is accumulating evidence that suggest pathology can arise in the gut. A hallmark pathology of PD is the accumulation of misfolded α-synuclein (α-syn) proteins in what are known as Lewy bodies. The enteric nervous system (ENS) facilitates bidirectional communication between the brain and the gut, mostly via the vagus nerve. Injection of recombinant α-syn has been shown to induce aggregate formation in the gut that is then transported to the brain via the vagal nerve. While these findings strongly support the hypothesis that central nervous system Lewy body pathology originates in the gut, the underlying mechanisms have yet to be fully understood. A well-known peripheral inducer of α-syn aggregation is the pesticide rotenone which has proven to be an excellent model for reproducing PD-like pathology in mice. The goal of this study is to gain insight into how rotenone contributes to accumulation of toxic α-syn aggregate pathologies in the gut as an intestinal model of PD development. Mice received intraperitoneal injections of rotenone once daily for 14 days. The gut was dissected from the stomach to the colon. Sections at a thickness of 50 µm were prepared from 1 to 3-mm sections of duodenum, jejunum, ileum, and colon cut from the whole intestine. Immunohistochemistry revealed changes in gut morphological integrity, α-syn aggregation, villi morphology, and epithelial cell number and molecular composition. Together, these results implicate the gut as a possible origin of PD pathogenesis.

Graduate Student / Biomedical Sciences

68. Differential transcript expression in compensatory limbs following DMM or sham surgery in male and female mice.

Ariel Timkovich, Lindsey Burton, Kelly Santangelo

Introduction: Post-traumatic osteoarthritis (PTOA) is a debilitating and degenerative condition. Clinical considerations and treatment primarily – and understandably – focus on the injured limb, with relatively little attention on the compensatory leg. Interestingly, mouse models of PTOA have utilized the contralateral limb in a variety of ways, including naïve internal controls, sham surgery internal controls, or bilateral surgery. To date, a comprehensive evaluation of contralateral limb changes following injury has been minimally investigated.

Purpose: We aimed to determine if there were significant transcript expression changes in key inflammatory genes in the compensatory limb following either sham surgery or DMM surgery as compared to a truly naïve animal. Sex was also considered. We hypothesized there would be increased inflammatory markers in the compensatory limb of both surgery groups, with more severe changes in the DMM group.

Methods: Male and female mice were randomly assigned to the following groups: naïve, sham surgery, or DMM surgery (right knee). The compensatory limb in all cases was left naïve. At 4- or 8-weeks post-injury, animals were harvested and total mRNA was extracted.

Results: Compared to naïve animals, compensatory limbs from either sham or DMM surgery groups demonstrated significant inflammatory transcript differences at both 4- and 8-weeks post-injury. Of note, very few differences were noted between compensatory limbs of the two surgery groups, implying that either DMM or sham surgeries appear to affect compensatory limbs relatively similarly.

Discussion: Regardless of the surgery that was performed (sham or DMM surgery), it would appear that contralateral limbs demonstrated a statistically significant difference in transcript expression relative to naïve knees.

Conclusion: When selecting/designating use of contralateral controls for mouse PTOA studies, the potential for compensatory limb changes should be carefully considered during conceptualization of the experimental design.

Graduate Student / Microbiology, Immunology and Pathology

69. Dried Blood Spots and Urine Metabolite Profiling reveals dietary biomarkers of increased cowpea consumption by young Children and Pregnant Women in Ghana.

Madison Tipton, Bridget Baxter, Kailey Berry, Brooke Sayre-Chavez, María Muñoz-Amatriaín, Corey D. Broeckling, Mark Manary and Elizabeth P. Ryan

Nearly 35.6% of all children under the age of five living in Ghana suffer from some type of malnutrition. Legumes are a high-quality source of dietary fiber, essential amino acids, prebiotics, and other bioactive secondary metabolites important for healthy growth. Cowpeas have evidence to combat malnutrition and have agronomic traits ideal to semi-arid regions. Dietary biomarkers of cowpea intake across varieties are needed to establish relationships with child growth. Dried blood spots (DBS) and urine were collected from a clinical feeding study assessing nutritional metabolite markers of cowpea intake in Ghana children and pregnant women using non-targeted metabolomics. The 24 children (9-21months of age) and 21 pregnant women (>18 years) had a washout period with no cowpeas, and then consumed a dose-escalation of cowpeas every five days for a total of 20 days. DBS, urine and the cowpea foods were analyzed by ultra-performance liquid chromatography tandem mass spectrometry. Two-way ANOVA was applied to compare metabolite profiles between timepoints whereby 675 metabolites in DBS and 930 metabolites in urine were identified. We identified 16 DBS metabolites and five urine metabolites that significantly increased in both children and pregnant women for both groups. Histidine, a marker for malnutrition in children, was significantly decreased 0.90-fold in children DBS who consumed the Tukara and Adua (TA) cowpeas. Palmitoylcarnitine (C16) was significantly increased (1.29-fold) in both children and pregnant women in this TA group. The Dagbantuya and Sangyi (DS) cowpeas showed a host derived metabolite N-delta-acetylornithine increased 1.88-fold in DBS for children and 1.86-fold in pregnant women. Gut microbial derived N-acetyltryptophan, present in urine, increased 1.56-fold in children and 2.02-fold in pregnant women who consumed DS cowpeas. Increased levels of S-methylcysteine and S-methylcysteine sulfoxide were identified by non-targeted relative abundance and with targeted quantification and was considered for utility as a legume exposure biomarker.

Undergraduate Student / Environmental and Radiological Health Sciences

70. Multiple myeloma with aberrant CD3 expression in a red-lored Amazon parrot (*Amazona autumnalis*).

Silvia G. Tovar-Lopez, Samantha Evans, Juan F. Muñoz Gutiérrez, A Russell Moore, Miranda J. Sadar,

: A 20-year-old, female, red-lored Amazon parrot (*Amazona autumnalis*) was presented for a 2-week history of weakness. On physical examination the bird was quiet, fluffed with generalized weakness, and the coelom was distended. Survey radiographs and coelomic ultrasound revealed coelomic distention, increased pulmonary parenchymal opacity, renomegaly, dilated intestines, and a thickened ventricular wall. A complete blood cell count revealed moderate anemia (28%) and intermediate to large lymphocytes with immature chromatin, which were suspected to be neoplastic. Immunocytochemistry on peripheral blood revealed that the suspected circulating neoplastic cells were CD3+ and occasionally expressed MUM-1. Plasma biochemistry revealed moderate hyperphosphatemia (6.8mg/dL), marked hyperproteinemia (13.6g/L), analbuminemia (0g/dL), and marked hyperglobulinemia (13.6g/dL). Agarose gel plasma protein electrophoresis documented the presence of albumin (1.2g/dL) and a monoclonal band which, on reduced Lithium dodecyl sulfate polyacrylamide gel electrophoresis (LDS-PAGE), resolved as 60KD and ~25KD bands consistent with IgY heavy chain and light chain. Based on these findings, multiple myeloma was diagnosed. Due to a poor prognosis, the bird was euthanized and a necropsy was performed. Postmortem bone marrow smears revealed 17.4% plasma cells and 24% large immature cells with occasional plasmacytoid features. Histopathologic findings included aggregates of neoplastic plasma cells in the bone marrow, spleen, kidney, liver, gastrointestinal tract, muscle, ovary, and brain. The neoplastic cells were strongly immunoreactive for MUM-1 and CD3, and negative for CD79a, PAX5, and CD20. This confirmed the clinical diagnosis of multiple myeloma. This is the first report of an avian IgY-secreting multiple myeloma with aberrant CD3 expression and pseudo-analbuminemia. Aberrant CD3 expression by avian multiple myeloma may explain previously published cases of birds with a monoclonal gammopathy and apparent T-cell lymphoma diagnosed by CD3 immunoreactivity.

Resident / Clinical Sciences

71. Neurotoxicity is seen in the aging cat brain by accumulation of misfolded proteins and glial inflammation.

Danielle F Weaver, Analeis Cofield, Amelia Hines, McKenzie Richards, Breonna R Kusick, Stephanie McGrath, Julie A Moreno

Medical advances in human and veterinary medicine are resulting in longer lifespans for people and their pets alike. Therefore, age-related medical concerns like Alzheimer's disease in humans and cognitive dysfunction syndrome in dogs and cats have become increasingly common and concerning. In this study, we seek to address the pathophysiologic processes in the brains of cats as they age. This knowledge will help with the understanding of feline cognitive dysfunction, a syndrome that afflicts 28% of cats over the age of 11 and up to 50% of cats over the age of 15. Feline cognitive dysfunction (FCD) syndrome presents with pathophysiology that includes amyloid-beta accumulation, tau protein hyperphosphorylation, gliosis, and neuronal loss. We hypothesize that the brain pathophysiology identified in FCD will be seen a proportion of an aged cat population. To assess this, we performed immunohistochemistry on sections of feline brain tissue from multiple cats of a wide variety of ages to look for hyperphosphorylation of tau, a protein found to aggregate and form fibrils, amyloid-b and glial inflammation, marked by GFAP for astrocytes and IBA1 for microglia. Our data show a significant increase in all protein markers when compared between young (less than four years of age) and older (10+). This study furthers the understanding of the pathophysiology that occurs naturally in the aging cat brain. Better understanding of the disease processes that occur during natural aging could lead to improved understanding to aging but also diseases of age like FCD and human Alzheimer's disease.

DVM Student / Environmental and Radiological Health Sciences

72. Comparative phenotypic and transcriptomic analysis of synovial macrophages and tissue macrophages from healthy horses and horses with osteoarthritis.

Meghan Webster, Steven Dow, Dylan Ammons, Laurie Goodrich, Lyndah Chow, Jade Kurihara, Nikki Phillips, Todd Bass, Lynn Pezzanite

Osteoarthritis (OA) represents a major source of disability in the United States, affecting an estimated 23% of adult humans and 33% of horses. The innate immune system, including particularly myeloid cells, the most common of which are synovial macrophages, plays an important role in regulating and perpetuating low-grade inflammation characteristic of OA. The objectives of this study are therefore to compare the phenotype and transcriptome of synovial macrophages from animals with healthy joints to 1) tissue macrophages from major organs (lung, liver, spleen) and 2) to macrophages isolated from the synovium of OA joints. We hypothesize that 1) endogenous synovial macrophages possess unique phenotypic properties (anti-inflammatory or M2-oriented) compared to macrophages from other tissue sources (M1-oriented), and that 2) macrophages isolated from OA joints will demonstrate a more pro-inflammatory (M1) phenotype than in healthy synovium. Synovial biopsies and tissues with well-defined macrophage populations (lung, liver, spleen) were obtained from 4 young (aged 2- to 5-year-old) horses without OA, and synovial biopsies will be obtained from 4 horses with OA. Macrophage populations will be characterized using immunohistochemistry for expression of M1 (iNOS) versus M2 (CD204, arginase) markers. Single cell RNA sequencing of blood leukocytes and synovial cell populations will also be used to analyze transcriptomic signatures of synovial macrophages and assign cell identity and function un-ambiguously. These studies will provide the first comprehensive assessment of equine synovial macrophages and their function in health and disease and will advance the development of new approaches to management of OA in horses and humans.

DVM Student / Clinical Sciences

73. Evaluation of prednisolone pharmacokinetics and toxicity in dogs with lymphoma or immune-mediated disease.

Madeleine Westbrook, Sarah Shropshire, Beck Ringdahl-Mayland, Steve Dow, Daniel Gustafson, Susan Lana

Despite being one of the most widely used drugs in veterinary medicine, little is known regarding the actual plasma concentrations achieved by current dosing recommendations of prednisolone in dogs with spontaneous disease. Our objective is to assess pharmacokinetic parameters and toxicity in dogs with lymphoma or immune-mediated disease before and after oral dosing of prednisolone using two commonly recommended doses. Ten patients in each disease group (n=20) will be randomly placed in two dosing groups. Plasma will be collected at pre-determined intervals post oral dosing. Free prednisolone levels will be measured by mass spectrometry to evaluate pharmacokinetic parameters. All adverse events (AE) will be graded according to the VCOG CTCAE v2.0. CBC, Chemistry panel, UA, and UPC will be assessed at predetermined intervals. To date, 10 dogs with lymphoma and 8 with immune-mediated disease have been enrolled. The current total number of drug attributable AEs is 73, with the average for the 1mg/kg group and the 2mg/kg group being 3.3 and 4.7 AEs respectively. The 2mg/kg group had more gr 2-4 AEs (11) than the 1mg/kg group (5). All cases had increases in ALP and ALT. PU and/or PD occurred in 7 cases. GI toxicity occurred in 5 cases, all of which were in the 2mg/kg group. USG decreased in 12 cases. Proteinuria was present pre dosing in 6 dogs and post in 4 dogs, one of which was a new development of proteinuria. Two patients developed evidence of infection (UTI, pneumonia) prior to day 7 assessment. Weight loss occurred in 15 patients with 8 losing >5% and <10% BW. These findings will be correlated with PK parameters (AUC, Cmax, CL, t1/2) and measures of immune response (cytokines, changes in gene expression) to be assessed in batch at the end of the study.

DVM Student / Clinical Sciences

74. *Campylobacter* prevalence differs across and within broiler houses with re-used poultry litter.

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Multiple interventions are recommended during the processing of broilers to reduce bacterial loads, but pathogens like *Campylobacter* remain a major foodborne disease linked to poultry consumption. Consequently, the poultry industry and regulatory agencies are looking for pre-harvest strategies that can reduce *Campylobacter* in broiler grow-out houses. To achieve this aim, it is critical we understand the ecology of *Campylobacter* in pre-harvest. In this study, a longitudinal sampling of the litter in four commercial broiler houses was conducted over three consecutive flocks to evaluate *Campylobacter* prevalence in litter. Prior to the start of the study, a complete house clean-out was done, and fresh peanut hull was used as the bedding. The second and third flock were raised in succession on the same litter without any litter clean-out between each grow-out cycle. Litter was sampled at the beginning of each grow-out cycle and at the end of the cycle. For each house section, three grab samples of litter were collected and pooled. Seventy-two pooled litter samples were taken from each house. *Campylobacter* was detected from litter samples by direct plating and enrichment. Statistical analysis was used to explore the relationship between *Campylobacter* prevalence and relevant physio-chemical parameters. *Campylobacter* was found to be most prevalent during the first flock and in houses 3 and 4. Early grow-out period had significantly less *Campylobacter* than the late grow-out period. Furthermore, the odds of finding *Campylobacter* increased when the house temperature was below or equal to 79ºF than higher or equal to 80ºF. We also observed higher litter moisture content (>25%) resulted in higher *Campylobacter* prevalence. Lastly, genetic analysis of isolates revealed that C. jejuni was the dominant species in the litter (39/44); C. coli (5/44). This study suggest that interventions to decrease litter moisture and grow-out house temperature control may reduce *Campylobacter* prevalence.

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