21ST ANNUAL RESEARCH DAY

SCIENTIFIC PROCEEDINGS

JAN. 25, 2020 | C. WAYNE MCILWRAITH TRANSLATIONAL MEDICINE INSTITUTE

COLLEGE OF VETERINARY MEDICINE
AND BIOMEDICAL SCIENCES
COLORADO STATE UNIVERSITY

OUR 21ST 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!

2020 CVMBS Research Day Organizing Committee

Kelly Santangelo – Faculty Chair
Microbiology, Immunology, and Pathology

Adam Chicco – Faculty Co-Chair
Biomedical Sciences

Aimee Oke – Committee Coordinator
CVMBS Dean's Office

Theresa Rulon – Committee Coordinator
CVMBS Dean's Office

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17 Veterinary Summer Scholars Program

18 Young Investigator Grant Program

19 Sponsors

SCHEDULE OF EVENTS

11-11:45 a.m.	Poster set up	TMI 1st Floor Lobby
Noon	Opening Remarks	TMI 331
12:10 p.m.	ZOETIS RESEARCH EXCELLENCE AWARD WINNER – Dr. Kelly Santangelo	TMI 331
12:50 p.m.	Break	
1-5 p.m.	ORAL SESSION 1: Clinical Sciences	TMI 331
1-5 p.m.	ORAL SESSION 2: Basic Sciences	TMI 222
1-5 p.m.	ORAL SESSION 3: Basic Sciences	TMI 101
1-2:45 p.m.	POSTER SESSION I JUDGING: Odd-Numbered Posters	1st Floor
2:45-3 p.m.	Break	
3-4:45 p.m.	POSTER SESSION II JUDGING: Even-Numbered Posters	1st Floor
5-6 p.m.	Social Hour	TMI Grand Hall, Room 330
6 p.m.	Awards	TMI Grand Hall, Room 330

DEPARTMENTAL ABBREVIATIONS

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

CONGRATULATIONS AGAIN TO 2019 CVMBS RESEARCH DAY WINNERS!

ORAL PRESENTATIONS

First Basic James Johnson, Graduate Student, Mech Eng, "Biomechanical property degradation accompanied with chronic rotator cuff degeneration: evaluation of two new ovine models." Mentor: Kirk McGilvray

Second Basic Nora Jean Nealon, DVM/PhD Student, CMB, "Lactobacillus spp. differentially metabolize rice bran to suppress antimicrobial-resistant salmonella growth." Mentor: Elizabeth Ryan

Third Basic Erin McCready, Staff, CS, "Structural, biochemical, and biomechanical characteristics of articular cartilage of the ovine humeral head." Mentor: Brad Nelson

First Clinical Alexander McFarland, DVM Student, CS, "Repeated use of a thiafentanil based anesthesia protocol in an okapi (Okapia johnstoni)." Mentor: Khursheed Mama

Second Clinical Megan Posukonis, DVM Student, CS, "Fracture Characterization via Computed Tomography in Thoroughbred Racehorses." Mentor: Chris Kawcak

Third Clinical Jennifer Kelley, DVM Student, CS, "Dentistry dogma: challenging the status quo and putting suture patterns to the (tension) test" Mentor: Jennifer Rawlinson

POSTER PRESENTATIONS

First April Regas, DVM Student, MIP,"Testing the detection of DNA barcoded microcrystals as a novel mosquito marking method." Mentor: Rebekah Kading

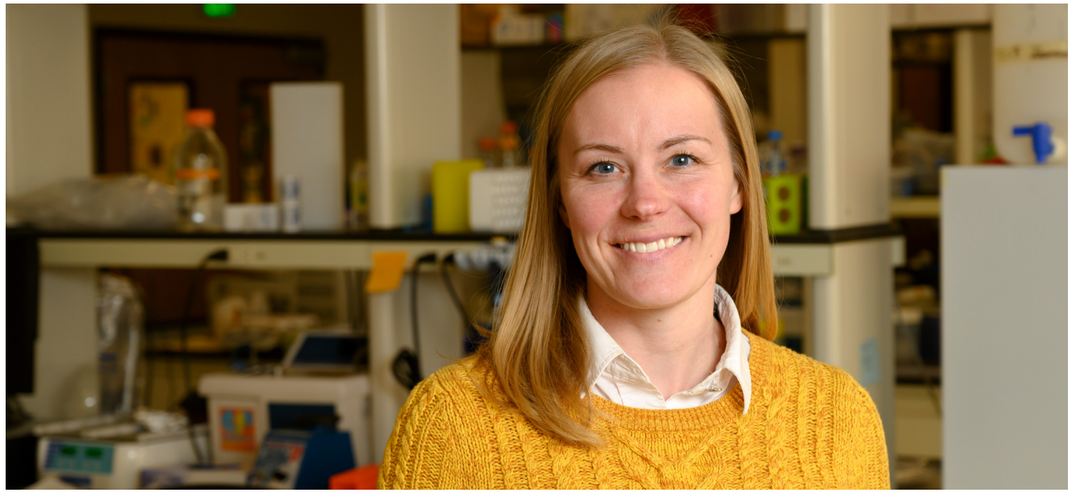
Second Steven Lakin, Graduate Student, MIP, "Statistical Supercomputing Improves Surveillance of Bacterial Pathogens and Antimicrobial Resistance." Mentor: Zaid Abdo

Third Katherine Redd, DVM Student, CS, "The effect of cannabidiol on seizure activity in uncontrolled canine epilepsy." Mentor: Stephanie McGrath

Golden Pipette Award Biomedical Sciences

ZOETIS AWARD RECIPIENT RECOGNIZES THE POWER OF PEOPLE

By Jessica Cox



REFUSING TO TAKE ALL THE CREDIT for her research projects, Dr. Kelly Santangelo attributes many of her accomplishments to collaborations with colleagues. An associate professor in the Department of Microbiology, Immunology, and Pathology, Santangelo seeks out collaboration opportunities beyond her home department, engaging faculty across campus. Even as she is recognized as the 2019 recipient of the Zoetis Award for Veterinary Research Excellence, her priority is acknowledging the human capital that contributes to the success of her work.

"This award allows me to showcase all of the people that I work with," Santangelo said. "It's just the perfect opportunity to say thank you to them."

The Zoetis Award fosters innovative research by recognizing outstanding research effort and productivity, and Santangelo's academic passion drives two main research themes: improving the efficiency and accuracy of diagnostics and providing better quality of life by maintaining mobility.

BETTER, FASTER DIAGNOSTICS

Empathetic to the challenges of owning an animal with medical issues, Santangelo strives to find ways that help clients make more informed decisions about their pets' health. Often, one of the hardest aspects of diagnosing a problem is how long it takes – owners struggle with not knowing what's wrong and having to wait for answers.

"If there's a way we can speed up the process, I want to be involved in that," Santangelo said. "Some diagnostics are not definitive, and I want to find a way to provide the highest confidence index possible."

With more accurate information about what issues their pet is experiencing and steps they can take to remediate medical problems, owners can make better decisions that make good financial sense and have a higher probability of improving their animal's overall health.

MAINTAINING MOBILITY

With a focus on the musculoskeletal system, Santangelo aims to find ways to maintain pain-free, independent mobility for humans and animals.

"I think people appreciate mobility but take it for granted until we can't do it anymore," Santangelo said. "Then we realize how much wellbeing is tied to independence."

Mobility is a marker of independence, and without it, quality of life decreases drastically. It's also dependent on different pieces of the body working together – joins, bones, nerves – and physical limitations or pain can both play a role in limiting an ability to get around.

"I will work on any project that supports keeping us mobile and independent, and with the ability to move as long as possible," Santangelo said.

SEEING THE PROMISE IN PEOPLE

While diagnostics and mobility are common threads that run throughout her work, Santangelo is most passionate about the people behind the science. There's inherent reward in seeing a research idea come to fruition, but there's power in the process, too.

"I've been blown away by what people are capable of accomplishing when you give them an opportunity," Santangelo said.

Leading by example, Santangelo's passion for research and collaboration is well recognized by her colleagues, including Associate Dean for Academic and Student Affairs Dr. Sandra Quackenbush.

"We are fortunate to have such a great colleague who is recognized for her mentorship and the impact of her research program," Quackenbush said. "She is an avid supporter of our students and dedicated to training the next generation of scientists who will be equipped to make significant contributions in their respective fields of study."

When she's not in the lab, Santangelo dedicates time to investing in the career development of peers and students, which also happens to be the most personally rewarding aspect of her job.

"I'm passionate about mentorship and seeing people reach their potential," said Santangelo. "When I retire one day what I will look back on as evidence of my success is what the people I worked with were able to accomplish."

CULTIVATING COLLABORATION

In recognition of her 2019 Zoetis Award for Veterinary Research Excellence, Dr. Kelly Santangelo will kick off the 21st annual CVMBS Research Day with a keynote address on Saturday, Jan. 25 at the C. Wayne McIlwraith Translational Medicine Institute. She'll talk about all the "little things" that have to come together to make a research project successful, and how at the end of the day, it's the people involved that bring the work together.

SESSION 1: Clinical Science

1–5 p.m. | TMI 331

Time	Presenter	Topic	Dept.
1:00	Chiu, Elliott	A Novel Test for Determination of Wild Felid-Domestic Cat Hybridization | VandeWoude	MIP
1:15	Drizen, Sienna	Effect of multi-dose Trazodone on arterial blood pressure in normal beagles | Ruch Gallie	CS
1:30	Ellis, Jayne	Comparison of a single versus staged two dart anesthesia induction protocol in Przewalski's horses | Mama	CS
1:45	Gitterman, Sarah	The pharmacokinetics of liposomal bupivacaine administered perineurally in the horse: a first investigation to improve postoperative and chronic pain management in horses | Griffenhagen	CS
2:00	Gross, Chase	Cannabis for The Cure: Cannabidiol Induces Apoptosis and Perturbs Mitochondrial Function in both Human and Canine Glioma Cells | McGrath	CS
2:15	Hoaglund, Elizabeth	Comparing the clinical success rate of the dorsolateral approach to the medial approach for equine distal intertarsal joint injection | Bass	CS
2:30	Hung, Fion	Correlational patterns between equine hoof conformation and midstance kinetics | Faramarzi	CS
2:45	BREAK		
3:00	Newman, Robert	Optimizing antibiotic selection in treatment of canine septic arthritis to minimize joint cytotoxicity | Dow	CS
3:15	Plenn, Johathan	Conformational variability of the pterygoid bone has no statistical correlation with acquired nasopharyngeal stenosis in dogs | Griffin	ERHS
3:30	Sapora, Joseph	Ventral femoral head ostectomy: Standard versus novel K-wire guided technique using a pre-measured ostectomy angle in canine cadavers | Goh	CS
3:45	Stewart, Holly	"Ewe have bone marrow lesions": experimental model development using the ovine stifle | Kawcak	CS
4:00	Slinkard, Powell	Retrospective analysis of the use of fluorine-18 fluorodeoxyglucose-positron emission tomography/computed tomography for the detection of metastatic lymph nodes in dogs diagnosed with appendicular osteosarcoma| Griffin/Randall	ERHS
4:15	Sullivan, Michelle	Preliminary evaluation of different anesthesia protocols in rabbits undergoing elective surgery | Boscan	CS
4:30	Ullal, Tarini	Treatment of idiopathic chronic hepatitis and copper associated hepatopathy in dogs | Shropshire	MIP
4:45	Zweck-Bronner, Carson	Incidence of shedding of equine herpes 2 and 5 in Colorado rescue horses | Landolt	CS

SESSION 2: Basic Science

1–5 p.m. | TMI 222

Time	Presenter	Topic	Dept.
1:00	Alfino, Lauren	In Vitro Effects of Integrin Signaling Inhibition in Canine and Human Osteosarcoma Cell Lines | Regan	MIP
1:15	Andrie, Kendra	Use of a novel Nrf2 activator to mitigate the progression of osteoarthritis in the Hartley guinea pig | Santangelo	MIP
1:30	Beaupied, Bonni	The effects of air pollutant levels on dairy cow health and production in Northern Colorado| Magzamen	ERHS
1:45	Dilisio, James	Targeting PKC Θ in T cells as an immunotherapy for autoimmune diabetes | Podell	MIP
2:00	Eklund, Bridget	Microbes in the Mucosa: A Probiotic-Based Vaccine and the Gut Microbiome | Abdo/Dean	MIP
2:15	Fox, Amy	Cyto-feature engineering: a flow cytometry analysis pipeline to uncover immune populations and association with disease | Henao-Tamayo	MIP
2:30	Gonzalez-Berrios, Carolina	Embryo mortality and peripheral transcriptome response in Holstein cows | Hansen	BMS
2:45	BREAK		
3:00	Harris, Macallister	Long term vitamin a deficient guinea pig model: a model for *mycobacterium tuberculosis* infections in vitamin A deficient humans | Podell	MIP
3:15	Hay, Arielle	Reverting prion disease in neuronal progenitor cells as a cell replacement therapy | Moreno	MIP
3:30	Jonas, Daniel	In silico mouse model of infection and immunity | Schenkel	MIP
3:45	Kraft, Caitlyn	Longitudinal Detection of Chronic Wasting Disease Prions in Nasal Swab Collections by Real-Time Quaking- Induced Conversion | Hoover	MIP
4:00	Kuiper, Grace	Validation of remote sensing and simulation methods for animal infectious disease modeling | Magzamen	ERHS
4:15	Labb, Samantha	Chemical separation of the minor actinides: towards a closed nuclear fuel cycle | Sudowe	ERHS
4:30	LiPuma, Lance	Mitochondrial PUFA Metabolism Regulates Myocardial Ischemic Tolerance | Chicco	BMS
4:45	Martenies, Sheena	Wildfire smoke may interfere with the use of black carbon as an indicator of traffic exposure | Magzamen	ERHS

SESSION 3: Basic Science

1–5 p.m. | TMI 101

Time	Presenter	Topic	Dept.
1:00	McCoy, Jasmine	Determination of the mechanism of oncolysis by MYXVorfC, a myxoma virus that expresses a pro-apoptotic protein | MacNeill	MIP
1:15	Merriman, Sean	Regionally-biased CNVs accompany large-scale chromosomal rearrangements in *S. cerevisiae* | Argueso	ERHS
1:30	Overton, Jessie	Novel identification of antimicrobial resistance markers among *Salmonella Typhimurium* across humans, bovine, and porcine hosts using metabolomics | Rao	CS
1:45	Parsons Aubone, Agata Maria	Novel identification of antimicrobial resistance markers among *Salmonella Typhimurium* across humans, bovine, and porcine hosts using metabolomics | Bouma	BMS
2:00	Personett, Alexa	Evaluation of electroacupuncture for symptom modification in a rodent model of spontaneous osteoarthritis | Santangelo	MIP
2:15	Petch, Raegan	Spillover of Feline Leukemia Virus from domestic cats to North American pumas | VandeWoude	MIP
2:30	Pires, Elena	Investigating the function of RAD51AP1 in homologous recombination DNA repair | Wiese	ERHS
2:45	BREAK		
3:00	Ragan, Izabela	Cold Blood: Reptiles and amphibians as reservoir and overwintering hosts for arboviruses | Bowen	BMS
3:15	Seidel, James	Does the attenuation of proinflammatory cytokines in the upper airways improve pulmonary function among dairy workers? | Schaeffer	ERHS
3:30	Selemenakis, Platon	Synergism between RAD51AP1 and RAD54 during the synaptic stage of homologous recombination DNA repair | Wiese	ERHS
3:45	Taloumis, James	Synergism between RAD51AP1 and RAD54 during the synaptic stage of homologous recombination DNA repair | Brazile	ERHS
4:00	Tanner, Amelia	In vivo impact of chorionic somatomammotropin RNA interference in the absence of intrauterine growth restriction | Anthony	BMS
4:15	Templeton, Hayley	Glucocorticoid receptor expression in placenta and brain tissue | Bouma	BMS
4:30	Vick, Zaria	Understanding the mechanism of a neurotoxic antibiotic | Moreno	MIP
4:45	Wagner, Kaitlyn	Identification of a novel strain of chronic wasting disease | Zabel	MIP

POSTER PRESENTATIONS

SESSION 1 | ODD-NUMBERED POSTERS | 1-2:45 p.m.
SESSION 2 | EVEN-NUMBERED POSTERS | 3-4:45 p.m.

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

No.	Presenter	Title | Mentor	Dept.
1	Adelmeyer, Lindsay	Correlation of two different fibrinogen assays in chelonian plasma | Johnston	CS
2	Ammons, Dylan	Investigating PD-L1 signaling in endothelial cells | Dow	MIP
3	Asatryan, Kathryn	Mesenchymal stem cells to reduce bacterial load and morbidity in an acute *Klebsiella pneumoniae* mouse model | Johnson	CS
4	Auerbach, Jeremy	Small changes in sidewalk connectivity can result in big gains for student walking | Magzamen	ERHS
5	Bergum, Nikolas	Assessing a novel therapeutic on drug-seeking behavior in mice | Hentges	BMS
6	Bisazza, Katherine	Using CRISPR-Cas9 to target PLS3 in ovine fibroblast cells | Easley	CS
7	Bork, Sydney	Efficacy of a Nrf2 agonist to increase long bone strength in a rodent model of osteoarthritis | Santangelo	MIP
8	Brock, Maria	Injection characteristics of autologous and commercial fibrin hydrogels for cartilage defects in horses using a modified Subchondroplasty® technique | Kawcak	CS
9	Brunk, Kaitlyn	Comprehensive evaluation of the circulating renin-angiotensin-aldosterone system in normal dogs and dogs with asymptomatic myxomatous mitral valve disease | Ames	CS
10	Burton, Lindsey	Systemic Iron Chelation Reduces Markers of Chondrocyte Hypertrophy and the Development of Cartilage Lesions in the Dunkin-Hartley Model of Primary Osteoarthritis | Santangelo	ERHS
11	Butler, Molly	Cyclin-Dependent Kinase 8 is a Transcriptional Regulator During Dengue Infection | Rovnak	MIP
12	Cao, Jennifer	EFGR As A Target For Solid Tumor Chimeric Antigen Receptor T Cell Therapy | Dow	MIP
13	Chornarm, Nida	A flow cytometric assessment of canine anti-erythrocyte antibodies in healthy dogs and dogs with anemic-associated diseases | Lappin	CS
14	Clark, Hayley	Effect of a commercially available probiotic with or without fiber supplementation on the incidence of diarrhea in shelter cats | Lappin	CS
15	Cooper, Sarah	Phenotyping immune cell populations in situ in *Mycobacterium tuberculosis* infected mouse lung tissue | Podell	MIP

No.	Presenter	Title | Mentor	Dept.
16	Davis, Lily	Evaluation of two maxillary block techniques in cats for painful dental procedures (Phase I, Cadaver Study) | Hoyer	CS
17	Deak, Anna	Radiocesium size categorization of suspend particles in Fukushima contaminated water | Johnson	ERHS
18	DeFilippo, Brian	Immune Modulatory Effects of Common Antibiotics on Canine Innate and Adaptive Immune Responses In Vitro | Dow	CS
19	Deluty, Sarah	The use of magnetic resonance spectroscopy to differentiate histologically confirmed canine brain masses: a retrospective analysis of 44 cases | Packer	CS
20	Dobesh, Kelsey	Serologic responses to oral administration of an intranasal FHV-1 and FCV containing vaccine | Lappin	CS
21	Donaghy, Dillon	Thrombin generation assay in healthy dogs receiving apixaban | Olver	MIP
22	Donkoh, Jasmine	The role of cyclin dependent kinase 8 in Zika virus control of host metabolism | Rovnak	MIP
23	Doster, Enrique	Antimicrobial drug use in beef feedlots; effects on microbiome and resistome dynamics | Morley	MIP
24	Edmonds, Marisa	Parkin mutations increase neuroinflammatory activation of glia and neuronal loss in the nigrostriatal pathway that is attenuated by genetic knockout of cGas-STING | Tjalkens	ERHS
25	Epstein, Sophia	Multiplex fluorescent immunocytochemistry: a novel approach to the diagnosis of Feline Infectious Peritonitis | Santangelo	MIP
26	Ericksen, Kelsea	Characterization of plumes generated from electrosurgical devices and mitigation strategies | Hackett	CS
27	Erlandson, Grant	Hypertonic saline rinse and its effect on nasal inflammation and microbiome in dairy workers | Schaeffer	ERHS
28	Estrada McDermott, Juan	Use of a macrophage culture system to assess inflammatory properties of cartilage degradation products and immune modulation by mesenchymal stromal cells in horses | Dow	CS
29	Evans, River	Testosterone binding to G protein-coupled receptor family C group 6 member A and syncytialization of human trophoblast cells | Bouma	BMS
30	Fuller, Dana	Development of a vitrification protocol for in vitro produced feline embryos | Barfield	BMS
31	Galloni, Allysa	The Impact of Sampling Method on Gut Bacterial Composition in Dogs and Cats | Webb	CS
32	Garrison, Emily	CD4+FoxP3+ T cells are elevated in two indolent canine hematologic cancers | Avery	MIP
33	Georges, Hanah	Don't judge an embryo by its cover | Hansen	BMS

No.	Presenter	Title | Mentor	Dept.
34	Gleason, Kathryn	Decellularized Biological Scaffolds for the Prevention Post-Operative Seroma Formation | Ehrhart	CS
35	Gray, Lyndsey	Something Old, Something New: Revisiting and novel applications for wing scale counting as a tool for age-grading An. Gambiae | Foy	MIP
36	Creigh, Ally	Effects of physostigmine on quality of recovery and post-operative gastrointestinal dysfunction following inhalant anesthesia in horses | Hassel	CS
37	Haugen Frenkel, Jessica	Metformin modulates T cell terminal differentiation following *M. tuberculosis* infection | Basaraba	MIP
38	Hicks, Jasmine	Synaptic Ultrastructure at the Drosophila Neuromuscular Junction | Reist	BMS
39	Hopkins, Leone	TLR agonist as a protectant against radiation toxicity | Dow	CS
40	Hord, Taylor	Approaches to study KDM1A in ovine placenta | Bouma	BMS
41	Hritz, Rachel	Effect of a single dose of oral trazodone on intraocular pressure in healthy horses | Wotman	CS
42	Johnson, James	Osteoporosis Correlated to Degradation of Tendon Mechanical Properties in an Ovine Model | McGilvray	CS
43	Jordan, Mariah	Myxoma ORFC virus detection in tissue | MacNeill	MIP
44	Khorsand, Matt	Effect of vaccination with commercial distemper-adenovirus-2-parainfluenza-parvo vaccines on platelet numbers and development of anti-platelet antibodies in healthy dogs | Shropshire	CS
45	Kiene, Jeremy	Use of a probiotic with supplemental fiber for treating antibiotic-induced diarrhea in cats | Lappin	BMS
46	Kline, Kyle	Effect of oral administration of trazodone on intraocular pressure in normal dogs | Wotman	CS
47	Korkis, Christian	Circadian Effects of Opioid Administration | Vigh	BMS
48	Krause, Danielle	Incidence of septic arthritis following arthroscopy and joint injection was independent of antibiotic protocol | Hendrickson	CS
49	Lakin, Steve	Are you counting correctly? Improving methods for categorical data analysis | Abdo	MIP
50	Liebig, Bethany	Preparation of platelet rich plasma in rats: a two-step centrifugation technique | Goodrich	CS
51	Litchford, Morgan	High-resolution tomography analysis of Drosophila vesicle priming | Reist	BMS
52	Lopes Sicupira Franco, Patricia Mara	Prevalence of Cryptosporidium spp. in scat samples from the Portland Watershed, Oregon | Scorza	CS

No.	Presenter	Title | Mentor	Dept.
53	MacNeill, Brooke	Making Colorado State University more green: implementation of a laboratory sustainability program | Duncan	MIP
54	Martinez, Heather	Cows as Canaries: Using a One-Health Approach to Investigate Air Pollution Effects | Magzamen	ERHS
55	Mathias, Alissa	The Role of Osteosarcoma Cell-Derived Exosomes in the Promotion of Lung Metastasis | Regan	MIP
56	McCann, Veronica	Determining risk factors of puma predation of domestic cats in an expanding urban landscape | VandeWoude	MIP
57	McCaw, Katherine	Veterinarians' Perspectives on the Health Impacts of Climate Change: Educational Gaps and Opportunities | Duncan	MIP
58	Mickelson, Alison	Localization of Nerve Growth Factor-β in the stallion reproductive tract | McCue	CS
59	Moawad, Adel	Comparison of in vitro production of bison and cattle embryos and effect of L-carnitine during maturation of bison oocytes | Barfield	BMS
60	Moskaluk, Alex	Pathogenesis and development of a rapid diagnostic test for *Microsporum canis* | VandeWoude	MIP
61	Nunez, Jessica	Source Attribution of MRSA Exposure and Carriage Among Dairy Workers | Schaeffer	ERHS
62	O'Doherty, Hazel	Identifying spatial drivers of avian influenza virus using true prevalence estimations | Webb	Other
63	Palmer, Eric	3D co-culture of human breast cancer cells with primary lung fibroblasts promotes drug and molecular subtype-specific differences in chemoresistance | Regan	MIP
64	Pearce, Camron	Inhaled tigecycline is highly effective against chronic pulmonary M. abscessus infection with promising clinical implications | Gonzalez-Juarrero	MIP
65	Perry, David	Measuring tumor oxygenation in veterinary radiotherapy patients using a novel optical spectroscopy device | Boss	ERHS
66	Pezzanite, Lynn	In vivo dose titration of amikacin in equine joints reveals sustained synovial fluid concentrations and dose-dependent cartilage toxicity | Dow	CS
67	Piquini, Gabriella	Antimicrobial selection for intra-articular administration may minimize cytotoxicity to equine chondrocytes and synovial cells | Goodrich	CS
68	Porter, Stephanie	Monkeypox virus transmission between small mammal species in an artificial ecosystem | Bowen	MIP
69	Posukonis, Megan	Evaluation of a Phantom-less Computed Tomography Protocol for Measurement of Bone Mineral Density | Kawcak	CS

No.	Presenter	Title | Mentor	Dept.
70	Riggs, Lauren	Coinfection of Epizootic Hemorrhagic Disease Virus and Bluetongue Virus in Culicoides Cell Culture | Mayo	MIP
71	Rosen, Sydney	Prospective evaluation of orthoses and prostheses in canine patients | Duerr	CS
72	Sanchez Rodriguez, Anais	An evaluation and comparison of phosphorus, calcium, magnesium, sodium, potassium, protein and fat in commercially available adult and senior cat diets | Stockman	CS
73	Schaeuble, Derek	Sexually-divergent effects of infralimbic cortex stimulation on endocrine and cardiovascular stress reactivity | Myers	BMS
74	Schiavone, Stephanie	Environmental Sustainability: The Role of Veterinary Teaching Hospitals in Global Health Education | Duncan	MIP
75	Schlein, Lisa	Parthenolide: a promising phytomedicine for deadly cancers in people and dogs | Thamm	CS
76	Schultz, Hal	In vitro cytotoxic effects of cannabidiol on canine melanoma cells | McGrath	CS
77	Schwerdtfeger, Luke	Vasoactive Intestinal Peptide Regulates Goblet Cell Production in the Small Intestine | Tobet	BMS
78	Scott, Daneille	The Value of Sustainability in Veterinary Practices: A Client Perspective | Duncan	MIP
79	Seebart, Cassie	Contribution of intra-abdominal immune dysfunction to musculoskeletal inflammaging | Santangelo	MIP
80	Seabolt, Rowan	Developing an environmental sustainability assessment tool for United States veterinary hospitals | Duncan	MIP
81	Stone, Cassidy	Determination of feline hepadnavirus infections in cats in the US | VandeWoude	MIP
82	Strnadova, Alena	Topically applied liposomal TLR ligand complexes to treat equine corneolimbal squamous cell carcinoma | Wotman	CS
83	Sun, Julianna	SPS1: A novel resistance factor to prion disease | Telling	MIP
84	Timkovich, Ariel	Manipulation of Toll-like receptor 4 ameliorates injury-induced osteoarthritis progression | Santangelo	MIP
85	Vahl, Bradleigh	Investigation of an educational veterinary resource on climate change that encourages positive action | Duncan	MIP
86	Wallace, Tyler	Sexual divergence in prefrontal regulation of depression-relevant behaviors | Myers	BMS
87	Warner, Shayna	Cardiac and inflammatory biomarkers in bovine pulmonary hypertension and congestive heart failure | Garry	CS

No.	Presenter	Title | Mentor	Dept.
88	Warren, Rebecca	The relationship between black carbon and polycyclic aromatic hydrocarbon exposures and mortality in Allegheny County, Pennsylvania | Magzamen	ERHS
89	Watkins, Jackson	Inactivation of *Mycobacterium tuberculosis* for Safe Use Outside of the BSL-3 Laboratory | Dobos	MIP
90	WeMott, Sherry	Developing a predictive model for indoor black carbon for the Denver, CO metropolitan area | Magzamen	ERHS
91	Winner, Anna	The role of *Streptococcus equi* subspecies *zooepidemicus* and influenza viruses in upper respiratory infections in shelter cats | Lappin	CS
92	Witter, Paige	Development of a Cerenkov imaging procedure as a lower-cost alternative for radiopharmaceutical imaging of positron emitters | Brandl	ERHS
93	Davis, Lily	Retrospective study of 240 dogs receiving gabapentin for chronic pain relief | Hellyer	CS
94	Zhang, Lei	Evaluation of select nutrients and vitamin D3 in senior cat foods | Stockman	CS

VETERINARY SUMMER SCHOLARS PROGRAM

DVM students dive into research with projects and field trips

APPLY BY FEB. 7, 2020!

Nearly 30 veterinary summer scholars traveled to Texas A&M with program director, Sue VandeWoude, this past August for the annual National Veterinary Scholar Symposium.

VETERINARY SUMMER SCHOLARS PROGRAM was initially to provide an opportunity for veterinary schools to expose first and second year veterinary students to biomedical research. The current CSU Veterinary Student Scholars Program provides veterinary students with hands-on exposure to veterinary medical research to introduce them to potential research careers. The application deadline is Feb. 7 for the summer 2020 program!

The College of Veterinary Medicine and Biomedical Sciences received funding from the National Institutes of Health in 2013 to expand an already successful program. Partnership with the Young Investigator Awards Program has further boosted participation

Last year, 42 veterinary students from CSU and abroad participated in the 2019 CSU Veterinary Summer Scholar Program. Students spent the summer working in research labs, attending weekly research seminars, and going on field trips to other CSU, federal, and state research facilities. Many of the projects conducted by CSU students last summer are being presented today at the CVMBS Research Day.

The National Institutes of Health and Boehringer Ingelheim, a multinational animal health company, support the program, along with several other organizations, the college, and faculty mentors who help provide stipends for program participants. We encourage students to apply for experiential learning in veterinary medical research!

To view the research of students funded in 2019, or to apply for the summer 2020 program, please visit the website at:
csu-cvmbs.colostate.edu/dvm-program/Pages/Veterinary-Scholars-Program.aspx

BY THE NUMBERS

- 42 scholars in the 2019 program, from CSU and other veterinary programs across the country and around the world. The scholars are selected through a competitive application process and receive financial support from program sponsors.
- 377 summer scholars since 2001
- 500+ total students mentored by CVMBS faculty in past 10 years
- 20 percent of student participants in past five years have been under-represented minorities
- Over 70 CVMBS faculty mentors

SPONSORS OF THE 2018 PROGRAM:

- National Institutes of Health
- Boehringer Ingelheim
- Morris Animal Foundation
- AVMA/ AVMF
- AKC Canine Health Foundation
- Petsmart Charities
- American Society of Lab Animal Practitioners
- United State Department of Agriculture
- CSU College of Veterinary Medicine and Biomedical Sciences

YOUNG INVESTIGATOR GRANT PROGRAM:
FUNDING RESEARCH AND BOOSTING VET STUDENTS

Center for Companion Animal Studies, Department of Clinical Sciences

Young Investigator grants help early-career researchers and DVM students (L to R; Hayley Clark, Anna Winner, Kelsey Dobesh, and Jeremy Kiene) pursue clinical projects, and improve the chances of securing complementary internships, graduate programs, and residencies.

THE YOUNG INVESTIGATOR GRANT PROGRAM provides funding to support research involving Colorado State veterinary students, and many of the recently funded projects are presented during Research Day.

In 2019, corporate and non-corporate sponsors donated more than $50,000 to the program. This funding was distributed to 21 research projects involving students in our DVM Program.

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The College of Veterinary Medicine and Biomedical Sciences thanks all program sponsors. These supporters are helping to advance veterinary science while also involving more DVM students in important clinical research. To view the grants funded in 2019 or to donate, please visit the Center for Companion Animal Studies website at *companionanimals.colostate.edu*.

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O-1. A Novel Test for Determination of Wild Felid-Domestic Cat Hybridization

Elliott S Chiu, Karen Fox, Lisa Wolfe, and Sue VandeWoude

In October 2018, Colorado Parks and Wildlife seized an animal believed to be an illegally possessed bobcat. The owner claimed the animal was a bobcat/domestic cat hybrid, exempted from license requirements. Burden of proof lay with CPW to determine the lineage of the animal. Commercial microsatellite arrays and DNA barcoding have not been developed for identification of bobcat/domestic cat hybrids, and limited time and resources prevented development of such tests for this application. Instead, we targeted endogenous feline leukemia virus (enFeLV) to quickly and inexpensively demonstrate the absence of domestic cat DNA in the contested animal. Using this assay, we were able to confirm that the contested animal lacked enFeLV, and therefore was not a domestic cat hybrid. **Funding**: NSF 1413925; NIH F30OD023386

DVM/PhD Student / Microbiology, Immunology and Pathology

O-2. Effect of multi-dose Trazodone on arterial blood pressure in normal beagles

Sienna Drizin, Kyle Kline, Kathryn Wotman, Michael R Lappin, Rebecca Ruch Gallie

Anxiety in canine companions is one of the most common causes of behavioral problems and veterinarians are increasingly prescribing anxiolytic medications to promote fear-free environments both at home and in the clinic. Specifically, trazodone, a serotonin antagonist reuptake inhibitor, is commonly prescribed to treat acute anxiety following surgical procedures, as well for a variety of chronic behavioral disorders, such as separation anxiety. Although trazodone is frequently utilized in veterinary patients, no studies have evaluated the effect of multi-dose trazodone on peripheral cardiovascular parameters. This randomized, double-blinded crossover study aims to determine the impact of multi-dose trazodone on canine arterial blood pressure over three days. Eight research beagles were entered into the study and randomly assigned to treatment and control groups. On Days 1-3 of the study, the treatment group received 50 mg (4.3-6.7 mg/kg) of trazodone orally and the control group received a placebo orally. Masked observers obtained systolic blood pressure via Doppler in lateral recumbency immediately before treatment (hour 0), and following treatment on hours 1, 4, 8, and 12. Following a 3-day wash out period, the groups crossed over and drug administration and data collection were repeated. Statistical analysis is pending and we hypothesize that multiple dose trazodone will decrease blood pressure in healthy beagles. **Funding**: Center for Companion Animal Studies Young Investigator Award

DVM Student / Clinical Sciences

O-3. Comparison of a single versus staged two dart anesthesia induction protocol in Przewalski's horses

Jayne Ellis, Sangeeta Rao, Matthew Kinney, Ryan Sadler, Matt Marincovich, Meredith Clancey, Lauren Howard, Nadine Lamberski, Khursheed Mama

Przewalski's horses (*Equus ferus spp. przewalski*) are an endangered equid. Immobilization using remote drug delivery is often needed to perform veterinary procedures. Behavioral and physiologic parameters were prospectively compared in 14 horses (7 males, 7 females, aged 5-19 years) after a single or staged two-dart anesthesia induction protocol. Seven horses were randomly assigned to each protocol. All horses received 0.06 mg/kg medetomidine, 0.05 mg/kg butorphanol, 0.02 mg/kg thiafentanil and 1 mg/kg ketamine. Horses assigned to the one dart protocol received all drugs in a single dart, while horses in the two dart protocol received medetomidine and butorphanol in the first dart and thiafentanil and ketamine 10 minutes later in a second dart. Induction and recovery quality were scored on a scale of 1-5 (worst to best). Video recordings were further assessed for frequency of specific behaviors. Need for supplemental propofol was also recorded. Median values for scores were compared using the Mann-Whitney test. Frequency of selected behaviors and propofol use were compared using Fishers Exact test. $p < 0.05$ considered significant. Median induction score was significantly better ($p = 0.01$) after two darts (3.9) compared to one (2.6). Degree of muscle fasciculation was also significantly ($p = 0.006$) less with the two versus one dart protocol. During transition to recumbency, 14% versus 71% of horses went down headfirst (undesirable) after two darts versus one dart, respectively ($p = 0.07$). Supplemental propofol administration was necessary to produce a working depth of anesthesia in 43% of horses after two darts and in 100% of horses after one dart ($p = 0.10$). There were no significant differences between groups for physiologic or recovery parameters. Clinically impactful improvement in quality of induction was observed using a staged two versus one dart protocol and should be considered when anesthetizing Przewalski's horses maintained in zoological institutions.

DVM Student / Clinical Sciences

O-4. The pharmacokinetics of liposomal bupivacaine administered perineurally in the horse: a first investigation to improve postoperative and chronic pain management in horses

Sarah Gitterman, Lynn Pezzanite, Dean Hendrickson, Valerie Moorman, Gregg M Griffenhagen

Treatment of equine distal limb pain can be challenging, and currently used analgesics have serious systemic side effects. Local anesthetics have fewer side effects, but their short duration of action (<12hrs) necessitates investigation of alternative therapies. The objective of this study was to determine the safety and pharmacokinetic profile of a long-acting liposomal bupivacaine in an induced model of equine lameness. Six normal horses had unilateral forelimb lameness induced, followed by peri-neural injection with either bupivacaine HCl or liposomal bupivacaine. Blood was drawn following perineural administration (0, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 60, and 72 hours). Following a one-week washout period, the procedure was repeated on the opposite limb with other treatment. Plasma bupivacaine concentrations were determined by high-pressure liquid chromatography/mass spectrometry. Pharmacokinetic variables were evaluated via non-compartmental and compartmental analysis, with the best model fit determined post-hoc based on criterion fit and visual analysis. The central hypothesis was that liposomal bupivicaine would maintain plasma concentrations below toxic levels for at least 72 hours following perineural administration. We further hypothesized that plasma pharmacokinetics would demonstrate biphasic peak concentrations as seen in other species, plasma concentrations would be above the lower limit of quantification for 72 hrs, and that peak concentrations of liposomal bupivicaine would be lower than those seen following administration of bupivicaine HCl. The results of this investigation will provide evidence to support the clinical use of peri-neural liposomal bupivacaine in equine distal limb pain. Funding was provided by CSU Young Investigator Grant.
Funding: Center for Companion Animal Studies Young Investigator Award, College Research Council

DVM Student / Clinical Sciences

O-5. Cannabis For The Cure: Cannabidiol Induces Apoptosis and Perturbs Mitochondrial Function in both Human and Canine Glioma Cells

Chase Gross, Mando Ramirez, Daniel Gustafson, and Stephanie McGrath

The use of cannabidiol (CBD), the major non-psychoactive compound derived from *Cannabis sativa*, as a nutraceutical in both human and veterinary medicine has increased within the past ten years despite a lack of scientific evidence for its effectiveness. Of the myriad of ailments that CBD supposedly treats, epilepsy, pain, and cancer remain prominent areas of interest to scientists and the general public. Basic cancer research has demonstrated that CBD is cytotoxic against breast, endometrial, glial, and blood cancers, and in all cases it appears that CBD triggers apoptosis through a cannabinoid receptor independent process. Because of their remarkable similarities, canine and human gliomas are of particular interest to the comparative oncology field. Regardless of species, gliomas are aggressive tumors that are notoriously resistant to multimodal treatment, thus creating a need for more effective therapeutics. We sought to characterize the mechanism of cell death in both human and canine glioma cells. We show that CBD is cytotoxic, anti-proliferative, and anti-migratory at concentrations which may be pharmacologically attainable. Using resazurin and the IncuCyte live cell imaging platform, we demonstrate that metabolic-based assays artificially increase cell line sensitivity to CBD compared to the IncuCyte, suggesting that non-cytotoxic concentrations of CBD perturb mitochondrial function. Furthermore, we show that CBD treatment results in the formation of large cellular non-lipid based vesicles whose existence is RIPK3-dependent. We also demonstrate that CBD-induced cell death is likely secondary to apoptosis; however, classically necroptotic protein, RIPK3, also appears to be involved. Lastly, combining CBD with the autophagy inhibitor hydroxychloroquine (HCQ) increases canine cell sensitivity to CBD, suggesting involvement of the autophagy pathway in CBD-induced cell death. These data demonstrate that human and canine glioma cells respond similarly to CBD, indicate a non-canonical function of RIPK3, and support the involvement of autophagy in CBD-induced cell death. **Funding**: NIH 5T35OD015130

Graduate Student

DVM Student / Clinical Sciences

O-6. Comparing the clinical success rate of the dorsolateral approach to the medial approach for equine distal intertarsal joint injection

Elizabeth L Hoaglund, Kathryn A Seabaugh, Kurt T Selberg, Ann Hess and Luke Bass

Distal intertarsal joint injection is an important component of lameness evaluation and treatment in horses. Successful injection is poor for the medial approach. The dorsolateral approach is an alternative but has not been validated with contrast medium. Radiograph-guidance has not been studied to determine its necessity or benefit for either approach. The objective was to determine if the dorsolateral approach to the distal intertarsal joint is more successful than the medial approach and to determine if radiograph-guidance is beneficial. This was a prospective, randomized study. Three operators injected 98 distal intertarsal joints in total, each horse served as its own control. In Phase 1, injections were performed by standard technique. In Phase 2, operators were allowed to use radiography to assist needle placement. Contrast deposition was evaluated by a single radiologist. Without radiographic assistance, 10/25 (40%) joints were successfully injected using either the medial or dorsolateral approach. With radiographic assistance, 19/24 (79%) joints were successfully injected using the medial approach, 11/24 (46%) joints were successfully injected using the dorsolateral approach. In summary, the dorsolateral approach was equivalent to the medial approach when traditional injection techniques were used. Radiograph-guidance improved success of the medial approach, but not the dorsolateral approach. Many injections performed from the dorsolateral approach (32/49; 65%) resulted in extensive perivascular subcutaneous contrast deposition after infiltration into the adjacent tarsal canal. Further research is needed to improve injection success of the distal intertarsal joint when using the dorsolateral approach. **Funding**: Zoetis Small Grant Programs

Resident / Clinical Sciences

O-7. Correlational patterns between equine hoof conformation and midstance kinetics

Fion Hung, Fanglong Dong, Babak Faramarzi

Variations in the anatomy of the hoof may alter stress distribution, predisposing horses to pathologies and lameness. However, current available experimental studies are scarce. The objective of this study was to investigate the correlation between midstance hoof kinetics at the walk and hoof anatomy in horses. Nine clinically sound, unshod athlete horses were walked across a pressure sensing system. Force, contact area, contact pressure, and peak contact pressure were recorded at the dorsal, palmar, medial, and lateral regions as well as for the whole hoof. Using digital radiography and digital pictures, 55 variables of internal and external anatomy of the hoof were measured. Correlations between biomechanical and anatomical measurements were investigated using Pearson correlation coefficient. P-values ≤ 0.05 and r values ≥ 0.5 were considered for analysis. Several anatomical variable showed correlations with biomechanical variables: toe angle was negatively correlated with contact area ($r = -0.72$); several heel height measurements were negatively correlation with dorsal force ($-0.59 \leq r \leq -0.50$); and the measurements of the dorsal hoof wall thickness and length/width of the distal phalanx showed correlations with force and contact pressure ($0.5 \leq r \leq 0.71$). The correlational patterns confirmed the relationship between hoof conformation and biomechanics. The results provide information on interactions between hoof shape and biomechanics that should be considered by veterinarians and farriers alike for routine hoof trimming and therapeutic manipulations. This study focused on midstance biomechanics at the walk; further investigation of other gaits and stance phases is warranted.
Funding: Arabian Horse Foundation; Western University of Health Sciences

DVM Student / Clinical Sciences

O-8. Optimizing antibiotic selection in treatment of canine septic arthritis to minimize joint cytotoxicity

Robert J Newman, Lynn M Pezzanite, Lyndah Chow, Laurie R Goodrich, Nicolaas E Lambrechts and Steven W Dow

Septic arthritis (SA) can cause debilitating lameness in dogs, necessitating long-term antimicrobial treatment. Intra-articular (IA) antibiotic therapy may shorten treatment time and reduce overall medical costs compared to oral treatment but the potential iatrogenic harm to joint tissues caused by certain types of antibiotics is rarely considered when selecting antibiotics for intra-articular administration. To address this question, we evaluated the cytotoxic effects of common classes of antibiotics on canine synovial and cartilage cells, using *in vitro* assays, to test the hypothesis that aminoglycoside antibiotics would be most toxic. Canine chondrocytes and synovial cells were collected, expanded in culture and plated in triplicate cultures for 48 hours in the presence of antibiotics at concentrations ranging from 25 to 0.39 mg/mL in complete media for 24 hours. Cell viability was assessed by trypan blue dye exclusion and 7-AAD uptake, using visual counting (trypan) and flow cytometric quantification (7-AAD). The inhibitory concentration for 50% cell viability (IC50) was determined for each antibiotic. Mechanism of cell death was investigated using caspase 3 staining. Induction of chondrocyte death was also assessed using cartilage explants and confocal microscopy. Antibiotic exposure decreased cell viability in monolayer culture and explant tissues in a dose-dependent fashion. Important differences in the relative cytotoxicity of antibiotics were identified. Amikacin and enrofloxacin were found to be the most toxic antibiotics while vancomycin and ampicillin-subactam demonstrated the least toxicity. Cell death was associated with caspase 3 induction, consistent with apoptotic cell death. These findings suggest that certain antibiotics (e.g. aminoglycosides) should be avoided when possible for IA administration in dogs, replaced by other, less-toxic antibiotics such as ampicillin-sulbactam or vancomycin.
Funding: CCTSI NIH/NCATS CTSA TL1TR002533 and the Shipley Foundation.

Staff / Clinical Sciences

O-9. Conformational variability of the pterygoid bone has no statistical correlation with acquired nasopharyngeal stenosis in dogs

Jonathan Plenn, Sangeeta Rao, Eric Monnet, and Lynn Griffin

Nasopharyngeal stenosis (NPS) is a rare disease characterized by narrowing within the nasopharynx resulting in inspiratory stridor that resolves when the mouth is held open. In domestic dogs and cats, the most common reported causes of NPS are idiopathic, chronic rhinitis and aspiration rhinitis in the peri-anesthetic period (secondary to gastroesophageal/nasopharyngeal reflux). At the authors' institution a case of NPS was diagnosed in a patient with a marked amount of irregularity and narrowing of the hamulus of the pterygoid. This finding suggested a possible correlation between patients with abnormal pterygoid anatomy and development of NPS.

Retrospective search of digital medical records was performed (May-2009 through September-2017). Selection criteria was limited to canines without congenital forms of NPS, confirmed nasopharygeal foreign bodies at the time of diagnosis, or neoplasia as the underlying etiology. Eight cases of NPS fit all inclusion criteria. Helical computed tomographic (CT) imaging studies for dogs affected with NPS were paired with control patients similar in size, breed and skull conformation for comparison of anatomy of the nasopharyngeal region. No statistical correlation between pterygoid conformation and development of nasopharyngeal stenosis was identified. All of the dogs in this study with NPS were young (less than 3.5yrs), consistent with prior literature. At least 50% (4/8) patients in this study implicate peri-anesthetic gastroesophageal reflux as the inciting cause of NPS, also consistent with prior literature. This study suggests that anatomic differences in the pterygoid do not pre-dispose dogs to NPS, and that a separate factor or factors are likely implicated.

Graduate Student / Environmental and Radiological Health Sciences

O-10. Ventral femoral head ostectomy: Standard versus novel K-wire guided technique using a pre-measured ostectomy angle in canine cadavers

Joseph Sapora, Clara S. Goh, and Ross H. Palmer

There are several proposed clinical benefits of a ventral FHO (vFHO). A previous study comparing vFHO to a standard craniolateral approach noted that removal of additional bone after initial ventral osteotomy was challenging. In this study a novel K-wire guide and pre-operative ideal FHO angle (iFHOA) were used to improve the efficiency and accuracy of vFHO, and the previously accepted 45° angle to guide vFHO osteotomies was assessed. A standard- and guided-vFHO were performed on each hip of 10 canine cadavers. A single unmodified osteotomy was performed in all cases. The pre- and post-operative iFHOA, and residual femoral neck were radiographically assessed. Subjective intraoperative palpation and postoperative radiographic scores were assigned. No significant difference was noted in subjective intraoperative osteotomy or radiographic scores ($p>0.63$), and guided vFHO were as good or better for 9/10 cadavers. Residual femoral neck measurements were similar for both groups ($p>0.75$). The average iFHOA in this study was 38.5°, with no significant difference between sides of the same cadaver ($p= 0.34$). Guided vFHO took significantly longer (294.5sec, $p=0.002$) than standard vFHO (166.7sec). Radiographic scores were as good or better for the guided vFHO group in 9/10 cadavers. Subjectively, the K-wire guide provided improved soft tissue retraction, neck visualization, and confidence in cut angulation. The mean iFHOA of 38.5° is less than the previously published 45° angulation described for vFHO procedures. Pre-operative iFHOA measurement may minimize risk of inappropriate vFHO osteotomy angle. **Funding**: CSU MacLaughlin fund

Resident / Clinical Sciences

O-11. “Ewe have bone marrow lesions”: experimental model development using the ovine stifle

Holly L. Stewart, Jeremiah T. Easley, Kurt T. Selberg, Brad B. Nelson, Christian M. Puttlitz, Chris E. Kawcak

Recent research has demonstrated that osteoarthritis is a disease of both cartilage and the underlying subchondral bone. Bone marrow lesions (BMLs), defined as increase fluid signal within the subchondral bone using magnetic resonance imaging (MRI), have been proposed as early indicators of progressive osteoarthritis. The purpose of this study was to develop a reproducible experimental model for BMLs using the ovine femorotibial joint. Six skeletally mature Dorper ewes were used for this study. A medial parapatellar arthrotomy was performed under general anesthesia and a 1.1 mm Steinmann pin was advanced to a depth of 8 mm in the central region of the medial femoral condyle. Computed tomography and MRI were performed biweekly for 90 days after surgery to monitor for development of BMLs and changes within the bone. BMLs were reliably generated in the medial femoral condyle of all sheep and observed using MRI within 14 days of surgery. The appearance of BMLs changed over the 90-day study period, reducing in size with a more defined region of signal intensity, similar to what is observed clinically. Histopathologic evaluation of experimentally-induced BMLs revealed local inflammation and remodeling. Taken together, this study confirms an experimental model for BMLs is possible using direct penetration of trabecular bone through the articular surface, and the appearance of BMLs on MRI mimic naturally-occurring pathologic states.
Funding: American College of Veterinary Surgeons Foundation Zoetis Dual Training Research Grant, College Research Council

Post-doctoral Fellow / Clinical Sciences

O-12. Retrospective analysis of the use of fluorine-18 fluorodeoxyglucose-positron emission tomography/computed tomography for the detection of metastatic lymph nodes in dogs diagnosed with appendicular osteosarcoma

Powell T Slinkard, Elissa K Randall, Lynn R Griffin

Fluorine 18 fluorodeoxyglucose positron emission tomography/ computed tomography (18F-FDG PET/CT) is a highly sensitive staging modality for detection of metastatic lymph nodes (LNs). For any type of cancer, the presence of metastasis to lymph nodes affects the prognosis. The primary objective of this retrospective analysis was to determine if 18F-FDG PET/CT is an accurate tool in the detection of metastatic lymph nodes in dogs diagnosed with appendicular osteosarcoma based on the measurement of maximum standard uptake values (SUVmax). A retrospective medical record search at the Colorado State University-Veterinary Teaching Hospital (CSU-VTH) was performed. Dogs were included in this study if they presented to the CSU-VTH for staging of a primary appendicular osteosarcoma with F18-FDG PET-CT between December 1, 2009 and May 8, 2019. Patients had to have the included lymph node(s) sampled cytologically or histologically following F18-FDG PET-CT. Patients were categorized based on lymph node status of either having metastatic or non-metastatic nodes. The SUVmax of the sampled lymph nodes were automatically measured by region-of-interest (ROI) analysis. There were 114 patients initially identified. A total 55/114 patients met the study inclusion criteria. There were 77 lymph nodes with diagnostic sampling. Of these, 3/77 (3.9%) were confirmed as metastatic. The median SUVmax of the lymph nodes (n = 77) was 2.2 (0.48- 11.35). The median SUVmax of the non-metastatic lymph nodes (n = 74) was 2.18 (range 0.48-11.35). The median SUVmax of the metastatic lymph nodes (n = 3) was 6.6 (range 2.2-8.91). A Mann Whitney U test revealed a statistical difference between the SUVmax of the metastatic versus non-metastatic lymph nodes (P-value=0.05 at a 95% confidence interval). This study showed a significant difference between metastatic and non-metastatic lymph nodes in dogs with appendicular osteosarcoma evaluated using F18-FDG PET-CT. **Funding**: Center for Companion Animal Studies Young Investigator Award

DVM Student / Environmental and Radiological Health Sciences

O-13. Preliminary evaluation of different anesthesia protocols in rabbits undergoing elective surgery

Michelle N. Sullivan, Miranda Sadar, Sangeeta Rao, Maya Grayck, Megan Gish, Laila Proenca, William Guerrera, and Pedro Boscan

Anesthesia-related morbidity and mortality rates in rabbits are at least three-fold higher than in cats and dogs. Rabbits are challenging to manage during anesthesia, and there are limited studies available comparing anesthetic quality in rabbits. The aim of the current study was to examine the effect of different drugs on anesthesia recovery quality. A total of 22 rabbits (12 male and 10 female) undergoing elective castration surgery were enrolled. Recovery quality was monitored using video surveillance viewed by three blinded reviewers. Parameters such as grooming, food/water consumption, and activity level were scored for post-operative hours 1, 6, and 12. Pain was also assessed using previously published scales (orbital tightening and ear position). Three different anesthetic drugs were compared: hydromorphone (0.2-0.3 mg/kg, n=7), dexmedetomidine (0.02-0.03 mg/kg, n=5), and butorphanol (0.2 mg/kg, n=15). We found that rabbits that received hydromorphone or dexmedetomidine groomed, ate or drank, and moved more than those that received butorphanol at post-operative hour 1 (28.6% vs. 20.0% vs. 0.0% groomed, 38.1% vs. 53.3% vs. 4.4% consumed, and 57.1% vs. 60.0% vs. 42.2% moved for hydromorphone, dexmedetomidine, and butorphanol, respectively) and hour 6 (47.6% vs. 46.7% vs. 22.2% groomed, 38.1% vs. 53.3% vs. 25.9% consumed, and 81.0% vs. 93.3% vs. 59.3% moved). Normal behaviors were exhibited by nearly all rabbits for each drug group by post-operative hour 12. Pain assessments were similar between groups for all timepoints. These data indicate that return of normal behaviors is faster with hydromorphone or dexmedetomidine relative to rabbits that received butorphanol. This suggests that 0.2 mg/kg butorphanol may not provide sufficient analgesia for elective surgery in rabbits, and traditional pain assessment methods may not correlate with return of normal behaviors. As this is a pilot study, enrollment of additional rabbits is necessary to elucidate statistical differences. **Funding**: Center for Companion Animal Studies Young Investigator Award, College Research Council

DVM Student / Clinical Sciences

O-14. Treatment of idiopathic chronic hepatitis and copper associated hepatopathy in dogs

Tarini V Ullal, David C Twedt, Sarah B Shropshire

Idiopathic chronic hepatitis (ICH) and copper associated hepatopathy (CAH) are common hepatic disorders in dogs. Treatment can prevent liver failure, but there is no established treatment protocol for either disease. To document the biochemical and histopathologic response in dogs diagnosed with ICH and CAH and treated with cyclosporine or penicillamine and a low copper diet, respectively. Six client-owned ICH and 3 CAH dogs were enrolled in a prospective case series. ICH dogs with interface hepatitis were treated with cyclosporine +/- low copper diet (if hepatic copper [Cu] > 1000 µg/g dry weight dw) for 6 months. CAH dogs with hepatic Cu > 1500 µg/g dw and absence of interface hepatitis were treated with penicillamine and a low Cu diet for 6 months. Clinical score (0-28), alanine aminotransferase (ALT), and hepatic cytology Cu grade (0-9) were monitored 1, 3, and 6 months into treatment. A laparoscopic liver biopsy was repeated at 6 months. Four of 5 ICH dogs and 2 of 3 CAH dogs completed the study or remain actively enrolled. All 7 dogs are alive with a median clinical score of 1, range 0-7. Two dogs were excluded due to adverse reactions to medications. In ICH dogs, ALT normalized in 1 and decreased from ≥ 15xULN (times upper limit of normal) to 1-2xULN in 3 dogs. Hepatic Cu normalized (< 400 µg/g dw) and Cu grade improved to ≤ 2/9 in both ICH dogs with elevated pre-treatment Cu that completed the study. In both CAH dogs, ALT improved mildly (5.3x to 4.3x, 4.2x to 3.2xULN) and hepatic Cu normalized in 1 dog that completed the study. Interface hepatitis resolved in all 3 re-biopsied ICH dogs. Cyclosporine or penicillamine with low Cu diet can improve ALT and normalize hepatic Cu in ICH and CAH dogs, respectively. **Funding**: American Kennel Club Foundation

Post-doctoral Fellow

Staff / Clinical Sciences

O-15. Incidence of shedding of equine herpes 2 and 5 in Colorado rescue horses

Carson Zweck-Bronner, Elsbeth O'Fallon, Erick Gagne, Gabriele Landolt

Up to 10,000 horses are housed at any given time at U.S. rescue facilities. As these horses are often malnourished, stressed and have a history of travel and comingling, they have a high risk of carrying contagious respiratory disease agents and are a likely source of transmission of contagious respiratory disease to other equids. This is a particular concern for infectious agents that can persist in hosts in the absence of clinical signs, such as the equine herpesviruses (EHV)-2 and -5. The goal of this study was to determine shedding frequencies of EHV-2 and -5 in nasal secretions of Colorado rescue horses and compare the presence or absence of virus shedding to clinical signs observed at the time of sampling. Nasal swab samples were collected from 49 rescue horses housed at 6 different facilities. The sample population was composed of mares, geldings, and stallions of different breeds, ages 9 months to 20 years. Clinical signs of infectious respiratory disease were defined as the presence of one or more of the following symptoms: lethargy, anorexia, nasal discharge, cough, or submandibular lymph node enlargement. Nasal swab samples were collected, placed in sterile saline, and stored at -80° C until analysis. Following DNA extraction, samples were analyzed by conventional PCR using primers designed to detect the EHV-2 and -5 glycoprotein B (gB) genes. Each assay included a positive control, consisting of DNA extracted from EHV-2 and -5 virus stocks, and a negative control consisting of saline. PCR results of nasal swab samples showed presence of viral DNA in nasal secretions of apparently healthy horses, consistent with previous reports of active shedding of EHV-2 and -5 in asymptomatic horses. These findings highlight the potential role of the rescue horse in the transmission and maintenance of equine contagious disease agents in the U.S. horse population. **Funding**: Center for Companion Animal Studies Young Investigator Award

DVM Student / Clinical Sciences

O-16. *In Vitro* Effects of Integrin Signaling Inhibition in Canine and Human Osteosarcoma Cell Lines

Lauren N. Alfino, Kai C. Wilczewski-Shirai, Makayla N. Risch, Eric P. Palmer, Dawn L. Duval, and Daniel P. Regan

Osteosarcoma (OSA) is the most common primary malignant bone tumor. New therapeutic strategies for OSA are desperately needed, as overall survival rates of OSA patients have remained unchanged for 30 years. Spontaneous OSA in pet dogs represents a valuable surrogate for evaluation of novel cancer therapies due to their many shared similarities including primary tumor location, overlapping genetic drivers, and the presence of pulmonary micrometastases at diagnosis. We assessed the in vitro effects of two integrin signaling-targeted drugs, Cilengitide, an αvβ3/5 inhibitor, and a focal adhesion kinase inhibitor (FAKi14) in canine and human OSA cell lines via western blot, flow cytometry, cell survival/proliferation and migration assays. FAKi14 demonstrated significant dose dependent inhibition of proliferation/survival in all cell lines at pharmacologically relevant concentrations. FAKi14 treatment was associated with decreased phospho-FAK, cell detachment and apoptosis in OSA cells. Cilengitide demonstrated significantly less anti-proliferative effects than FAKi14, with clinically relevant IC50 values not reached in half of the cell lines; however, Cilengitide mediated significant dose dependent inhibition of OSA cell migration in the majority of tested lines. Cilengitide also resulted in decreased phospho-FAK and OSA cell detachment similar to FAK inhibition but this was not associated with cell death, and instead promoted a spheroid morphology of OSA cells. Overall, these data suggest the potential for complimentary anti-neoplastic effects of combined FAK and αvβ3/5 signaling inhibition and warrant further in vitro and *in vivo* investigation of these drugs as novel therapeutic strategies for canine and human OSA. **Funding**: NIH 5T35OD015130, NIH 5K01OD022982-04

Graduate Student

DVM Student / Microbiology, Immunology and Pathology

O-17. Use of a novel Nrf2 activator to mitigate the progression of osteoarthritis in the Hartley guinea pig

Kendra. M. Andrie, Rob Musci, Sydney Bork, Daniel Palmer, Christian M. Puttlitz, Benjamin. F. Miller, Karyn. L. Hamilton, Kelly. S. Santangelo

Chronic inflammation and oxidative stress are key contributors to the pathogenesis of osteoarthritis (OA). Nuclear factor-erythroid 2-related factor-2 (Nrf2) is a transcription factor that serves as a master regulator of anti-inflammatory, phase I xenobiotic, and phase II antioxidant enzyme gene expression. Our central hypothesis is that innate downregulation in Nrf2/ARE signaling during aging serves as a central driver for persistent low-grade inflammation, dysregulation of redox homeostasis, mitochondrial dysfunction, and protein dyshomeostasis – all of which contribute to OA. The aim of this study was to demonstrate that treatment with a Nrf2 activator delays the progression of primary OA. Male (N=28) and female (N=28) Hartley guinea pigs, aged 5 months, were randomly assigned to receive daily oral treatment with either the Nrf2 activator, PB125, or vehicle control. Animals were treated for 10 months and sacrificed at 15 months, when advanced OA was expected in control animals. Gait analysis, whole joint histology, NanoString mRNA gene expression, micro-computed tomography, and femoral four-point bending mechanics were utilized as outcome measures to test the use of PB125 in delaying OA. Treatment with PB125 resulted in: increased stride length in females (P=0.02) and males (P=0.01) when run at 55 cm/sec and 75 cm/sec, respectively; decreased total knee joint histology score for OA (P=0.003); and increased long bone strength as determined by ultimate bending stress (P=0.006). Further, Nanostring gene expression profiling unveiled 10 month treatment with PB125 resulted in a significant decrease at the transcriptional level of: Nrf2 shutdown and repressor transcripts (KEAP-1, GSK3b), catabolic (MMP-13, MMP-9), anabolic (COL10A1, TGFB), inflammatory (IL1B, IL-6, PTGS-1, PTGS-2, NFkB, p65), pro-apoptotic (BAX, BIM, CASP 8, CASP9) and necroptosis (HMGB1, RIPK1) transcripts. Collectively, this work provides insights into the pathogenesis of OA and proof of concept for translational clinical trials using Nrf2 activation as a novel therapeutic target for managing disease. **Funding**: T32 OD010437, R21 AG054713

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

O-18. The effects of air pollutant levels on dairy cow health and production in Northern Colorado

Bonni L Beaupied, Heather Martinez, Colleen Duncan, Craig S McConnel, Sheena Martenies, Sheryl Magzamen

Exposure to air pollution, including criteria pollutants such as ozone (O_3) and aerosolized fine particulate matter ($PM_{2.5}$), has been associated with increased morbidity and mortality in mammals. These effects have primarily been studied in the laboratory or in humans living in urban settings. Situated in a controlled environment that facilitates data collection, dairy cattle present a unique, yet unexplored, opportunity to assess the correlation between subtle shifts in air pollution and mammalian health. Furthermore, ground-level O_3 peaks during the hot summer months, when dairy production is lowest, and should therefore be evaluated as a possible contributing factor in reduced milk production. This study aims to assess the effects of air pollutants on dairy cow health by comparing O_3 and $PM_{2.5}$ levels recorded by local US EPA air quality monitors to daily production data and bulk tank somatic cell counts. Initial results have supported the hypothesis that O_3 exposure is associated with reduced dairy yield. The results of this study may uncover areas for intervention to improve these impacts at the dairy level. These data will also contribute to a translational model comparing cattle health shifts to human health, particularly in rural settings or other regions with limited air quality data. **Funding**: One Health Institute, Office of the Vice President for Research, Colorado State University

DVM Student / Environmental and Radiological Health Sciences

O-19. Targeting PKC Θ in T cells as an immunotherapy for autoimmune diabetes

James Dilisio, Liane Sehrt, Noah Mishkin, Michael Mangalea, David Ackart, Andres Obregon-Henao, Marcela Henao-Tamayo, Brendan Podell

Protein kinase C (PKC) is a signaling molecule involved in a diversity of cellular actions with implications in diabetes. The novel PKC isoform, PKC-theta, is particularly enriched in T lymphocyte populations, serving a critical role in signal transduction after CD3/CD28 T cell receptor stimulation. PKC-theta is known to be involved in promoting T cell mediated immunity and has been implicated in autoimmune diseases in particular, where its activation impairs the generation of important regulatory T cell phenotypes. Since PKC-theta-null mice have increased populations of regulatory T cells, we hypothesized that inhibition of PKC-theta would increase regulatory T cell phenotypes, promote anti-inflammatory environments, and lead to delayed onset of type 1 diabetes in the NOD mouse model. A cell permeable pseudosubstrate peptide inhibitor was identified with potent suppression of IL-2 secretion at <15 mg in T cell enriched populations stimulated in vitro with CD3 and CD28 antibodies and a significant reduction in stimulated IL-2 secretion after 7 days of intraperitoneal treatment with 50 mg of pseudosubstrate inhibitor. Five week-old prediabetic NOD mice were treated daily with 50 mg of inhibitor or mock treated with saline for 4 weeks, then allowed to progress until 17 weeks of age. An 80% conversion to diabetes, based on IP glucose tolerance tests, was observed in saline mice compared to 30% conversion in inhibitor-treated mice, which correlated with a reduction of insulitis in inhibitor-treated mice. Inhibitor-treated mice had increased frequencies of IL-10 secreting cells by ELISPOT, and increased FoxP3+, CD25+, CD4+ Tregs in spleen at 17 weeks of age. Furthermore, reduced frequencies of IL-17+ CD4+ T cells were present in spleen of inhibitor-treated mice. Our data demonstrate that PKC theta activation contributes to the progression of beta cell destruction in NOD mice, where inhibition of this isoform may confer an increase in regulatory T cell. **Funding**: NIH ORIP 5K01OD016997 (BKP) and NIH/NCATS Colorado CTSI grant number UL1TR001082 (BKP)

Graduate Student

Staff / Microbiology, Immunology and Pathology

O-20. Microbes in the Mucosa: A Probiotic-Based Vaccine and the Gut Microbiome

Bridget Eklund, Darby Gilfillan, Alora LaVoy, Gregg Dean, and Zaid Abdo

The gut microbiome is a collection of microbes found along the gastrointestinal tract. However, the impact the immune response has on the microbiome is not well characterized, especially when the immune system is activated during vaccination. Understanding this relationship is important as the gut microbiome influences many aspects of an individual's health, including maintenance of intestinal homeostasis, nutrient processing, and maturation of the mucosal immune system. We are investigating two changes within the host after vaccination with a *Lactobacillus acidophilus* vector: the host's immune response to the vaccine and shifts in the microbiome bacterial community. This study uses a murine model to show the ability of this probiotic bacterium to deliver an HIV antigen (MPER) to relevant mucosal immune sites. The immune response to the vaccine and longitudinal shifts in microbiome are shown. We demonstrate antibody-specific induction of the immune response after vaccination, thus validating the bacterium *Lactobacillus acidophilus* is a suitable vector for delivering target antigens to mucosal effector sites. The microbiome will be analyzed further by sorting bacteria that are bound with mucosal IgA antibodies to further define the relationship between the microbiome and the mucosal immune system during oral vaccination. **Funding**: NFS GRFP

Graduate Student / Microbiology, Immunology and Pathology

O-21. Cyto-feature engineering: a flow cytometry analysis pipeline to uncover immune populations and association with disease

Amy Fox, Taru S. Dutt, Burton Karger, Mauricio Rojas López, Andrés Obregón-Henao, G. Brooke Anderson and Marcela Henao-Tamayo

Flow cytometers can now analyze up to 50 parameters per cell and millions of cells per sample; however, conventional methods to analyze data are subjective and time-consuming. To address these issues, we have developed a novel analysis pipeline to identify a plethora of cell populations efficiently. Coupled with feature engineering and immunological context, researchers can immediately extrapolate novel discoveries through easy-to-understand plots. The R-based pipeline uses Fluorescence Minus One controls or distinct population differences to develop thresholds for positive and negative marker expression. The continuous data is transformed into binary data, capturing a positive/negative biological dichotomy often of interest in characterizing cells. Next, a filtering step refines the data, from all identified cell phenotypes to populations of interest. The data can be partitioned by immune lineages and statistically correlated to other experimental measurements. The pipeline's modular nature allows customization of statistical testing, adoption of alternative initial gating steps, and incorporation of other datasets. Validation of this pipeline through manual gating of two datasets (murine splenocytes and human whole blood) confirmed its accuracy in identifying even rare subsets. Lastly, this pipeline can work on both small and large flow cytometry panels, can input clinical and research samples from different types of flow cytometers (e.g., conventional cytometers and spectral cytometers), and can use either Fluorescence Minus One controls or data that has clear population separation. **Funding**: NIH grant 1R01 AI127475-01A1 and National Science Foundation grant (DGE-1450032)

Graduate Student / Microbiology, Immunology and Pathology

O-22. Embryo mortality and peripheral transcriptome response in Holstein cows

Carolina L. Gonzalez-Berrios, Hanah M. Georges, Jeanette V.Bishop, Hana Van Campen, Milton G. Thomas, Thomas R. Hansen

Interuptions in conceptus-endometrial signaling on day 16 of pregnancy in dairy cows may cause embryo mortality (EM); such physiological mechanisms are unknown. It was hypothesized that EM pregnancies are associated with an impaired endocrine action of Interferon tau (IFNT) from the conceptus on the corpus luteum (CL) and peripheral blood mononuclear cells (PBMC), additional to paracrine failure in preventing the up-regulation of estradiol-mediated luteolytic signals in the endometrium. The study consisted of control cows on day 16 estrous cycle (EC; n = 7) and two other groups (n=15; inseminated) that had conceptuses flushed on day 16 of pregnancy and were sorted based on conceptus quality. EM conceptuses (n = 6) lacked elongation, were pink, red and/or opaque, while normal (N, n=9) were elongated and translucent. RNA extraction of conceptuses, endometrium, CL, and PBMC were submitted to RNA-Seq analysis. Data was trimmed, aligned and controlled for false discovery rate using DESeq2 package in R. Submission of data into Ingenuity Pathway Analysis (IPA) had fold change ≥1.5 and P≤0.05. Previously, we reported N were longer (P<0.006) and had greater (P<0.0001) IFNT mRNA transcript raw counts (from RNA-Seq) than EM conceptuses. IFNT protein concentrations (ELISA) in uterine flushings were greater (P< 0.004) in N and EM tended (P < 0.07) to be greater than EC cows. The key upregulated biological pathway in IPA for EMvsN conceptuses was T helper cell activation. Sixty-percent of endometrial genes were upregulated and functioned in estradiol signaling for EMvsN. Ten genes in CL were upregulated and function in pro-inflammatory, calcium binding, and inhibition of adenylate cyclase responses in EMvsN. No differences in transcripome were found between EMvsN for PBMC. In summary, compromised conceptuses have upregulated calcium-mediated responses that promote a localized overproduction of immune cells, disruption of steroidogenesis in the CL and thus, pregnancy loss. **Funding**: USDA-AFRI-NNF 2016-38420-25289

Graduate Student / Biomedical Sciences

O-23. Long term vitamin a deficient guinea pig model: a model for *mycobacterium tuberculosis* infections in vitamin a deficient humans

Macallister C. Harris, , James E. Dilisio, Randall Basaraba, Noah Mishkin, Kody Armann, Brendan Podell

Two recent studies demonstrated that subclinical vitamin A deficiency (<200 ug/L) in humans increases the risk of infection with mycobacterium tuberculosis (TB). Previously our lab demonstrated that the guinea pig is a valid model for vitamin A deficiency for short term (21 day) TB infections. The current work investigates this model during a long term TB infection study (60 days). 9 to 13 day old guinea pigs were separated in vitamin A sufficient (VAS), vitamin A deficient (VAD), and vitamin A partially deficient (VA Supp) groups. Vitamin A deficiency was achieved in the VAD group between 4 to 8 weeks. Once deficient, all groups were inoculated with TB, and monitored via weight, blood and urine collection for 60 days. On day 60, tissues were collected. Results demonstrated that VAD guinea pigs had a significantly higher splenic lesion burden with similar lesion burden and CFU counts in the lung. However, pulmonary lesions in VAS pigs demonstrated well-delineated organized type 1 granulomas with necrosis vs. VAD pigs which had poorly delineated and organized histiocytic lesions. On a cellular level the VAD group had markedly decreased CD4+ and increased CD8+ T-cells (ratio 1:1). Nanostring was performed to evaluate the expression of vitamin A metabolic genes and immunomodulatory genes. This analysis demonstrated increased expression of vitamin A related genes in the VAS guinea pigs and increased of immunosuppressive genes in the VAD guinea pigs. These results indicate that the VAD group had shifted pathologic, cellular and genetic responses to TB compared to the VAS group. Additionally this shifted response led to higher splenic pathology, potentially indicating loss of local control of TB in the VAD guinea pigs leading to greater systemic spread. This model serves as a potential model for the role of vitamin A in the pathogenesis of Mycobacterium tuberculosis. **Funding**: U19AI111224 supplement to BKP, R21AI144662 to BKP

Graduate Student

Resident / Microbiology, Immunology and Pathology

O-24. Reverting prion disease in neuronal progenitor cells as a cell replacement therapy

Arielle Hay, Lindsay Parrie, Mark Zabel, Julie A. Moreno

We propose the use of gene edited stem cells as therapy for protein misfolding neurodegenerative diseases. This therapy will be modeled by prion infected mice, which display the typical features of neurodegenerative disease. Prion protein (PrP) is highly expressed in neurons, and its misfolding results in disease. Early signs of this disease include glial inflammation, followed by the irreversible loss of neurons. Mesenchymal stem cells (MSCs) can be derived from adipocytes of adult mice and further differentiated to neural stem cells (NSCs). These cells express immunomodulatory and anti-inflammatory mediators. Olfactory neuronal progenitors (ONPs) can differentiate into neurons in adulthood. We hypothesize that together these cells will reduce neuroinflammation and regenerate neurons that have been lost due to aggregation of disease-associated proteins. PrP knockout mice are resistant to prion diseases. To ensure that the ONPs are resistant to disease, CRISPR/Cas9 gene editing will be used to delete the *prnp* gene. Additionally, we will edit the *prnp* gene in M/NSCs to be resistant to infection by expressing a secretable, dominant-negative PrP. Single cell sorting will be used to produce homogenous populations of edited cells, which will be sequenced to identify relevant mutations. Gene edited ONPs and M/NSCs will be instilled intranasally or engrafted into the brains of prion infected mice. We hypothesize that these cells will resist prion infection. ONPs will migrate throughout the brain to restore damaged neurons and M/NSCs will secrete dominant negative PrP and anti-inflammatory cytokines.

Graduate Student / Microbiology, Immunology and Pathology

O-25. In silico mouse model of infection and immunity

Daniel Jonas, Michael J Kirby, Alan R Schenkel

An organism's immune system tries to protect it primarily by identifying the presence of pathogens and attempting to eliminate them. The defense is twofold: innate immune cells mobilize rapidly, while acquired immune cells slowly develop into pathogen-killing specialists. These responses incur collateral tissue damage, which anti-inflammatory mediators seek to control. This system of checks and balances is responsible for host survival. Experimental research has demonstrated how vastly complex these interactions are, indicating a place for theoretical and computational study. In this work we develop a comprehensive dynamic model of the immune system by considering the interactions between major immune system components in the presence of pathogen or tissue trauma. Through this step-by-step construction we explore the dependence of the anti-inflammatory mediators on pathogen levels, and also how they temper the immune responses at the tail end of an infection, by considering different growth and death terms. We then challenge the virtual mouse with typical pathogens of varying virulence. Our model indicates that if the anti-inflammatory mediators depend on the presence of pathogen, then they must do so via an inverse relationship to avoid over-suppressing the immune responses. We observe that anti-inflammation can downregulate the activation and proliferation of immune cells or promote apoptosis as cessation mechanisms, suggesting the need for experimental work to shed light on this dynamic. Finally, we find that initial insult and pathogen growth rate play important roles in determining whether the in silico mouse overcomes the infection. In future work we will incorporate measurable variables into our model to validate it with data and estimate parameters; model atypical pathogens, and investigate the effects of immune system primers such as vaccinations. **Funding**: College of Natural Sciences

Graduate Student / Microbiology, Immunology and Pathology

O-26. Longitudinal Detection of Chronic Wasting Disease Prions in Nasal Swab Collections by Real-Time Quaking- Induced Conversion

Caitlyn N Kraft, Nathaniel D Denkers, Candace K Mathiason, and Edward A Hoover.

Chronic wasting disease (CWD) is an emergent transmissible spongiform encephalopathy of cervids now identified in North America, South Korea, and Scandinavia. The exact mechanisms of CWD transmission remain unknown; thus efforts to identify the pathways of prion shedding and transmission remain a high priority. Previous studies have shown that nasal olfactory epithelium can contain CWD seeding activity, yet the temporal profile of prion shedding in nasal secretions has not been identified. In this study, we characterize CWD pathogenesis using longitudinal nasal swab samples from twenty (20) CWD-inoculated deer by iron-oxide magnetic extraction coupled with real-time quaking induced conversion (IOME-RT-QuIC) protocol. We correlated these assay findings with those from longitudinal tonsil biopsies and terminal tissue samples from the same deer assayed by immunohistochemistry and RT-QuIC. Our results demonstrate prion shedding in nasal secretions in 11 of 20 deer as early as two months after first tonsil biopsy positivity (mean=10.3 months; range: 2-17 months). Once detected, persistent prion shedding in nasal fluid occurred of 9 of the 11 deer. Prion seeding activity in frontal cortex and olfactory bulb regions of the brain was demonstrated in 15 of 20 deer, suggesting brain involvement precedes nasal shedding. We conclude that CWD-infected deer shed prions in nasal secretions throughout mid to late stages of pathogenesis. In that direct nasal contact is common in social interactions, it is likely that nasal fluids play a significant role in the horizontal transmission. Moreover, nasal swabs may also constitute an accessible sample for antemortem diagnosis using the RT-QuIC assay. **Funding**: NIH R01-NS-061902, P01-AI-077774, F30-ODO-118143, T32-OD0-10437

Staff / Microbiology, Immunology and Pathology

O-27. Validation of remote sensing and simulation methods for animal infectious disease modeling

Grace Kuiper, David South, Susan Maroney, Chris Burdett, Andrew Fox, Kelly Patyk, Mary Jane McCool-Eye, and Sheryl Magzamen

Comprehensive location data are not available for the United States poultry industry, which limits geospatial analyses of zoonotic and livestock-associated infectious diseases. A novel "hybrid" method for locating operations was developed that leverages both remote sensing spatial accuracy and simulation modeling feasibility. We conducted a validation was conducted to compare accuracy of the hybrid model and a fully-simulated model (Farm Location and Animal Population Simulator, i.e. FLAPS). For spatial accuracy, buffers generated around each ground truth operation were assigned "Yes/No" for the inclusion of a hybrid or FLAPS poultry operation. Additionally, root mean square errors (RMSE) of the difference in farm count per grid cell were used to compare clustering patterns of the hybrid and FLAPS models to true distributions of poultry operations. For all radii tested, the proportion of true farm buffers that captures at least one hybrid model operation is greater than the proportion that captures a FLAPS operation. Approximately 57% of the 1,000-meter buffers capture hybrid operations, which is significantly greater than FLAPS (28%, $p<0.001$). RMSE values suggest that, at all grid sizes, the distribution of hybrid model operations is more similar to truth compared to FLAPS data. For example, RMSE is 0.93 for the hybrid model and 1.18 for FLAPS for a 3,000-meter grid. Compared to FLAPS, the hybrid model demonstrated greater locational accuracy and more closely resembled true poultry operation distributions. Future adaptations of this method may mitigate the analytical challenges of modeling infectious disease transmission and support control of high priority livestock-associated animal diseases. **Funding**: USDA

Staff / Environmental and Radiological Health Sciences

O-28. Chemical separation of the minor actinides: towards a closed nuclear fuel cycle

Samantha A. Labb, Ralf Sudowe

Modern civilization has been made possible through the use of heat energy provided from fossil fuels. However, in doing so, natural resources have been expended and the environment has suffered. An alternative source for clean and reliable energy is essential and nuclear energy needs to be considered. While nuclear power has numerous advantages, the long-term disposal of the radioactive byproducts created through nuclear fission remains a predominant environmental and political issue. Spent fuel contains mainly inert materials, but the trace amount of the minor actinides (americium and curium) contribute to its increased heat load, radiotoxicity, and shelf life. The Partitioning and Transmutation (P&T) of spent fuel involves the separation of these components from the waste and their transformation into less hazardous elements. Thus, finding ways to efficiently separate these transplutonium elements is crucial in helping overcome resistance to nuclear power. The significant scientific and technological challenge in achieving these separations stems from 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 is able to 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. Three oxidizing agents, sodium bismuthate, copper(III) periodate, and sodium persulfate, have been shown to have the ability to oxidize americium. The goal of this research is to compare the oxidative action of these three candidates. In addition, the potential of these oxidizing agents as a driving force for the separation on DGA extraction chromatographic resin will be explored. As a result, new potential candidate systems for minor actinide separation can be studied. **Funding**: Nuclear Regulatory Commission (NRC)

Graduate Student / Environmental and Radiological Health Sciences

O-29. Mitochondrial PUFA Metabolism Regulates Myocardial Ischemic Tolerance

Lance C Li Puma and Adam J Chicco

Common polymorphisms of the *FADS2* gene associated with hyperactivity of its product, delta-6-desaturase, predict cardiovascular morbidity and mortality in humans. To investigate this, we generated mice with global (CMV promoter) transgenic overexpression of *Fads2* (TG). These mice exhibit elevated serum omega-6 product/precursor PUFA ratios characteristic of human *FADS2* polymorphisms. TG mice have no overt cardiac pathology under basal conditions, but exhibit 60% larger myocardial infarcts following ischemia/reperfusion *ex vivo* compared to wild-type (WT) controls. Interestingly, cardiac mitchondria from TG mice were found to have greater membrane levels of arachidonic acid compared to WT, which were selectively depeleted following a non-lethal ischemia/reperfusion protocol. To investigate this further, we evaluated TG and WT cardiac mitochondrial responses to progressive titrations of 25 µM free Ca^{2+} by high-resolution respirometry and fluorometry, modeling the cardiomyocyte calcium overload conditions experienced during ischemia/reperfusion. OXPHOS-linked respiration declined in TG at a lower cumulative Ca^{2+} concentration than WT mitochondria, while H_2O_2 released per O_2 consumed and the extent of mitochondral swelling were higher in TG. Preincubation of mitochondria with pharmacological inhibitors towards arachidonic acid release or metabolism attenuated Ca^{2+} induced damage in both WT and TG mitochondria, with TG mirroring WT levels. Taken together, these studies establish an important role of *Fads2* in modulating cardiac ischemic tolerance and membrane PUFA composition, and suggest a mechanistic role of mitochondrial membrane arachidonic acid hydrolysis on cardiac responses to Ca^{2+}-overload in these contexts. This work was supported by grants from the American Heart Association. **Funding**: American Heart Association

Graduate Student / Biomedical Sciences

O-30. Wildfire smoke may interfere with the use of black carbon as an indicator of traffic exposure

Sheena E Martenies, Sherry WeMott, Grace Kuiper, Kacy Lorber, Cody Dawson, Kevin Andresen, William B Allshouse, Anne P Starling, John L Adgate, Dana Dabelea, Sheryl Magzamen

Black carbon (BC) has been used to characterize traffic-related air pollution (TRAP) exposure. However, BC has multiple sources, including wildfire smoke (WFS). We examined the potential for WFS to bias TRAP exposure assessments during wildfire events impacting Denver, Colorado. Weekly integrated BC samples were collected May-November, 2018. For each filter we calculated a time-weighted average BC concentration and assessed the length of major roads in a 300-m buffer around the sample location as a comparative measure of TRAP. A filter was considered impacted by WFS if the closest network monitor recorded a weekly mean concentration at least one standard deviation (SD) above the 10-year monthly mean and if a smoke plume was present within 50 km. We used the Kruskal-Wallis test and correlation to compare BC concentrations by roadway length. We collected 624 filters from > 50 locations across the region, 36% of which were WFS-impacted. The mean (SD) BC concentration was 1.15 (0.25) µg/m^3. Median BC concentrations were 12% higher for WFS-impacted filters (1.16 µg/m^3) than non-impacted filters (1.02 µg/m^3) and urban gradients during wildfire events were reduced. Similarly, the median absolute deviation for WFS-impacted filters (0.19 µg/m^3) was slightly higher than for non-impacted filters (0.16 µg/m^3), suggesting increased variability in concentrations during wildfire events. For each roadway-length quartile, median concentrations for WFS-impacted filters were higher than for non-impacted filters: Q1: 1.13 vs. 0.97 ($p < 0.01$); Q2: 1.14 vs 0.98 ($p < 0.01$); Q3: 1.16 vs 1.01 ($p < 0.01$); and Q4: 1.19 vs 1.15 µg/m^3 ($p = 0.1$). Correlations between BC concentration and roadway-length were similar for WFS-impacted filters (r_s=0.24) and non-impacted filters (r_s=0.29). Exposure assessments relying on BC as a proxy for TRAP may be biased by wildfire events. Future work will identify the extent to which this bias may affect epidemiology studies. **Funding**: National Institutes of Health Office of the Director

Post-doctoral Fellow / Environmental and Radiological Health Sciences

O-31. Determination of the mechanism of oncolysis by MYXVorfC, a myxoma virus that expresses a pro-apoptotic protein

Jasmine L. McCoy, Laura V. Ashton, Garin C. Wilson, Mariah S. Jordan, and Amy L. MacNeill

MYXVorfC is a newly engineered myxoma virus (MYXV) that expresses a pro-apoptotic protein and has the potential to be used as an oncolytic virotherapy. The goal of this study is to determine the mechanism of cell death induced in MYXVorfC-infected cancer cells. We hypothesize that the insertion of the orfC gene will allow MYXVorfC to induce high levels of apoptosis by activation of the intrinsic pathway. We infected canine osteosarcoma and soft tissue sarcoma cells, and a rabbit kidney cell line with MYXVorfC or wild type MYXV. We then looked at processes involved in the pathway including: change in mitochondrial cell membrane potential, activation of cytochrome C oxidase, caspases 9 and 3, and detection of phosphatidylserine on the cell surface. The results showed that mitochondrial membrane potential is being disrupted by 24 hours post-inoculation (hpi), cytochrome C oxidase is being activated as early as 12 hpi, and activation of caspase 9 and 3 peak between 12 and 18 hpi. Detection of phosphatidylserine peaks and cell viability is significantly decreased 48 hpi with MYXVorfC as compared to MYXV. This supports that MYXVorfC uses the intrinsic pathway and may be a more effective cancer treatment than MYXV. Future experiments will assess the extrinsic pathway. Currently, we are evaluating the safety of MYXVorfC in rabbits (the natural host of MYXV). If the virus is safe, we plan to test its efficacy in dogs with cancer. **Funding**: College Research Council, NIH 5T35OD015130

DVM Student / Microbiology, Immunology and Pathology

O-32. Regionally-biased CNVs accompany large-scale chromosomal rearrangements in S. cerevisiae

Sean A Merriman, Ane F. B. Zeidler, Matthew Dilsaver, Ruthie Watson, and J. Lucas Argueso

In the last decade the field of genomic medicine has experienced unprecedented growth made possible by massive improvements in DNA sequencing technologies. One of the main breakthroughs that followed was the discovery that many of the genetic differences that exist between healthy and cancer cells are variations in the number of copies of their genes. Such gene copy number variations (CNVs) are a particularly important component of the altered genomes of breast and ovarian cancer cells. Despite the importance of CNVs to cancer development, our understanding of the mechanisms that trigger these large-scale mutations is still very limited. Using yeast as a model in which to study such CNV-generating mutations, the J.L. Argueso lab has discovered that a specific region of *S. cerevisiae* genome (the right arm of chromosome 7; Chr7R) is much more susceptible to the formation of chromosomal rearrangements leading to large deletions or translocations than other apparently similar segments of the genome. To further illuminate the nature of these rearrangements, we are utilizing cell-based genetic assays, pulsed-field gel electrophoresis, and array comparative genomic hybridization, techniques which facilitate detection of both novel fusion chromosomes as well as genome-wide CNVs. We are hopeful that our findings will open a window into the fundamental cellular processes that are responsible for CNVs found in eukaryotic genomes, and inform translational implications for modeling this class of mutation in cancer. **Funding**: NIH Grant 5R35GM119788, qCMB T32 Training Grant T32GM13205

Graduate Student / Environmental and Radiological Health Sciences

O-33. Novel identification of antimicrobial resistance markers among *Salmonella* Typhimurium across humans, bovine, and porcine hosts using metabolomics

Jessie M Overton, Roberta Magnuson, Elizabeth P Ryan, Corey Broeckling, and Sangeeta Rao

Antimicrobial resistance (AMR) in bacteria of animal origin is a major, global public health threat. Tools for detecting phenotypic resistance patterns are limited and require advanced molecular methods to reveal associations with the AMR patterns. The metabolomics approach will produce metabolite profiles and help provide scientific evidence if there are differences in metabolite regulation between *Salmonella* Typhimurium from various hosts. This could lead to advancements in drug discoveries and act as indicators of cellular effects of AMR. This research has two specific aims 1) to evaluate metabolomic profiles of *Salmonella* Typhimurium indicating AMR 2) to evaluate homogeneity or differences in metabolomic profiles of *Salmonella* Typhimurium across various host species. Three samples each from bovine, porcine and humans were selectively chosen from a library to undergo an antimicrobial drug growth challenge using an ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfisoxazole, tetracycline) panel in one set of the 9 isolates and no drug on the other set of the same 9 isolates. This method was followed by a non-targeted metabolomic analysis. All 9 isolates were previously determined to be resistant to each of the ACSSuT drugs. Metabolomic profiling was conducted using Ultra Performance Liquid Chromatography-Mass Spectrometry and Gas Chromatography-Mass Spectrometry. MetaboAnalyst 4.0 was used to conduct Analysis of Variance (ANOVA), 2-way ANOVA, Principal Component Analysis, and post-hoc analysis on the data. Metabolite regulation was more effected by the antibiotic exposure irrespective of the host species. Of all metabolites, 26.2% and 14.6% were upregulated and downregulated, respectively, when exposed to antibiotics. Glycerophospholipid metabolism and Pantothenate/CoA biosynthesis were the most effected pathways. The biomarkers that were identified to be upregulated of those pathways were phosphatidylethanolamine, phosphatidylcholine, and LPC(18:1(9Z)). These three biomarkers have known associations with antibiotic-resistance. Targeting these biomarkers and their associated pathways could be a stepping stone to lead to new drug discoveries. **Funding**: USDA Animal Health & Disease Formula Funds

DVM Student
DVM/MPH Student / Clinical Sciences

O-34. Presence of Clock genes in equine full-term placenta

Agata M. Parsons Aubone, Christian M. Bisiau, Patrick M. McCue, Gerrit J. Bouma

Mammals have a circadian rhythm which is synchronized by a master clock located in the hypothalamic suprachiasmatic nucleus (SCN). The SCN regulates additional clocks located in peripheral tissues, including some involved in endocrine or reproductive functions. Studies in humans and mice report that molecular clocks also exist in the placenta. However, little is known about the presence of "Clock genes" in equine placenta. Pregnancy length in mares varies and shows fluctuations in hormone concentrations throughout pregnancy. We postulate that similar to humans and mice, "Clock genes" are present in the horse placentas. Our goal was to determine if relative levels were different between placentas associated with male and female fetuses or correlated with gestational length. Placenta tissue samples were obtained from twenty-one pregnant mares following normal unassisted foaling by vaginal delivery. Placental biopsy samples were collected from four different areas; uterine body, pregnant horn, none pregnant horn and cervical star. From each area one section was immediately fixed overnight in 4% paraformaldehyde (PFA) and a second section was snap frozen in liquid nitrogen and stored at -80°C until processed. We used PCR and immunofluorescence to study the presence of Circadian Locomotor Output Cycles Kaput (CLOCK), Brain and Muscle Arnt-Like 1 (BMAL1), Period1 (PER1), Period2 (PER2), Cryptochrome 1 (CRY1) and Cryptochrome 2 (CRY2) in full-term mare placentas. "Clock genes" were present in horse all placentas. Student T-test analysis was performed to determine difference between female and male placentas, revealing lower relative levels of CRY2 and CLOCK in placentas associated with male fetuses. A Pearson correlation analysis showed no association between relative levels of "Clock genes" and gestational length. In conclusion, "Clock genes" are present in term equine placentas, and possibly play a role in sex-different regulation of placental function and pregnancy. **Funding**: College Research Council

Graduate Student / Biomedical Sciences

O-35. Evaluation of electroacupuncture for symptom modification in a rodent model of spontaneous osteoarthritis

Alexa R Personett, Maryam F Afzali, Richard B Martinez, Lauren A Culver, Sarah E Leavell, Ariel Timkovich, Joseph L Sanford, Melinda R Story, and Kelly S Santangelo

When faced with the frustration of chronic discomfort and restricted mobility due to osteoarthritis, many individuals have turned to acupuncture. Acupuncture is a traditional Chinese practice for pain alleviation that involves needle insertion into the skin followed by manual or electrical stimulation. However, the efficacy of acupuncture is uncertain, as much of the evidence is of questionable quality. The purpose of this study was to evaluate electroacupuncture in a rodent model of osteoarthritis such that unbiased conclusions regarding its efficacy for symptom modification could be drawn. Ten 11-month-old, male Dunkin Hartley guinea pigs, which characteristically have moderate osteoarthritis at this age, were randomly assigned to receive electroacupuncture (n=5) or anesthesia only (n=5). Gait analysis and enclosure monitoring were performed bi-weekly to evaluate changes in movement. Serum was collected for inflammatory biomarker testing, and knee joints were collected for histology and gene expression. Statistical analyses were performed with unpaired t-tests for normally distributed data or Mann-Whitney tests for non-normally distributed data. Animals receiving electroacupuncture had significantly greater changes in movement parameters compared to those receiving anesthesia only. There was a trend toward decreased serum C3 and TNF protein concentrations in the electroacupuncture group compared to the anesthesia group. COL2A1, FGF18, TIMP1, TGFβ1, and SOD2 gene transcripts in articular cartilage were significantly increased by electroacupuncture. There was not a significant difference in total joint histology scores between groups. This study provides evidence that electroacupuncture had a positive effect on symptom, but not disease, modification in a rodent model of osteoarthritis. Further investigations into mechanistic pathways that may explain the efficacy of electroacupuncture in this animal model are needed. **Funding**: College Research Council

Graduate Student
Resident / Microbiology, Immunology and Pathology

O-36. Spillover of Feline Leukemia Virus from domestic cats to North American pumas

Raegan J Petch, Erick B Gagne, Elliott S Chiu, and Sue VandeWoude

Feline Leukemia Virus (FeLV) is a gammaretrovirus that is horizontally transmissible between many species of felids. Approximately 25% of animals infected with FeLV experience progressive infections, which can result in mortality in wildlife and domestic cats, as evidenced by the outbreak of FeLV that occurred in the Florida panther population during the early 2000's. Previous studies have documented that FeLV is transmitted from domestic cats to pumas. We conducted an extensive survey of FeLV infection using a large biobank of samples collected from free-ranging puma and domestic cats presented to shelters from California, Colorado, and Florida to assess risk of this infection in each species. Samples from 651 puma and 307 domestic cats were tested for the presence of FeLV using a standardized quantitative PCR assay. Proviral load was quantified in positive samples by normalizing with the puma CCR5 gene, and an FeLV *env* genome segment was amplified by PCR, cloned, and sequenced to assess unique identify of individual infections. We identified 39 positive samples in pumas (6.0% prevalence) and 23 positive samples in domestic cats (7.5% prevalence). Regional differences were detected, with the highest prevalence in both cats and puma occurring in Florida. Preliminary data obtained from genomic analysis of the *env* region of the viral genome demonstrates variation between viral sequences in different samples. A seven-log variation was detected in proviral load across samples, indicating different infection outcomes following exposure, similar to what has been described in domestic cats. Our data provides evidence that FeLV is relatively common in pumas, potentially indicating interactions with domestic cats which may vary by region. Future studies will assess genotypic relationships of FeLV in the two species and further characterize the relationship of infection outcomes to demographic and ecological conditions. This research was supported by NSF (DEB-1413925) and the Felidae fund. **Funding**: NSF (DEB-1413925) and the Felidae fund

Undergraduate Student / Microbiology, Immunology and Pathology

O-37. Investigating the function of RAD51AP1 in homologous recombination DNA repair

Elena Pires, Neelam Sharma, Wiexing Zhao, Patrick Sung, Claudia Wiese

DNA double-strand breaks (DSBs) are considered the most detrimental DNA lesions induced by ionizing radiation (IR). Cells respond to DSBs by activating DNA damage response pathways that includes DNA damage repair. Flawless DNA repair through homologous recombination (HR) is essential for correcting DSBs, maintaining genome integrity, and preventing cancer. A pivotal step in HR involves the joining of homologous DNA strands via formation of a presynaptic RAD51 nucleoprotein filament and subsequent D-loop. During this step of the HR reaction, RAD51 interacts with RAD51-Associated Protein 1 (RAD51AP1). Nonetheless, how exactly RAD51AP1 functions during the HR reaction is still poorly understood. Our main objective is to define key attributes of RAD51AP1 in the HR reaction by further characterizing the DNA-binding properties of recombinant human RAD51AP1. Using the electrophoretic mobility shift assay, we find that RAD51AP1 avidly associates with both naked and chromatinized double-stranded (ds)DNA. Deletional and mutational analyses were used to further define the DNA- and chromatin-binding regions in RAD51AP1 via division of the protein into three fragments: F1 (residues 1-94), F2 (residues 95-187), and F3 (residues 188-335). We show that the N-terminal 94 residues of RAD51AP1, which are able to associate with naked dsDNA avidly, are devoid of binding to chromatinized dsDNA. In contrast, the C-terminal 148 residues of RAD51AP1 show affinity for both naked and chromatinized dsDNA. Two post-translational modification sites that lie within its C-terminal DNA-binding region were also evaluated and showed decreased affinity to chromatinized dsDNA. Based on these findings and other results, we propose a model in which RAD51AP1 guides homology search and hetero-duplex formation after presynaptic filament formation in the HR reaction. By understanding the biology of this important HR protein, we expect to have direct clinical relevance via the optimization of targeted cancer therapies. **Funding**: NIH T32OD012201

DVM/PhD Student / Environmental and Radiological Health Sciences

O-38. Cold Blood: Reptiles and amphibians as reservoir and overwintering hosts for arboviruses

Izabela K Ragan, Stephanie Porter, Airn Hartwig, and Richard A Bowen

Arthropod-borne pathogens continue to have a significant threat to animal and human health. A majority of these pathogens that affect humans are maintained in enzootic cycles where the pathogen cycles between an animal reservoir and hematophagous arthropods. Animal reservoirs like ectothermic vertebrates are ubiquitous and may be important in the maintenance and overwintering of viral pathogens. Viremia in ectothermic vertebrates has been demonstrated for emerging arthropod-borne viruses (arboviruses) like Zika, chikungunya, and West Nile virus. However, there is a limited understanding of the viral pathogenesis in ectothermic vertebrates under various environmental conditions. Our objective is to better understand the role of amphibians and reptiles in the maintenance, overwintering, and amplification of arboviruses. We first screened a diverse range of arboviruses (Zika, Dengue, La Cross, Mayaro, yellow fever, Venezuelan equine encephalitis, and Rift Valley fever viruses) for viremia and antibody production in four ectotherm vertebrate species (frogs, toads, snakes, and iguanas). To date our results show that infectious virus was detected in blood from frogs infected with Zika and Venezuela equine encephalitis viruses, toads and snakes with Venezuelan equine encephalitis and Mayaro viruses, and iguanas with Venezuelan equine encephalitis virus. Additionally, antibodies were detected in sera against yellow fever virus in frogs, Rift Valley fever virus in iguanas and frogs, and Zika virus in iguanas. Next, we will investigate the influence of temperature changes on viremia production for select arboviruses in ectotherms. Lastly, an artificial ecosystem will be used to evaluate arbovirus transmission cycles among ectothermic vertebrate hosts and mosquitos. The knowledge gained from these studies will significantly enhance our understanding of ectothermic vertebrates as reservoir hosts for several arboviruses with significant public health consequences. This in turn will aid in developing global measures to reduce or eliminate the disease risk associated with these pathogens. **Funding**: NIH grant 1R21AI137954-01A1

Post-doctoral Fellow / Biomedical Sciences

O-39. Does the attenuation of proinflammatory cytokines in the upper airways improve pulmonary function among dairy workers?

James Seidel, Grant Erlandson, Julia D. Labadie, Sheryl Magzamen, Julia Sharp, Ken Jones, Matthew Nonnenmann, Stephen J. Reynolds, Joshua W. Schaeffer

Purpose: Dairy workers are exposed to bioaerosols, which typically contain high abundances of bacteria and associated pro-inflammatory constituents (e.g., endotoxin). Repeated exposures have led to an increased burden of respiratory inflammation among dairy workers. Very few studies have evaluated control strategies to reduce exposure and improve health. Given challenges and limitations of implementing engineering controls in a dynamic industry, we propose the use of a low-cost and low-burden intervention. Hypertonic saline (HTS) was recently shown to attenuate inflammation in trauma patients. Here, we conducted an intervention to administer HTS in a nasal lavage to mitigate inflammation and improve airway function. Methods: Ten participants were recruited from a large herd dairy and randomly assigned to treatment (n=5) and control groups (n=5). Each participant received normotonic saline nasal lavage (NL) before their shift over five consecutive days. After each shift, the treatment group received HTS while the control group received normotonic. All samples were analyzed for pro-inflammatory cytokines. Spirometry was performed pre- and post-shift to examine forced expiratory volume in one second, forced vital capacity and the subsequent ratio and peak expiratory flow rate (PEFR). Statistical analyses and models were performed using SAS. Results: Differences in pro-inflammatory cytokines (IL-6 and IL-8) were observed between hypertonic and control groups. There was an interaction effect on IL-10 between treatment, day and time, indicating promotion of anti-inflammatory effect. The treatment group experienced an increase in PEFR change (0.76 L, p=0.12) near statistical significance; no statistically significant differences were observed in the other spirometry measures.
Funding: High Plains Intermountain Center for Agricultural Health & Safety

Graduate Student / Environmental and Radiological Health Sciences

O-40. Synergism between RAD51AP1 and RAD54 during the synaptic stage of homologous recombination DNA repair

Platon Selemenakis, Neelam Sharma, David Maranon, Weixing Zhao, Patrick Sung, Claudia Wiese

Radiation therapy (RT) uses ionizing radiation (IR) to produce a plethora of DNA lesions, the most toxic one of which is a DNA double-strand break (DSB). One of the pathways that can resolve DSBs is Homologous Recombination (HR), a relatively faithful DSB repair pathway. However, HR is up-regulated in some tumor types, rendering these more resistant to RT. Hence, targeted inhibition of HR during RT may lead to improved treatment outcome. The main focus of this study is to investigate synergism between two HR proteins, RAD51AP1 and RAD54, to improve RT. In human cells, RAD51-mediated HR is supported by the DNA motor protein RAD54 and by the RAD51 activator RAD51-Associated Protein 1 (RAD51AP1). Whether these two proteins work independently or together in HR has remained unclear. We hypothesize that simultaneous inactivation of both RAD54 and RAD51AP1 may lead to a synthetic phenotype in HR impairment and increased cellular sensitivity to IR. We have generated RAD51AP1 and RAD54 single knockout (KO) and RAD51AP1/RAD54 double KO HeLa cells using CRISPR/Cas9. We find that, compared to single KO cells, double KO cells show reduced growth rates under unperturbed conditions, indicative of their more pronounced defect in repairing endogenously occurring DNA damage. Similarly, following exposure to MMC, a higher fraction of double KO cells than of single KO cells arrest in G2 phase, suggesting that double KO cells overcome damage less well. Double KO cells also are more sensitive to the cytotoxic effects of mitomycin C exposure, a chemotherapeutic agent that induces inter-strand DNA crosslinks which are repaired by HR. Taken together, our results show that RAD51AP1 and RAD54 largely function independently of each other in the HR reaction. Hence, targeted inhibition of both RAD54 and RAD51AP1 during RT may increase therapeutic gain and improve the survival rates of cancer patients. **Funding**: NIH: ES021454, ES029206

Graduate Student / Environmental and Radiological Health Sciences

O-41. Evaluation of the effectiveness of firefighter clean cab decontamination procedures

James L Taloumis, Katelyn N Craft, William J Brazile

It is estimated that there are currently 1,056,200 local firefighters working in the United States. In this profession, firefighters are exposed to carcinogens while fighting fires, conducting overhaul activities, and from contacting the exteriors of self-contained breathing apparatuses (SCBA's) and protective gear. The National Institute for Occupational Health and Safety (NIOSH) found that firefighters are at increased risk to a number of cancers, particularly digestive and oral cancers. During fire suppression, particles containing heavy metals and semi-volatile polycyclic aromatic hydrocarbons (PAH's) can be deposited on the firefighters PPE and skin. When riding in the cabs, there is the potential that firefighters receive skin and oral exposure to PAH's and heavy metals even after the cab interior and SCBA's are wiped down. In recent years, the idea of the "clean cab concept" has become prevalent in fire department response and decontamination procedures. The concept focuses around minimizing the exposure to the interior of engines to contaminants typically encountered fighting fires by keeping SCBA's out of the cab of the engine by storing the SCBA's in a cabinet behind the cab of the engine. This study focuses on the collection and evaluation of carcinogenic contaminants within the cabs of fire engines at two different fire protection districts that follow differing procedures on allowing SCBA's in the cab while on route to fires. The researchers will perform surface-wipe sampling for PAH's and for arsenic, cadmium, and lead. On each day of sampling, the researchers will mobilize to the station location following a fire. Once the engine has been decontaminated following fire department procedures, the researchers will take two wipe samples for PAH's and two wipe samples for metals. The two wipes will be taken from the steering wheel and the rear door near the door handle. **Funding**: South Adams County Fire Department

Graduate Student / Environmental and Radiological Health Sciences

O-42. *In vivo* impact of chorionic somatomammotropin RNA interference in the absence of intrauterine growth restriction

Amelia R Tanner, Ashgar Ali, Quinton A Winger, Paul J Rozance, Russell V Anthony

Reductions in maternal chorionic somatomammotropin (CSH) have been reported for intrauterine growth restricted (IUGR) pregnancies, yet the exact biological function of CSH remains elusive. Previously, we reported the use of *in vivo* RNA interference (RNAi) to assess the impact of CSH deficiency on placental and fetal growth at 50 and 135 days of gestational age (dGA) in sheep. At 135 dGA, there are two distinct phenotypes: 1) pregnancies with placental growth restriction and IUGR, and 2) pregnancies where placental and fetal size were not impacted. Herein, we report the *in vivo* steady-state changes resulting from CSH RNAi in the absence of IUGR. The trophectoderm of hatched blastocysts (9 dGA) were infected with our lentiviral-constructs expressing either a scrambled control (NTS) or CSH-specific shRNA (tg6), prior to transfer into recipient sheep. At 120 dGA, 6 NTS and 6 tg6 pregnancies were fitted with maternal and fetal cannulas. Uterine and umbilical blood flows (132±0.25 dGA) were measured utilizing the 3H2O transplacental diffusion technique and nutrient uptakes were calculated using the Fick principle. Data were analyzed by Student's T-test. In this cohort, CSH RNAi (tg6) tended ($P \leq 0.10$) to reduce placenta weight with no effect on fetal weight. While uterine blood flow was reduced ($P \leq 0.05$) in CSH RNAi pregnancies, the uterine artery-to-vein gradient for both O2 content and glucose were significantly ($P \leq 0.05$) increased. Furthermore, glucose utilization by the placenta was increased 27% ($P \leq 0.05$). Umbilical blood flows, O2 content and glucose concentrations were not impacted by CSH RNAi. With the present cohort in the absence of IUGR, uteroplacental blood flow was reduced while uteroplacental glucose utilization was increased, which could infer a compensatory response to CSH RNAi. Future investigation of CSH RNAi pregnancies exhibiting both phenotypes may help determine the direct effects of CSH deficiency. Supported by NIH R01HD093701. **Funding**: NIH R01HD093701

Graduate Student / Biomedical Sciences

O-43. Glucocorticoid receptor expression in placenta and brain tissue

Hayley N Templeton, River Evans, Agata Parsons, and Gerrit J Bouma

Glucocorticoid (GC) hormones play an essential role in maintaining homeostasis during exposure to stress. However, prolonged GC exposure during prenatal stress has been shown to increase the risk of developing neuroendocrine, metabolic, and psychiatric disorders. For GCs to exert their effects, they must bind glucocorticoid receptors (GR). With increased GC exposure, GR signaling may be permanently altered. An organ that acts to protect the fetus by limiting GC exposure is the placenta. There appears to be a sex specific placental response to prenatal stress where females are impacted less than males. The purpose of this preliminary study is to determine if GR is located in brain and placenta tissue of ewe and mice; and second, to determine if there are sex specific differences in the relative amount and location of GR in the brain and placenta. All tissue samples in this study were collected as part of different experiments. Ewe placental tissue was collected near term from 4 pregnant ewes, and mouse placental tissues were collected during mid-gestation. Mouse brain tissue was collected from two female mice in estrus and two male mice. RNA was isolated, reverse transcribed into cDNA, and used for real time PCR to analyze relative GR transcript abundance. Immunofluorescence was performed to examine localization of GR in both placenta and brain. Data revealed that GR is present in the brain and placenta of both sheep and mice. In ewe and mouse placenta, GR was localized in the cytosol and nuclei of fetal trophoblast cells. In mouse brain, GR was ubiquitously present with increased expression in the paraventricular nucleus (PVN) and anterior nucleus. In conclusion, GR is present in male and female brains and placenta providing the basis for ongoing experiments to uncover sex differences in localization and relative amounts. **Funding**: College Research Council

Graduate Student / Biomedical Sciences

O-44. Understanding the mechanism of a neurotoxic antibiotic

Zaria D. Vick, William H. Hanneman, and Julie A. Moreno

Metronidazole is a well-known broad-spectrum and broad-use antibiotic utilized in human and veterinary medicine. However, in rare instances, neurotoxicity has been reported as an off-target side effect with the normal use of this drug. The mechanism of this unintended toxicity is largely unknown however clinical cases have shown the toxicity to be reversed with discontinued use. We have utilized computational modeling to reassess dosing regimens in human and equine schemes via physiologically based pharmacokinetic models. To aid in our understanding of this neurotoxicity, we have assessed a human neuronal cell model SY-5Ys with the treatment of metronidazole or the vehicle control with and without differentiation of the cells. In a cell viability assay, our results conclude metronidazole induces insult but not neuronal cell death. Using these exposed cells, we have explored various mechanisms of cellular toxicity such as cell survival, calcium signaling and mitochondria integrity via flow cytometry. These results will add to human and veterinary medicine by enabling us to understand the proposed mechanisms of neurotoxicity for metronidazole. The ultimate goal of our work is to understand metronidazole as a neurotoxin, and its potential link to neurodegenerative diseases. **Funding**: Center for Environmental Medicine

Graduate Student / Microbiology, Immunology and Pathology

O-45. Identification of a novel strain of chronic wasting disease

Kaitlyn M Wagner, Robyn Pierce, Caitlin Ott-Conn, Kelly Straka, Bob Dittmar, Jasmine Batten, Mercedes Hennessey, Elizabeth Gordon, Brett Israel, Jenn Ballard and Mark D Zabel

Chronic wasting disease (CWD) is an invariably fatal prion disease affecting captive and free-ranging cervids, including white-tailed deer, mule deer, moose, elk and reindeer. Since the initial description of the disease in the 1960's, CWD has spread to 23 states, 3 Canadian Provinces, South Korea, Norway, Finland and Sweden. While some outbreaks of CWD were caused by transport of infected animals from endemic regions, the origin of CWD in other epizootics is unclear and has not been characterized. Previous studies have shown that there are two distinct strains of CWD that are independent of genotype. However, the continuous spread and the unclear origin of several outbreaks warrant continued surveillance and further characterization of strain diversity. For this study, we used biochemical techniques to examine strain characteristics from two captive white-tailed deer from a deer farm in Texas with an outbreak of CWD of unknown origin. Interestingly, when testing our CWD isolates from Texas to analyze electrophoretic mobility and glycoform ratio, we found that these samples did not exhibit the characteristic band shift when treated with proteinase K (PK), but PK resistant material remained. Additionally, we observed a unique relationship between our isolates and different detergent classes, which has not been previously reported. Our data indicate that we have found a novel strain of CWD. These findings have important implications for understanding the emergence of CWD and highlight the importance of continued surveillance for novel strains. Ongoing experiments are examining the zoonotic potential of this novel strain. **Funding**: Boone and Crockett Club

Graduate Student / Microbiology, Immunology and Pathology

1. Correlation of two different fibrinogen assays in chelonian plasma

Lindsay M Adelmeyer, Matthew S Johnston

Diagnostics in chelonian medicine are currently limited, especially for recognition of systemic inflammation. In mammals, two assays are commonly used to evaluate plasma fibrinogen concentrations as an indicator for systemic inflammation. These tests are referred to as the Qualitative and Quantitative Fibrinogen Assays. The Qualitative Fibrinogen Assay grossly measures the amount of fibrinogen that precipitates when plasma is heated. The Quantitative assay uses the Clauss Clotting method to measure the time for fibrin polymerization to occur in excess thrombin. The Qualitative method is widely preferred by clinicians over the Quantitative method due to its relatively low cost, simplicity, and accessibility. Correlation of the two methods would support the investigation of the Qualitative method as a measure for systemic inflammation in chelonian patients. Therefore, the null hypothesis for this study is that no significant correlation exists between the results of the two methods. To test this, a pilot study consisting of 12 chelonian patients was conducted. Chelonian patients that presented to the Colorado State University Veterinary Teaching Hospital Avian and Exotics Service with a pre-determined need for blood collection were included in the study. The plasma fibrinogen concentrations of each patient were measured with both techniques and the results were evaluated using a point-biserial correlation coefficient. This analysis demonstrated an inverse relationship between the results of the two assays, thus rejecting the null hypothesis. Furthermore, evidence of an inverse relationship indicates the Qualitative Fibrinogen Assay is not interchangeable with the Quantitative Fibrinogen Assay for evaluating plasma fibrinogen concentrations in chelonian patients and suggests that further investigation is needed to evaluate the utility of either method in chelonian medicine. **Funding**: Center for Companion Animal Studies Young Investigator Award

DVM Student / Clinical Sciences

2. Investigating PD-L1 signaling in endothelial cells

Dylan Ammons, Gen Hartley, Lyndah Chow, and Steven Dow

Host immune responses play an important role in combating disease processes. Cancerous pathologies are no exception. Therefore, immunotherapies have been developed to bolster anti-tumor immune responses. One immunotherapy, called checkpoint blockade, uses monoclonal antibodies to interfere with immune suppressive signals. The programmed cell death signaling axis (PD-1/PD-L1) transmits inhibitory signals to T cells and has been implicated in many cancers. Checkpoint antibodies have been developed to bind PD-1 (expressed on T cells) or PD-L1 (expressed on macrophages, endothelial cells, and tumor cells) to promote T cell activation. Both anti-PD-1 and anti-PD-L1 antibodies can induce complete tumor regression and have been approved as a first line treatment. Despite unprecedented tumor regression, only a fraction of treated individuals respond to PD-1/PD-L1 checkpoint blockade. Variable outcomes have pushed scientists to identify prognostic markers and ideal combination therapies. Here, we document that PD-L1 signaling can directly influence endothelial cell function. We show that the ligation of PD-1 to PD-L1 in endothelial cells induces signal transduction. Current studies aim to investigate how anti-PD-L1 antibodies influence function. Future studies will investigate anti-PD-L1 mediated changes to intratumoral oxygen tension, number of immune cell infiltrates, metastatic potential, and vascular integrity. We hypothesize that anti-PD-L1 and anti-PD-1 immunotherapy differentially impact the tumor stroma. If supported, these widely used immunotherapies should be considered distinct entities when evaluating combination therapies. **Funding**: Shipley Foundation

DVM/PhD Student / Microbiology, Immunology and Pathology

3. Mesenchymal stem cells to reduce bacterial load and morbidity in an acute Klebsiella pneumoniae mouse model

Kathryn Asatryan, Valerie Johnson

Klebsiella pneumoniae induces high rates of morbidity and mortality in human patients worldwide. Conventional antibiotic therapy has led to development of multidrug resistant strains of this pathogen contributing to increased mortality. A novel strategy is to reduce pharmacological dependence by utilizing immunotherapy to assist the immune system in combatting infection. Mesenchymal stem cells (MSC) have been successfully utilized to treat acute lung injury and decrease inflammation in the lung. Additionally, pre-activating cells with Poly I:C increases their antimicrobial properties and ability to track to sites of inflammation. Therefore, we hypothesize that pre-activated MSC delivered intravenously following infection in an acute pneumonia mouse model will decrease bacterial burden and improve survival. To test this model, CD-1 mice will be infected with $3.5x\ 10^4$ CFU intratracheally which will induce a rapid pneumonia culminating in severe clinical signs such as weight loss and tachypnea within 48 hours. Four groups of inoculated mice (sham inoculation n=5, sham treatment with PBS n=5, treatment with resting MSC n=5, treatment with pre-activated MSC n=5). Treatments will consist of IV injection of $1x10^6$ MSC 8 hours post inoculation. Weights and clinical signs will be monitored daily and at 48 hours mice will be sacrificed. Bronchoalveolar lavage will be performed with quantitative bacterial counts. The right cranial lung lobe will be harvested and bacterial counts performed on tissue and right caudal lung lobe will be preserved for immunohistochemistry and histopathology. This therapy is a novel treatment that could lead to greatly improved outcomes in this devastating and widespread disease process. **Funding**: USDA-NIFA-10207-AHDRXXXX-19-0001

DVM Student / Clinical Sciences

4. Small changes in sidewalk connectivity can result in big gains for student walking

Jeremy Auerbach, Kelly Haworth, Liz Young, Sheryl Magzamen

Increased thoroughfare connectivity between and within residential developments and schools can overcome several barriers to student active commuting and foster student walking. This study conducts a cost-benefi t analysis of increased sidewalk connectivity around elementary and middle schools in a U.S. school system. Benefits, which include the potential increased time of physical activity from walking for the students, are compared to the financial costs of the new connections. Advanced network optimization techniques were applied to schools from a representative U.S. school system to locate the optimal new connections that maximize student walking to a nearby school. Results from this case study showed that short and inexpensive connections could lead to a large increase of potential student active commuters, provide a signicant increase of physical activity for those potential walkers, and reduce busing costs. Increased thoroughfare connectivity can increase the potential for more student active commuters, thereby providing more U.S. children needed physical activity, and help reduce the burden of busing costs on school systems. **Funding**: Larimer County Health Department

Post-doctoral Fellow / Environmental and Radiological Health Sciences

5. Assessing a novel therapeutic on drug-seeking behavior in mice

Nikolas Bergum, Andrew R Rau, Shane T Hentges

Opioid drugs exert their rewarding properties by acting at the mu opioid receptor (MOR) and mice lacking the MOR fail to exhibit conditioned place preference (CPP), a well-established assay of drug seeking behavior. Despite the known roles for the MOR, the possibility of targeting MORs to therapeutically interfere with opioid use has not been heavily explored. Here, we set out to test the hypothesis that inhibiting the expression of MORs will reduce the acquisition of drug-seeking behavior and lessen reinstatement of drug seeking after abstinence. To test this hypothesis, we first established CPP for morphine in mice. CPP is performed in a cage with two chambers that have different contextual cues and a removable divider between them. After determining that the mice to did not naturally have a preference for either side, mice received daily injections of either saline or morphine and were selectively placed in one side of the divided cage for 30 minutes. This went on for 10 days and then mice were placed in the cage with the divider removed. In general, mice spend about 20% more time in the morphine-paired environment following conditioning. Subsequent cohorts of mice will be injected with an antisense oligonucleotide (ASO) designed to decrease MOR expression or a control oligonucleotide. If the hypothesis is correct, ASO-injected mice will fail to acquire preference for the morphine-paired context. Our studies may indicate the utility in using ASO-mediated knock down of MORs as a potential therapeutic for opioid use disorder in people. **Funding**: DK-078749

Graduate Student / Biomedical Sciences

6. Using CRISPR-Cas9 to target PLS3 in ovine fibroblast cells

Katie Bisazza, Russ Anthony, Kimberly Jeckel, Kirk McGilvray, and Jeremiah Easley

Osteoporotic fractures constitute the majority of reported fractures in the U.S. and are becoming an increasing burden on the medical industry. Current large animal models of osteoporosis do not accurately represent the level of bone loss observed clinically and are not naturally occurring. Mutations of the gene *PLS3* have been identified as a potential cause of an X-linked form of juvenile osteoporosis, resulting in patients with a low bone density phenotype. The main objectives of this study were to design single target guide-RNAs (sgRNA) for *PLS3*, transfect ovine fetal fibroblast (oFF) cells with targeting lentivirus and validate successful mutation and downregulation of the *PLS3* gene *in vitro*. To generate a functional mutation of PLS3 in ovine cells, sgRNAs were designed and synthesized to separately target a selected exon (exons 4, 8, and 9) in the coding region of the ovine *PLS3* gene. Three sgRNA oligonucleotide sequences were designed, integrated into a plasmid vector, and targeting lentiviruses were generated. Western blotting and Sanger sequencing reveal that CRISPR-Cas9 targeting via lentiviral transfection in oFF cells results in *PLS3* disruption and genomic mutation at the intended exons. These results demonstrate successful integration of a lentiviral vector into oFF cells to generate a functional knockout of *PLS3* utilizing a designed sgRNA target. Future research will determine the efficiency of each single target lentivirus *in vivo* utilizing one-cell embryos, with the ultimate goal of developing a more translational large animal model of osteoporosis. **Funding**: CSU Office of the Vice President of Research (OVPR) and the Translational Reproductive Biotechnology (TRB) Grant

Graduate Student / Clinical Sciences

7. Efficacy of a Nrf2 agonist to increase long bone strength in a rodent model of osteoarthritis

Sydney Bork, Daniel Palmer, Owen Wahl, Kendra Andrie, Rob Musci, Maggie Campbell, Joseph Sanford, Sara Wist, Karyn Hamilton, Benjamin Miller, Christian Puttlitz, Kelly Santangelo

Primary osteoarthritis (OA) is a multi-factorial, age-related disease that causes progressive damage to structural components within a joint, particularly bone. OA pathology is associated with chronic production of inflammatory mediators resulting in tissue remodeling, pain, and decreased mobility in affected individuals. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor present in many cell types and considered a master up-regulator of antioxidant/anti-inflammatory enzymes. Our study aimed to investigate a novel Nrf2 activator, PB125, as a therapeutic option for treatment of OA. We hypothesized that one potential benefit of PB125 would be the mitigation of OA through increased strength of long bones associated with knee joints. Five-month-old male and female Dunkin-Hartley guinea pigs, an animal model of primary OA, were orally administered PB125 or vehicle control for ten months. Femoral bones were collected and four-point bending tests were performed to measure ultimate bending stress (UBS), which is correlated to bone strength. Statistical analyses between groups were performed using a student's t-test. PB125 treated females showed a statistically significant ($p=0.005$) increase in UBS compared to control females. In contrast, there was no difference between PB125 treatment and control males. Additionally, PB125 treated females had a greater UBS ($p=0.05$) than PB125 treated males. PB125 treated females had stronger bones than control females, as well as PB125 treated males. Increased bone strength could result in better joint stability, thereby mitigating degenerative lesions from OA. We are currently evaluating knee joints to determine where OA was influenced from the treatment. **Funding**: AVMA/AVMF 2nd Opportunity Research Scholar Program

DVM Student / Microbiology, Immunology and Pathology

8. Injection characteristics of autologous and commercial fibrin hydrogels for cartilage defects in horses using a modified Subchondroplasty® technique

Maria Brock, Lauren Smanik, and Christopher Kawcak

Addressing changes in both the subchondral bone and articular cartilage has become increasingly important for the treatment of joint disease, as the relationship between the two plays a significant role in osteoarthritis progression. Current management options for joint injury in horses are limited and often fail to re-establish normal osteochondral architecture. Subchondroplasty, a technique involving injection of bone substitute material into compromised subchondral bone, was developed to treat bone marrow lesions associated with osteoarthritis in humans, with significant improvement in pain and function. We developed a modified Subchondroplasty technique (MST), providing access of injectable materials to both the bone and articular cartilage defects. Our objective was to evaluate the injection characteristics and effect of fibrin concentration of autologous and commercial fibrin hydrogels for use with the MST as a potential treatment for osteochondral disease in horses. Two full-thickness cartilage defects were created on the medial trochlear ridge of the femur in twelve equine cadaver stifles. Both hydrogels were prepared at concentrations of 25%, 50%, 75% and 100%, each used to treat 3 randomly assigned defects using the MST. Hydrogels were subjectively graded for ease of injection, firmness, defect fill, injectate volume and retention, both at the time of injection and after 60 minutes. Data was analyzed using ANOVA with post hoc Tukey-Kramer HSD testing. Preliminary results showed reduced retention and firmness with 25% autologous fibrin compared to the 100% and 75% concentrations, respectively. 100% commercial fibrin showed reduced defect fill compared to the 75% concentration. Concentrations of 50% and 75% fibrin, autologous or commercial, may be ideal for maximizing defect fill and injectate retention without sacrificing ease of injection or the potential for cellular migration during tissue healing. These results will contribute to future cartilage repair studies comparing MST to more commonly used techniques. **Funding**: Center for Companion Animal Studies Young Investigator Award

DVM Student / Clinical Sciences

9. Comprehensive evaluation of the circulating renin-angiotensin-aldosterone system in normal dogs and dogs with asymptomatic myxomatous mitral valve disease.

Kaitlyn E Brunk and Marisa K Ames

Activation of the renin-angiotensin-aldosterone system (RAAS) contributes to cardiovascular and kidney disease progression via increased sodium and water retention, vasoconstriction, and pathologic remodeling of the myocardium, vasculature, and kidneys. We hypothesized that RAAS activity will be significantly greater in dogs with stage B2 myxomatous mitral valve disease (MMVD) when compared to normal dogs. Furthermore, we hypothesized that RAAS activity of dogs with stage B1 MMVD will not differ significantly from normal dogs.. Healthy dogs and dogs with stage B1 and B2 MMVD without concurrent systemic disease, were prospectively recruited. A physical exam, minimum database, systemic blood pressure measurement, and echocardiogram were performed on all dogs. Serum was submitted for comprehensive RAAS peptide quantification via high performance liquid chromatography – mass spectrometry. Data were analyzed using the Kruskal Wallis test with Dunn's multiple comparison test if indicated. To date, 15/120 dogs have been enrolled. Preliminary analysis shows that there is no difference in RAAS activity between the three groups (Figure 1). RAAS activity is increased in some outwardly healthy dogs. Overall, marked individual variation in RAAS activity was seen within all groups. The clinical implications of an activated RAAS system in normal dogs and dogs with asymptomatic MMVD requires further investigation. The RAAS Fingerprint should be used to identify cohorts with increased RAAS activity and study the causes and implications of this increased activity. These data are preliminary and this study is ongoing. **Funding**: Boehringer Ingelheim Veterinary Scholars Program

DVM Student / Clinical Sciences

10. Systemic Iron Chelation Reduces Markers of Chondrocyte Hypertrophy and the Development of Cartilage Lesions in the Dunkin-Hartley Model of Primary Osteoarthritis

Lindsey H Burton, Maryam F Afzali, Lauren B Radakovich, Margaret A Campbell, Lauren A Culver, Angela J Marolf, Kelly S Santangelo

Osteoarthritis (OA) is a debilitating condition affecting millions of individuals worldwide. OA is characterized by the progressive loss of articular cartilage from the joint. Unfortunately, the molecular mechanisms driving disease onset and/or progression remain poorly understood. It has been documented that chondrocytes, the only cell type present in cartilage, undergo improper dedifferentiation to a hypertrophic phenotype when affected by OA; thus, it is postulated that this process contributes to OA pathogenesis. One theory for why this hypertrophic state occurs is that oxidative stress from reactive oxygen species (ROS) contributes to this shift in cell phenotype. We had previously demonstrated the ability of systemic iron reduction, achieved by an iron deficient diet, to decrease ROS production and the development of OA-associated cartilage lesions in an animal model of primary OA. Given the success of this study, we hypothesized that systemic iron chelation—achieved by administration of the pharmacologic iron chelator deferoxamine (DFO)—would reduce markers of chondrocyte hypertrophy and the development of knee OA in this animal model. Results indicated that administration of DFO was successful in reducing iron levels both systemically and within the knee joint, which was associated with a reduced development of OA-associated cartilage lesions. Gene transcript analysis of cartilage tissue revealed that DFO treated animals exhibited decreased expression of select markers for chondrocyte hypertrophy and genes promoting chondrocyte dedifferentiation, while displaying an increased expression of the anti-apoptotic gene BCL-2. Overhead enclosure monitoring indicated that animals receiving iron chelation therapy were able to maintain mobility throughout the study, while the movement of control animals declined by study termination. Collectively, these results indicate that removal of excess iron may help prevent chondrocyte hypertrophy and the development of OA-associated changes within the cartilage of knee joints. **Funding**: NIH

Graduate Student

DVM/PhD Student / Environmental and Radiological Health Sciences

11. Cyclin-Dependent Kinase 8 is a Transcriptional Regulator During Dengue Infection

Molly Butler, Nunya Chotiwan, Jasmine Donkoh, Connie Brewster, Rushika Perera, Sandra Quackenbush , and Joel Rovnak

Dengue virus infection alters the gene expression landscape within host cells. Some of these changes, including upregulation of metabolic pathways, are necessary to meet the demands of viral replication. Metabolic upregulation supports viral genome replication and infectious particle production. Conversely, some changes in gene expression, such as activation of the type I interferon response, are critical for protecting cells from infection and limiting viral replication. We were interested in the transcriptional regulation of these changes in gene expression. We investigated the role of a host transcriptional regulator, cyclin-dependent kinase 8 (CDK8), during dengue infection. Here we show that CDK8 is not only a transcriptional regulator of select metabolic gene expression but also the type I interferon response. This surprising contradiction demonstrates that in the context of dengue infection, CDK8 is a transcriptional regulator of both pro-viral and anti-viral processes. Further, we show that manipulation of CDK8 activity by chemical inhibition, and CDK8 expression by lentivirus-mediated shRNA knockdown, changes the outcome of dengue infection by reducing viral genome replication and infectious particle production. CDK8 is therefore a transcriptional hub for induced gene expression during dengue infection and can be manipulated to alter the course of viral infection. **Funding**: College Research Council (CRC)

Graduate Student / Microbiology, Immunology and Pathology

12. EFGR As A Target For Solid Tumor Chimeric Antigen Receptor T Cell Therapy

Jennifer W. Cao, Tyler Shank, Jade Kurihara, Lyndah Chow, Michael Verneris, and Steven Dow

The development of chimeric antigen receptor (CAR) T cell therapy has been a game changer in the treatment of lymphoma and other liquid tumors. CAR T cell therapy has the potential to cause life-long remission and surveillance against metastatic disease. The most impressive to date have been seen with CD19 CAR T cell therapies in humans for B cell lymphoma and acute lymphoblastic leukemia. Contrarily there has much less success in implementing CAR T cell therapy towards solid tumor cancers. The challenges for solid tumor CAR T cell therapy lies both in choosing a safe yet efficacious target and trafficking and migration of the engineered T-cells into tumor tissues. Epidermal growth factor receptor variant III (EGFRvIII) is a mutated ligand independent receptor that allows malignant cells to grow abhorrently and is highly expressed in a number of human tumors, including glioblastoma and osteosarcoma. Early trials have seen signs of efficacy, but issues with targeting and CAR T cell exhaustion remain. Therefore, we propose dogs with osteosarcoma and brain cancer can be a valuable model in improving the efficacy of solid tumor CAR T cell therapy targeting EGFRvIII. To assess the suitability of dogs with OS and bone cancer as models for EGFR CAR T cell therapy, we assessed EGFR expression by canine OS and glioma cell lines. In addition, we assessed EGFR CAR T recognition of dog OS cell lines in vitro. We found that the majority of dog OS lines expressed EGFR, and were recognized by human EGFR CAR T cells, and glioma testing is in progress. These early results indicate that dogs with OS and brain cancer can be an effective large animal model for evaluating CAR T cell treatment of solid tumors, and strategies to improve CAR T efficacy.

DVM/PhD Student / Microbiology, Immunology and Pathology

13. A flow cytometric assessment of canine anti-erythrocyte antibodies in healthy dogs and dogs with anemic-associated diseases

Nida Chornarm, Sarah B Shropshire, Jennifer Hawley, Melissa Brewer, Arianne Morris, Jonathan Coy, and Michael R Lappin

Immune-mediated hemolytic anemia (IMHA) is a fatal autoimmune disease in dogs resulting in red blood cell rupture (hemolysis). IMHA can be primary (idiopathic) or secondary from underlying conditions such as infectious disease or cancer. A characteristic of IMHA is the detection of anti-erythrocyte immunoglobulin (percent IgG) bound to the red blood cell surface; the Coombs test (direct agglutination test or DAT) is currently the test of choice. However, studies have shown that flow cytometry can provide both higher sensitivity and specificity for the diagnosis of IMHA. The objectives of this pilot study were to develop a flow cytometric assay for detection of anti-erythrocyte antibodies and to assess validity of results out to 72 hours and to compare the percent IgG between healthy and anemic dogs. In 11 healthy dogs and 10 idiopathic IMHA dogs, there were no significant differences between fresh and 24, 48, and 72 hour samples. From the healthy dog results, a cut-off of ≤ 15% was defined as negative. The mean percentage of anti-erythrocyte antibodies in healthy dogs was 3.46%, range 1.24% – 10.4% whereas in anemic dogs due to idiopathic IMHA (n=10), neoplasia (n=38), infectious disease and other etiologies (n=39) it was 31.74% (3.75%-97.70%), 1.18% (0.08%-16.85%) and 1.80% (0.12%-8.38%) respectively. ANOVA analysis of the mean percentages between dogs with idiopathic IMHA and other groups was significantly different at $p<0.05$ with IMHA dogs having statistically higher levels of antibodies. The results support that this flow cytometric assay provides reliable results up to 72 hours after blood collection and that dogs with idiopathic IMHA have significantly higher levels of anti-erythrocyte antibodies compared to dogs with other causes of anemia. **Funding**: Center for Companion Animal Studies

Graduate Student / Clinical Sciences

14. Effect of a commercially available probiotic with or without fiber supplementation on the incidence of diarrhea in shelter cats

Hayley Clark, Anna Winner, Kelsey Dobesh, Jeremy Kiene, and Michael R Lappin

Diarrhea is a common clinical sign in cats living in shelters with several, often difficult to diagnose, etiologies. Diet supplementation with some probiotics or fiber has been shown to decrease the occurrence, duration, and severity of diarrhea in multiple species. In this study, we hypothesize that dietary supplementation with a commercially available probiotic with added fiber (proprietary) will decrease the incidence and duration of diarrhea compared to the probiotic alone or a placebo. All otherwise healthy adult cats entering the shelter and housed in the cat stray room qualify for this ongoing study. All cats are fed the same dry food ad libitum and the first fecal sample passed is evaluated for the presence of enteric parasites. The cats are randomly assigned into one of three groups receiving either; 15 g of canned food alone, 15 g of canned food with the probiotic, or 15 g of canned food with the probiotic and additional fiber. Fecal scores for each cat are determined daily by use of a standardized scale applied by masked observers. The study is ongoing and researchers are still masked so only a preliminary analysis has been performed. There were numerical differences in proportions of cats that were normal on entry and then developed diarrhea (Group 1 = 16.7%; Group 2 = 27.3%, Group 3 = 40%). Of the cats that entered the study with diarrhea, there were numerical differences in proportions of abnormal stools over the course of the study amongst the groups (Group 1 = 30%; Group 2 = 43.3%, Group 3 = 50%). Based on these findings, a power calculation showed that if these trends are true, 15 more cats per group is likely to show significance and so the study is ongoing. **Funding**: Purina PetCare

DVM Student / Clinical Sciences

15. Phenotyping immune cell populations *in situ* in Mycobacterium tuberculosis infected mouse lung tissue

Sarah Cooper, James DiLisio, Hadley Gary, Alexander Grover, Brendan Podell

During *Mycobacterium tuberculosis* infection, granuloma formation in the lung plays a critical role in orchestrating complex host-pathogen interactions. While immune cell populations involved in *M. tuberculosis* infection have been extensively studied using flow cytometry and RNAseq, these interactions during granuloma formation *in situ* remains poorly understood. Understanding contextual and spatial relationships as well as the unique populations in and around granulomas in various disease states can give insight into immunopathogenesis to understand the immune correlates of protection afforded by vaccination. Here, we use multiplex fluorescent immunohistochemistry (IHC) and in situ hybridization (ISH) on fixed mouse lung tissue to simultaneously visualize *M. tuberculosis*, immune cell surface markers, and important cytokines involved during infection. We use Visiopharm image analysis software and machine learning to detect and quantify these targets within *M. tuberculosis* infected lungs of Bacillus Calmette–Guérin (BCG) vaccinated and unvaccinated mice. Our preliminary data demonstrates differences in B-cell and T-cell populations, bacterial load, and cytokine profiles. We show increased numbers of B-cells and T-cells in BCG vaccinated mice, with lower bacterial load, and increased levels of cytokines IFNg and TGFb production. In conclusion, we were able to successfully identify and quantify *M. tuberculosis*, immune cell populations, and cytokines present in granulomas of mouse infected lungs *in situ* using multiplex fluorescent IHC, ISH, and machine learning. **Funding**: NIH AI75N93019C00072

Graduate Student / Microbiology, Immunology and Pathology

16. Evaluation of two maxillary block techniques in cats for painful dental procedures (Phase I, Cadaver Study)

Lily Davis, Jennifer E Rawlinson, Sangeeta Rao, Pedro Boscan and Naomi Hoyer

Regional maxillofacial nerve blocks are an essential component of safe, balanced anesthesia, particularly for painful procedures such as tooth extractions. They are used daily in small animal practice, but there is little published on the distribution of oral local anesthetic agents. Several publications have discovered anatomic variability in the maxillofacial skeletal and neuroanatomy of dogs and horses that may be clinically relevant to variability of regional anesthesia techniques. Several methods have been published demonstrating regional anesthesia of the maxillary nerve in cats. There are also numerous reports of complications from inappropriately administered regional anesthesia, including orbital penetration. This Phase I study utilizes computed tomography (CT) reconstruction technology to visualize anatomic variability among the infra-orbital canal of cat skulls. There was significant anatomic variability between brachycephalic and mesocephalic skulls. New methylene blue dye and contrast material were injected using two different published techniques for regional anesthesia of the maxillary nerve into cat cadaver specimens. CT and gross dissection were used to assess the distribution of dye along the nerve, comparing the two different techniques. There were significant differences in diffusion of dye and contrast between injection methods. The significant anatomic differences we found in both their anatomy and the diffusion of injectate will lead to a better understanding of possibly the most efficacious way to block the maxillary nerve in cats. **Funding**: Center for Companion Animal Studies Young Investigator Award and Boehringer Ingelheim Veterinary Scholars Program

DVM Student / Clinical Sciences

17. Radiocesium size categorization of suspend particles in Fukushima contaminated water

Anna T Deak, Alexei Konoplev, Thomas E Johnson, and Ralf Sudowe

High levels of radioactive cesium contamination in the environment presents complications with soil and water utilization. Wide areas were contaminated to varying degrees with radiocesium after the Fukushima Dai-ichi Nuclear Power Plant accident. However, certain rivers within the Fukushima exclusion zone exhibited 1-2 times higher levels of suspended radiocesium compared to rivers in Chernobyl. Theories about the type and size of microparticles and their respective affinity for radiocesium are currently being tested. This project attempts to distinguish radiocesium carrying particles by their size in order to study how size affects migration and deposition behavior. Water-soil samples from ponds and rivers within the exclusion zone will be analyzed. The contaminated water will be filtered through a multilayer cascade to separate the radiocesium particles and then through a sorbent in order to determine the amount of dissolved cesium. The cascade will contain micro-pore membranes of varying size, in descending order, such that larger particles are separated first. An ANFEZH (iron ferrocyanide) sorbent will be used at the end of the separation cascade to retain dissolved cesium. Each filter will then be dried and counted for activity. The amount of activity on each filter is assumed to be proportional to the amount of cesium bound to a specific size range of particles. This research was supported by the grant T42OH009229 funded by the National Institute for Occupational Safety and Health (NIOSH) in the Centers for Disease Control and Prevention (CDC). **Funding**: National Institute for Occupational Safety and Health (NIOSH) in the Centers for Disease Control and Prevention (CDC) grant number T42OH009229

Graduate Student / Environmental and Radiological Health Sciences

18. Immune Modulatory Effects of Common Antibiotics on Canine Innate and Adaptive Immune Responses *In Vitro*

Brian M. DeFilippo and Steven Dow

Antimicrobial drug use has been indicated in modulating host innate and adaptive immune responses, in vivo and in vitro. Due to current prescribing practices of high doses and/or long courses of antibiotics in dogs, this study investigates the potential immune suppression resulting from these treatments. The inhibition of systemic immune function could be contraindicated in treating diseases of bacterial pathogen etiology. The objective of this experiment is to determine the effect in vitro of commonly prescribed oral antibiotics in canines on T cell proliferation and pro-inflammatory cytokine production, Interferon gamma (IFN-y) and Interleukin-6 (IL-6). Understanding more about the immune response to antibiotics will aide in determining the effect these treatments can have on the host in acute and chronic disease states. Thus, this study aims to evaluate the major classes of antibiotics, as represented by the following common veterinary antibiotic compounds used in dogs: Doxycycline, Enrofloxacin, Azithromycin, Amoxicillin, Metronidazole, and Cefazolin. We hypothesize that these antibiotics will exhibit varying degrees of suppression on T cell proliferation and the production of IFNy and IL-6 in vitro. The anticipated variegated proliferation rates and cytokine production by canine T cells will be evaluated via flow cytometry, MTT assays, and enzyme-linked immunosorbent assays (ELISAs). Mononuclear cells were isolated from canine peripheral blood in EDTA via single density gradient centrifugation. Following isolation, proliferation assays were incubated with a dilution range of clinically achievable serum antibiotic concentrations, following oral drug administration, of 0.1 ug/ml, 1 ug/ml, and 10 ug/ml. Among the antibiotic compounds evaluated, only Doxycycline exhibited immunosupressive effects on canine T cells at achievable serum concentrations after oral administration. ELISAs also indicated that these Doxycycline concentrations result in a decreased IFN-y production of canine T cells in vitro. **Funding**: NIH 5T35OD015130-07

DVM Student / Clinical Sciences

19. The use of magnetic resonance spectroscopy to differentiate histologically confirmed canine brain masses: a retrospective analysis of 44 cases

Sarah B. Deluty, Lynn R. Griffin, and Rebecca A. Packer

Magnetic Resonance Imaging (MRI) is a common modality used to aid in the diagnosis of canine brain tumors. MR spectroscopy (MRS) can be obtained with the diagnostic MRI procedure, and is a quantitative measure of brain metabolism that may eventually provide information to help predict histologic type, grade, outcome, and individualized treatment targets. The aim of this study was to evaluate the use of MRS in the context of canine brain masses, and evaluate whether or not MRS could be used to differentiate neoplastic from non-neoplastic masses, as well as differentiate histologic type of tumor. Data from 144 clinical cases of naturally-occurring canine brain tumors and inflammatory lesions that presented to the Colorado State University Veterinary Teaching Hospital from 2006-2019 were available for analyses. 44 of these cases provided paired MR spectroscopy and histological data for correlative study. Choline (Ch), creatine (Cr), lactate (LL), myoinositol (mI) and NAA brain metabolites, and calculated values of Cr+Ch, Ch/Cr, Cr/Ch, NAA/Cr, NAA/Ch, Cr/NAA and Ch/NAA, were explored for their correlation to the final etiology of brain dysfunction. NAA/Ch differed significantly between normal vs. glioma (P=0.0082) and glioma vs. inflammatory (P=0.0118). Based on our data, MRS may be used as an additional evidence to differentiate glioma from inflammatory brain lesions, providing and alternative non-invasive diagnostic tool to clinicians. **Funding**: Boehringer Ingelheim Veterinary Scholars Program

DVM Student / Clinical Sciences

20. Serologic responses to oral administration of an intranasal FHV-1 and FCV containing vaccine

Kelsey C Dobesh, Jeremy Kiene, Sienna Drizin, Jennifer Hawley and Michael R Lappin

Feline herpesvirus 1 (FHV-1) and feline caliciviruses (FCV) are the most common causes of viral upper respiratory disease in cats. Effective vaccines for these viruses are commercially available for subcutaneous (SC) or intranasal (IN) administration. However, SC administration of vaccines has been associated with vaccine site sarcomas and feline chronic kidney disease. While IN administration of FHV-1 and FCV vaccines are not associated with either of these problems, this route of administration can be difficult with cats. Recently, oral vaccines have lessened disease associated with canine *Bordetella bronchiseptica* infection on challenge. If effective, oral administration might be preferred in cats. The study hypothesis was that previously unvaccinated healthy young adult cats would develop increased FHV-1 and FCV serum antibody titers after a vaccine licensed for IN administration was administered orally at days 0 and 14. Prior to vaccination blood was collected from 5 cats and evaluated for FHV-1 and FCV antibodies by ELISA. After vaccination of these 5 cats FHV-1 and FCV antibody absorbance values were determined on days 0, 14 and 28. FCV antibody absorbance values were over 1000% greater than day 0 in all of the vaccinated cats and FHV-1 antibody absorbance values were over 200% greater than day 0 in 3 of the vaccinated cats. Local and cell mediated immunity were not measured in this pilot study. Since there was some evidence for induction of primary immunity by this route of administration, a FHV-1 challenge study is scheduled to begin in January. **Funding**: Center for Companion Animal Studies Young Investigator Award

DVM Student / Clinical Sciences

21. Thrombin generation assay in healthy dogs receiving apixaban

Dillon Donaghy, Christine Olver, Allison Gagnon, Brian Scansen , E. Chris Orton, and Sarah Shropshire

Apixaban is a drug used to treat or prevent thrombosis by inhibiting the function of Factor Xa in the coagulation cascade. Inhibition of this major player in coagulation prevents the conversion of prothrombin to thrombin, ultimately limiting the formation of fibrin clots. Over the course of four separate dose-escalations, apixaban was administered to healthy beagles (3 dogs/dose) using a distinct dosage (dose 1-4) for each trial for 8 days. The apixaban dose was 5 mg PO q12h, 5 mg PO q8h, 10 mg PO q12h, and 10 mg PO q8h respectively. Blood was collected at each designated time point (0hr, 3hr, 8hr) on days 1, 3, and 8. Each blood sample was tested using a fluorescence-based Thrombin Generation Assay measuring the area under the curve (AUC), peak height, time to peak, and lag phase. The data showed no significant differences from baseline for any variable at dose 1 ($P>0.05$). Compared to baseline, lag phase and time to peak height were longer and the peak height and AUC were smaller for doses 2, 3 and 4 (all $P< 0.05$). These results show that apixaban does significantly inhibit thrombin generation and clot formation in healthy dogs. Further work is currently underway to measure the overall hemostatic potential and coagulation kinetics using these samples that will further elucidate the effect of apixaban on healthy dogs.

Undergraduate Student / Microbiology, Immunology and Pathology

22. The role of cyclin dependent kinase 8 in Zika virus control of host metabolism

Jasmine Donkoh, Molly Butler, Connie Brewster, Rushika Perera, Sandra Quackenbush and Joel Rovnak

Zika virus (ZIKV) is a mosquito borne flavivirus that is associated with outbreaks of microcephaly in South America in 2016-2017. ZIKV is linked to cases of Guillain-Barre syndrome, fatal encephalitis and myelitis as well as other neurological complications. DENV, a close relative of ZIKV, has been shown to alter cellular metabolism, resulting in an alteration in metabolites and host proteins. We have found that ZIKV infection leads to induced expression of the host mediator complex protein, cyclin dependent kinase 8 (CDK8) and that CDK8 promotes virus replication. CDK8 is a transcriptional co-factor that regulates gene expression in multiple metabolic pathways. We show that CDK8, as well as the glycolytic enzyme hexokinase 2 (HK2), are increased during ZIKV infection. Using the CDK8 kinase inhibitor, Senexin A, we demonstrate that CDK8 is required for increased expression of HK2. We also show that ZIKV genomic RNA and particles decrease. The attenuated ZIKV replication may be due to decreased availability of metabolites necessary for nucleic acid and fatty acid synthesis. **Funding**: College Research Council (CRC)

Graduate Student / Microbiology, Immunology and Pathology

23. Antimicrobial drug use in beef feedlots; effects on microbiome and resistome dynamics

Enrique Doster , Jennifer K. Parker, Cameron A. Anderson, Steven M. Lakin, Noelle R. Noyes, Maggie Weinroth, Calvin W. Booker, Sherry J. Hannon, Sheryl P. Gow, Tim A. McAllister, Keith E. Belk, and Paul S. Morley

Antimicrobial drugs (AMD) are used in beef production to treat clinical disease and to control disease in groups of cattle. Previously, investigations regarding AMD and AMR have focused largely on AMR phenotypes of selected pathogens and indicator bacteria, but genes that confer AMR are known to be distributed and shared throughout microbial communities (microbiome). Use of high-throughput metagenomic sequencing enables a holistic perspective into AMR ecology by sequencing DNA from the entire microbiome. The objective of this study was to employ metagenomic sequencing to investigate the effects of antimicrobial drug use on the microbiome and resistome in beef feedlot cattle. During a 3-year longitudinal study of Canadian beef feedlot operations, 30% of newly arriving pens of cattle and 10% of animals were randomly selected for inclusion in the study. Fecal samples were collected as composite-fecal samples from pen floors and per-rectum from individual cattle cattle. Samples were collected during arrival at the feedlot and at a second date (re-handling) during the feeding period. All antimicrobial drug use was recorded and characterized across different drug classes using animal defined daily dose (ADD) metrics. Samples were analyzed using 16S rRNA sequencing to characterize the microbiome and AMR target-enriched shotgun sequencing to characterize the fecal resistome. Overall, resistome composition in composite pen samples and individual animals was dominated by alignments to gene accessions conferring resistance to tetracycline and macrolide-lincosamide-streptogramin (MLS) drug classes. The diversity of bacterial phyla was greater early in the feeding period and decreased over time as the microbiome shifted toward a similar composition dominated by the phyla, Proteobacteria and Firmicutes. The strongest association between AMD treatment and resistome composition was related to sampling time for individual cattle, which accounted for 2.5% of the variation in the resistome and 1.2% in the microbiome. **Funding**: USDA NIFA grant 2015-68003-23048

DVM/PhD Student / Microbiology, Immunology and Pathology

24. Parkin mutations increase neuroinflammatory activation of glia and neuronal loss in the nigrostriatal pathway that is attenuated by genetic knockout of cGas-STING.

Mary Stischer, Marisa Edmonds, and Ronal Tjalkens

The prevalence of the neurodegenerative disease Parkinson's disease (PD) is a significant public health issue. Neurological symptoms of PD include rigidity, gait imbalance, and resting tremor, due to loss of dopamine (DA) neurons in the substantia nigra. Pathological processes associated with loss of DA neurons include α-synuclein protein-aggregation, neuroinflammatory glial activation, and mitochondrial dysfunction. Evidence suggests that genetic susceptibility, environmental exposures, and viral infections are possible risk-factors for PD but the cause is still unknown. Neuroinflammatory activation of glial cells is a consistent factor of PD and likely contributes to neuronal loss. Genes that may modulate this inflammatory phenotype include Parkin, an E3 ubiquitin ligase, and PINK1, a ubiquitin kinase, both of which are crucial in the process of mitophagy. Disruption of mitophagy can lead to the activation of the cGas-STING (STING) inflammatory pathway in neurons and glial cells. Also, humans with Parkin mutations have elevated levels of inflammatory cytokines, suggesting that mitophagy dysfunction may modulate inflammatory activation of glia and contribute to DA neuronal loss. In order to examine the role of defective mitophagy, we used the mitochondrial mutator mouse (Polga), which develops spontaneous mutations in mitochondrial DNA, crossed with a Parkin knock out (Prkn-/-) mouse that either had STING knocked out or functional STING expression. These results show that the neuromuscular deficits, nigrostriatal damage, and glial activation in Polga+Prkn-/- is attenuated with inactivation of STING, suggesting that neuroinflammatory activation facilitates the neurodegenerative phenotype seen in Polga+ Prkn-/- mice. Parkin-mediated mitophagy could play a role in restraining activation of glia, and neuroinflammation could play a causative role in genetic PD. Thus, inhibition of glial inflammatory responses may represent a possible therapy for genetic PD. **Funding**: The anti-aging foundation

Undergraduate Student / Environmental and Radiological Health Sciences

25. Multiplex fluorescent immunocytochemistry: a novel approach to the diagnosis of Feline Infectious Peritonitis

Sophie Epstein, Samantha Evans, Margaret Howell, Ben Curtis, Gregg Dean, Kelly Santangelo

Feline Infectious Peritonitis (FIP) is a devastating disease affecting feline populations worldwide. The causative agent of FIP is Feline Infectious Peritonitis Virus (FIPV), a mutated form of the endemic Feline Enteric Coronavirus present in most healthy cat populations. FIPV shows a unique tropism for macrophages, and often manifests as a fatal fibrinous and granulomatous serositis with the presence of protein-rich serous body cavity effusion. There are currently no ante-mortem confirmatory diagnostic tests, leaving owners and veterinarians with uncertainty in regard to prognosis, treatment and quality of life for their pets and patients respectively. The current gold standard diagnostic for FIP is immunohistochemical (IHC) staining for viral antigen within macrophages of gross granulomatous lesions. However, this test is typically done post-mortem and has limitations in terms of sensitivity and sample quality. Other diagnostics, including indirect ELISA and qt-PCR show similar limitations. We proposed multiplex fluorescent immunocytochemistry (MF-ICC) as a feasible, rapid, and cost-effective diagnostic that can be performed on effusion samples taken ante-mortem. Fluorescently-labeled antibodies were used to selectively tag FIPV within macrophages present in effusion and tissue samples from patients confirmed to have FIP via necropsy and IHC. Preliminary results show a sensitivity of 80%, specificity of 100%, PPV of 100% and NPV of 70%. While the absence of false positives is a promising finding, the sensitivity of 80% leaves room for improvement. We are currently adapting our protocol for flow cytometry, with the aim of significantly increasing the number of recorded cellular events and thus, the sensitivity. **Funding**: NIH 5T35OD015130-07

DVM Student / Microbiology, Immunology and Pathology

26. Characterization of plumes generated from electrosurgical devices and mitigation strategies

Kelsea Ericksen, Arsineh Hecobian, Amy Sullivan, Jeffrey Collett, Joshua Schaeffer, Eileen Hackett

Cautery, the most commonly used electrosurgical device used in veterinary medicine, produces a substantial amount of smoke when applied to tissues. Various studies have determined carcinogenic, viral and bacterial components in smoke produced by cautery devices in human medicine. Local evacuation ventilation (LEV) systems have been shown to decrease smoke in the operating room thus mitigating exposure to the deleterious components of smoke. Despite this fact, across the country veterinariany practices largely lack the use of LEV systems and to this day its ability to capture smoke has never been evaluated in veterinary medicine. In this study a cautery pencil equipped with an attached vacuum (Medtronics) activated upon activation of the pencil was used to determine its ability to mitigate smoke during surgery. Collection of operating air within surgeon distance of surgery site either in the presence of absence of the LEV system and was tested for organic and elemental carbon (OCEC), respirable particulate matter under 2.5 microns (PM2.5) and endotoxins. These three parameters will be able to determine to what extent an LEV system has the ability to decrease smoke exposure in the veterinary operating room. **Funding**: NIH 5T35OD015130-07

DVM Student / Clinical Sciences

27. Hypertonic saline rinse and its effect on nasal inflammation and microbiome in dairy workers

Grant Erlandson, Sheryl Magzamen , Julia Sharp, Ken Jones, Matthew Nonnenmann, Stephen J. Reynolds, Joshua W. Schaeffer

Livestock workers experience an increased burden of bioaerosol-induced respiratory disease. We have previously demonstrated that dairy operations generate bioaerosols spanning the inhalable size fraction. These aerosols contained complex bacterial communities and inflammagens. Because those particles with an aerodynamic diameter between 10-100 µm are known to deposit in the nasopharyngeal region, we believe that exposure health outcomes in the upper respiratory tract need consideration, especially in the context of the nasal microbiome (i.e., collection of bacterial communities suggested to play a key role in health and disease). We evaluated the effectiveness of a hypertonic saline nasal lavage in reducing inflammatory responses in dairy workers from a high-volume dairy operation. We collected inhalable personal breathing zone (PBZ) samples and pre-/post-shift nasal lavage samples from each participant over five consecutive days. The treatment group (n=5) received hypertonic saline while the control group (n=5) received normotonic saline. PBZ samples were analyzed for particulate concentrations and endotoxin using gravimetric and enzymatic methods, respectively. Pro- and anti-inflammatory cytokines (i.e., IL-8, IL-10, and TNF-a) were measured from nasal lavage samples using a multiplex assay. To measure the air and nasal microbiomes, lavage and dust samples were analyzed using 16S rRNA sequencing. Inhalable dust concentrations ranged from 0.15 to 1.9mg/m3. Significantly higher pro-inflammatory cytokines were observed in the treatment compared to the control group for IL-6. However, the treatment group had significantly higher IL-10 (anti-inflammatory) concentrations than the control group. Distinct bacterial communities were observed in pre and post-shift lavage samples with post-shift communities more closely resembling those found in dust samples. Based on pilot results, the intervention was successful in upregulating anti-inflammatory cytokines and promoting changes in the nasal microbiome. Therefore, more research is warranted to determine if a hypertonic nasal rinse is an effective intervention that limits adverse perturbations to the microbiome. **Funding**: National Institute for Occupational Safety and Health U01 grant #: 1U01OH010840.

Graduate Student
DVM/PhD Student / Environmental and Radiological Health Sciences

28. Use of a macrophage culture system to assess inflammatory properties of cartilage degradation products and immune modulation by mesenchymal stromal cells in horses

Estrada-McDermott, J.M., Wheat, W., Goodrich, L and Dow, S.

Background and Rationale. Osteoarthritis (OA) is a common condition in horses, dogs, and humans. Repair and regeneration of full-thickness and functional articular cartilage defects remains a significant challenge to orthopedic surgeons, and there is still an incomplete understanding of the factors that drive progressive joint cartilage destruction in OA. Macrophages are the most numerous immune cells in the joint capable of responding to cartilage degeneration, and therefore, we developed an in vitro system to assess macrophage responses and immune modulation by mesenchymal stromal cells (MSC). Objective: Demonstrate that monocyte derived macrophages can be used to assess inflammatory responses to cartilage degradation products (CDP) in horses. Hypothesis: Exposure to CDP will trigger macrophage activation, which will be suppressed by exposure to MSC secreted factors. Methods: Macrophages will be generated from adherent monocytes by culture in M-CSF, and then exposed to graded concentrations of CDP, which will be generated from equine cartilage biopsies, or to synovial fluid from animals with OA. Macrophage immune responses will include cytokine release and upregulation of co-stimulatory markers. Effects of MSC conditioned medium on CDP induced inflammation will be assessed as well. Results: In preliminary studies, we have found that equine CDP and synovial fluid from animals with OA both induce macrophage activation, as assessed by TNFa release and upregulation of MHCII expression. Exposure to MSC CM appears to suppress these inflammatory responses. Summary: Our preliminary studies indicate that the macrophage culture system can be used effectively to evaluate and identify the inflammatory factors present in CDP, and to assess novel strategies such as treatment with MSC for their ability to suppress these responses. **Funding**: ACC / TMI

Graduate Student / Clinical Sciences

29. Testosterone binding to G protein-coupled receptor family C group 6 member A and syncytialization of human trophoblast cells

River Evans, Erin S McWhorter, Natascha Heise, Quinton A Winger, Gerrit J Bouma

Testosterone is widely known to enter cells, bind to a cytoplasmic androgen receptor, dimerize and translocate into the nucleus where it binds to an androgen response element on DNA, altering transcription. Testosterone is also known to bind to G-protein coupled receptors (GPCRs) on cell membranes known as membrane androgen receptors. More recently, a GPCR known as G protein-coupled receptor family C group 6 member A (GPRC6A) has been investigated for its potential interaction with testosterone. This project is investigating the effect of testosterone binding to GPRC6A in human trophoblast cells in order to further establish the relationship between androgens and placental development and angiogenesis. ACH3P cells were used as a model for human trophoblast cell function, and treated with cell membrane impermeable testosterone-BSA-FITC conjugate. Human chorionic gonadotropin (hCG) and cyclic adenosine monophosphate (cAMP) enzyme-linked immunosorbent assays (ELISA) were performed after treating ACH3P cells for 48 hours with 100 μM testosterone-BSA-FITC conjugate to determine if trophoblast cell differentiation occurred. Negative control treatment included 100 μM BSA-FITC conjugate whereas two positive control treatments included forskolin (40 μM; known to induce trophoblast cell differentiation) and of L-lysine (0.013M; a known ligand for GPRC6A). Control cells were treated with 0.1% DMSO. In addition, immunofluorescence was performed to analyze GPRC6A localization and testosterone-BSA-FITC localization in trophoblast cells. The hCG and cAMP ELISA assays showed a statistically significant increase in hCG and cAMP levels in cell media and cell lysate, respectively, compared to the FITC-BSA only and non-treatment controls. In addition, immunofluorescence analysis revealed GPRC6A and testosterone-BSA-FITC staining in a similar staining pattern in trophoblast cells. While the results are preliminary, our data suggests that testosterone binding to GPRC6A causes differentiation of ACH3P cells. **Funding**: College Research Council (CRC)

Staff / Biomedical Sciences

30. Development of a vitrification protocol for *in vitro* produced feline embryos

Dana W. Fuller, Jason R. Herrick, James K. Graham, Sue Vandewoude, Jennifer Barfield

This study was designed to evaluate in-vitro-produced feline embryo viability post-vitrification using DMSO plus equi-molar propanediol (PrOH) or ethylene glycol (EG). Oocytes and frozen/thawed epididymal sperm from spay-neuter clinics were used to produce embryos. Day 7 early to expanded blastocysts of quality grade 1 or 2 were randomly assigned to 3 treatments: vitrification with PrOH (n=32), vitrification with EG (n=31), or non-vitrified control (n=47). The base medium for vitrification media was HEPES-buffered feline-optimized-culture-medium (FOCMH). Embryos were placed in 0.5mL of equilibration medium (7.5% DMSO + 7.5% PrOH or EG, 0.5M sucrose, 10% Ficoll, and 20% Fetal Calf Serum (FCS)) for 5min at room temperature (RT) and then moved to drops of vitrification medium (15% DMSO + 15% PrOH or EG, 0.5M sucrose, 10% Ficoll, and 20% FCS) at RT for 30sec before being loaded onto Cryolock® devices and plunged into liquid nitrogen (LN2). Warming was a 3-step process: (1) embryos were moved from LN2 to 0.5mL of 1M sucrose, 10% Ficoll, and 20% FCS at 37°C for 1min; (2) then to 0.5mL of 0.5 M sucrose, 10% Ficoll, and 20% FCS at 20°C for 3min; (3) then to 0.5mL of FOCMH for 5min at 37°C. Embryos were cultured in FOCMH+5% FCS and evaluated at 24 and 48h for re-expansion and development progression. From 5 replicates, viability of embryos with EG exhibited higher viability (84%) 24h after warming, than embryos vitrified with PrOH (59%; $p < 0.05$; control 100%). At 48h, developmental rates were at 96% for EG and 100% for PrOH-treated embryos ($p > 0.05$). Viability of embryos post-warming was higher than a previous study using similar protocols, possibly due to our shorter exposure time to the cryoprotectants (CP) during vitrification. DMSO+EG or PrOH are effective CP's for Day 7 feline IVP embryos. Funding provided by OVPR, CVMBS, and MIP. **Funding**: OVRP, CVMBS, MIP

Staff / Biomedical Sciences

31. The Impact of Sampling Method on Gut Bacterial Composition in Dogs and Cats

Allysa M Galloni, Stacie C Summers, Rachael K Isdale, and Craig B Webb

The gastrointestinal microbiome is an important research area in veterinary medicine. Feces, rectal swabs, and colonic mucosal biopsies can be used to characterize the bacterial profiles of the descending colon using 16S ribosomal RNA (16S rRNA) gene sequencing. To date, there are no studies comparing the potential bacterial variation between these three sampling techniques in dogs and cats. The study objective was to compare the bacterial composition between feces, rectal swabs, and colonic biopsies in healthy cats and dogs as well as cats and dogs diagnosed with a chronic enteropathy (CE). Three samples (feces, rectal swab, and colonic mucosal biopsy) were collected from clinically healthy dogs (n=9), dogs with CE (n=8), and cats with CE (n=9). Rectal swab and feces were collected from clinically healthy cats (n=8). DNA extraction and 16S rRNA gene sequencing was performed on each sample. Analysis of similarity (ANOSIM) was performed to determine the difference in the bacterial community structure between fecal, rectal swab, and colonic biopsy samples for each study group. For healthy dogs, the bacterial communities were not significantly different amongst the three sampling techniques. For healthy cats, the fecal bacterial community was significantly different when compared to rectal swabs (R=0.815; P=0.001). For dogs with CE, the fecal bacterial community was significantly different when compared to the colonic biopsy samples (R=0.251; P=0.022). There was no significant difference in the bacterial community between rectal swabs when compared to feces and colonic biopsies. For cats with CE, the fecal bacterial community was significantly different when compared to rectal swab samples (R=0.272; P=0.001) and colonic biopsy samples (R=0.192; P=0.020). There was no significant difference between rectal swabs and colonic biopsies. In conclusion, rectal swabs may be an alternate option to collection of fecal samples in dogs and to collection of colonic biopsy in both dogs and cats. **Funding**: Comparative Gastroenterology Society, Boehringer Ingelheim Veterinary Scholars Program

DVM Student / Clinical Sciences

32. CD4+FoxP3+ T cells are elevated in two indolent canine hematologic cancers

Emily A. Garrison, Janna A. Yoshimoto, Lauren J. Harris, and Anne C. Avery

T regulatory cells (Tregs) are an important part of the healthy immune system, but cancerous tissues can take advantage of these cells to diminish the antitumor immune response. Immunosuppression is known to be an important sequela of indolent hematologic malignancies, but the exact cause of this process is unknown. Immunosuppression has been observed in canine T zone lymphoma (TZL) and human B cell chronic lymphocytic leukemia (B cell CLL) but has not yet been studied in canine B cell CLL. Previous studies have found that Treg levels are elevated in other human and canine cancers which may be the underlying cause of immune suppression. We hypothesized that CD4+FoxP3+ T cells would be increased in peripheral blood from dogs with B cell CLL and lymph node aspirates from TZL cases. To evaluate this, flow cytometry and immunohistochemistry (IHC) were performed on tissues from 69 dogs. A Mann-Whitney U test was then used to compare the median levels of CD4+FoxP3+ cells between normal tissues and dogs diagnosed with one of these indolent hematologic cancers. B cell CLL and CD8+ TZL both had significantly elevated CD4+FoxP3+ T cells, while CD4-CD8- TZL did not. These results provide insight into the pathogenesis of these cancers and related immunosuppression. We will continue to investigate the significance of these findings by performing cell cultures to assess the immunosuppressive capabilities of these CD4+FoxP3+ cells and gathering individual clinical signs to look for manifestations of immune suppression. **Funding**: Georgia Norris Scholarship/Canine Lymphoma Foundation

DVM Student / Microbiology, Immunology and Pathology

33. Don’t judge an embryo by its cover

Hanah M Georges, Jeanette V Bishop, Hana Van Campen, Jennifer P Barfield, and Thomas R Hansen

Early embryonic mortality accounts for 75-80% of the current decline in bovine fertility, costing the industry over $1.28 trillion worldwide. Despite advancements in assisted reproductive technologies, many transferred embryos do not survive past day 24 of gestation. It was hypothesized that visually lower quality *in-vitro* fertilized (IVF) day 7 blastocysts were developmentally delayed as a result of altered mitotic signaling, increasing the risk of embryo mortality. To identify potential causes for early embryo mortality in IVF embryos, RNA-Seq was performed on 6 categories of day 7 blastocysts: stages (S) 5 (early), 6 (full), and 7 (expanded) with quality scores (Q) of 1 (healthy appearing) or 2 (some defects in morphology). Oocytes were matured, fertilized *in vitro*, cultured for 7 days, classified/ graded into the 6 categories above, and subjected to Pronase digestion of the zona pellucida. RNA was extracted from three biological replicates of each blastocyst group and submitted for RNA-Seq. Secondary bioinformatic analyses were performed using R to determine differentially expressed genes. When S7.Q1 blastocysts were compared to other categories, 55 genes were differentially expressed ($p<0.05$) in S5.Q1/2 and S6.Q2. The 5 top common upregulated genes were *BTG4*, *ARGFX*, *GPC4*, *BOC*, and *CNTNAP2*. The top 5 common downregulated genes included *MUC1*, *HSD3B1*, *ADAM19*, *EVPL*, and *TGM1*. EVPL and TGM1, associated with cell barrier permeability, may be limiting communication between blastomeres. This limited communication might delay embryonic gene expression at the 4-8 cell stage and the maternal zygotic gene expression transition, as supported by an increase in *ARGFX* and *BTG4* mRNA concentrations. This work was supported by USDA NNF 2016-38420-25289 and Zoetis, Inc. **Funding**: USDA NNF 2016-38420-25289 and Zoetis, Inc

Graduate Student / Biomedical Sciences

34. Decellularized Biological Scaffolds for the Prevention Post-Operative Seroma Formation

Kathryn A. Gleason, Ruth J. Rose, Laura Chubb, Nicole Ehrhart

Introduction: Seroma is a frequent and frustrating post-operative complication. Decellularized biological scaffolds, which are known to be immunomodulatory, have been shown to have beneficial effects on wound healing. We hypothesized that decellularized freeze fractured tissue scaffolds (DFFTP) would prevent or reduce the formation of post-operative seromas in a rodent model of seroma formation. Materials and Methods: A previously validated latissimus dorsi (LD) resection model was used for the study. 56 Sprague Dawley rats were randomly assigned to receive either 1) no DFFTP, 2) skeletal muscle DFFTP, 3) adipose DFFTP, 4) dermis DFFTP. Seroma diameter was monitored and measured with calipers weekly by a single blinded observer. Rats were euthanized at either 28 days or 60 days and the surgical sites were collected for histological evaluation. Results: All DFFTP treated groups had a higher number of seromas and greater seroma volume as compared to controls (no DFFTP) ($p <0.001$). When compared to the other DFFTP groups, skeletal muscle decellularized tissue products had reduced seroma volumes and more rapid seroma resolution. Histology is pending. Discussion/Conclusions: Decellularized freeze fractured tissue powders did not prevent acute seroma formation and therefore our hypothesis was rejected. The muscle-derived DFFTP had the most rapid decrease in volume and number of resolved seromas when compared to the other DTTFPs. While DTTFP may not be efficacious at preventing seroma, further studies will focus on their use to treat chronic persistent seromas. **Funding**: Gift of Hope Foundation, Limb Preservation Foundation and Boehringer Ingelheim

DVM Student / Clinical Sciences

35. Something Old, Something New: Revisiting and novel applications for wing scale counting as a tool for age-grading *An. gambiae*

Lyndsey Gray, Bryce Asay, Emmanuel D Sougué, Anyirekun F Some, Roch K Dabiré, Sunil Parikh, Brian D Foy

Malaria transmission is successfully prevented when *Anopheles gambiae* mosquitoes are killed before Plasmodium parasites complete their developmental cycle. To measure this outcome, researchers need efficient and accurate methods to determine mosquito age. Our research investigates scale loss along posterior wing edges, a revised technique first used in the early 1900s, as a simple and accurate means of determining mosquito age on both the individual- and population-level scale. To begin, colony-raised *An. gambiae* mosquitoes were reared through four blood feedings in standard biocages. Adult females were dissected and wing scales were counted. Linear correlation analyses demonstrated that loss of wing scales could be used to predict female *An. gambiae* age (p-value for R2s <0.01). Secondly, images of dissected wings were used to train a mosquito age predicting machine learning model. This model found that wing analysis predicted mosquito age with 90% accuracy. Lastly, *An. gambiae* were reared in two separate mesocosms for 10 weeks to replicate semi-field environments. One mesocosm was exposed to four blood meals that contained a sub-lethal concentration of ivermectin, a mosquitocidal drug, to determine if shifts in the mesocosm population age structure could be detected via wing scale counting. Results indicated that wing scales were lost as mosquitoes aged (p-value for R2<0.001) and population-level differences in age structure following ivermectin treatment were detected via wing scale counting (all two-way t-test p-values <0.01). Our findings may provide an alternative field method for detecting age shifts in malaria mosquito population structures, as well as opportunities for novel biotechnology development based on mosquito wing scale counting. **Funding**: NIH, International Vector Control Consortium

Graduate Student / Microbiology, Immunology and Pathology

36. Effects of physostigmine on quality of recovery and post-operative gastrointestinal dysfunction following inhalant anesthesia in horses

Alexandra Creigh, Drew Koch, Diana M Hassel

The number of adverse events from undergoing general anesthesia are significantly higher in horses than humans or companion animals, with death rates up to 1.8%. A third of those deaths are associated with the anesthetic recovery period, many through catastrophic injuries associated with poor quality of recovery. Current thinking assigns this post-anesthetic disorientation to residual low circulating levels of inhalant anesthetics that produce anticholinergic effects. Based on previous studies, it was hypothesized that physostigmine, an acetylcholinesterase inhibiting drug, would combat this disorientation and increase both recovery quality and gastrointestinal motility during the post-operative recovery period. This study sought to confirm promising earlier work on a small population of horses demonstrating a positive effect of physostigmine on post-operative recovery and gastrointestinal motility. A randomized, double-blind, clinical trial was conducted on 32 horses undergoing elective arthroscopy and 9 horses undergoing emergency ventral midline celiotomy for treatment of colic using both objective and subjective measurements. Data analyzed by Student t-tests demonstrated a statistically significant increase in fecal output ($P < 0.05$) during the early post-operative period in horses that received physostigmine compared with controls with no effects identified on quality of anesthetic recovery. This study did not support previous findings suggesting that physostigmine improved recovery scores in horses, although it does suggest that gastrointestinal motility may be improved using physostigmine post-operatively. Considering the high prevalence of gastrointestinal stasis in horses undergoing both elective and emergency general anesthesia, physostigmine may serve as a promising preventative therapy for this common complication. **Funding**: USDA Animal Health & Disease Program, Center for Companion Animal Studies Young Investigator Award

DVM Student / Clinical Sciences

37. Metformin modulates T cell terminal differentiation following *M. tuberculosis* infection

Jessica D Haugen Frenkel, Marissa Quilici, Amanda Latham, David F Ackart, Alexandra K Todd, Andrés Obregón-Henao, Randall J Basaraba

Despite major efforts to elucidate what constitutes a protective immune response against Mycobacterium tuberculosis (Mtb) infection, knowledge gaps exist that hinder our ability to prevent or treat Tuberculosis disease (TB). Our data show that metformin has in vitro and in vivo immunomodulatory activity and enhances protection against Mtb in guinea pigs. The mechanisms by which metformin alters T cell function following activation and whether these cells are responsible for the protection observed against TB is currently unknown. We hypothesize that metformin promotes the development of more-protective, less differentiated T cells that are capable of self-renewal, multipotency, and persistence by targeting mitochondrial function. To test this, we used an in vitro model system that generates different levels of T cell differentiation through anti-CD3/anti-CD28 activation. In response to metformin treatment, we find that T cells have dramatically reduced mitochondrial respiration and membrane potential following activation, which corresponds to decreased expression of the terminal differentiation marker KLRG-1 and transcription factor Bcl-6. Moreover, metformin significantly enhanced the expression of the activation-induced endothelial adhesion ligand CD44 and decreased the IL-2 receptor alpha chain, CD25. Taken together, these data demonstrate that metformin preserves mitochondrial function following T cell activation, thereby limiting their level of differentiation. Future studies will evaluate the corresponding protective efficacy of these "less-differentiated" T cells against Mtb infection through adoptive transfer experiments, which will help guide the development of novel vaccines and host-directed therapies against TB. **Funding**: 1U19AI111224-01, 1R21AI107254, 1R01AI106733

Graduate Student / Microbiology, Immunology and Pathology

38. Synaptic Ultrastructure at the Drosophila Neuromuscular Junction

Jasmin A Hicks, U. J. McMahan, Noreen E Reist

Fast and efficient neurotransmission is orchestrated through the release of neurotransmitter from a presynaptic nerve terminal, which travels across the synapse and reaches an adjacent cell. The "active zone", a specific presynaptic specialization of the neuromuscular junction (NMJ), is the primary mediator for transmitter release during cell-to-cell communication. Synaptic vesicles dock, prime, and ultimately fuse with the presynaptic membrane at the active zone. Proteins required for each of these steps are highly concentrated and specifically organized into active zone material (AZM). Previous experimentation has revealed functional roles of several active zone proteins. However, the location of these essential proteins remains unknown. Determining the synaptic ultrastructure of the AZM will help us determine the functional relationships of active zone proteins and decipher the molecular mechanisms mediating the vesicle cycle. However, resolution limitations of standard electron microscopy (~50 nm) has limited our ability to identify the spatial relationships between individual proteins and vesicles. Recently, through the use of electron tomography, the 3D active zone ultrastructure of mouse and frog has been determined at a sufficient level of resolution to begin assessing functional relationships. This technique permits the analysis of 0.5 nm virtual slices through a single 50-70 nm sample, to construct a 3D rendering of the sample. I have started to exploit the fast, cost effective, genetic system of *Drosophila* and high resolution of electron tomography to further elucidate the ultrastructural makeup at the neuromuscular junction. **Funding**: Graduate Research Fellowship Program, NSF

Graduate Student / Biomedical Sciences

39. TLR agonist as a protectant against radiation toxicity

Leone Hopkins, Lauren Harrison, Mary-Keara Boss, Steve Dow

Radiation therapy is widely used to treat malignant neoplasia in dogs, particularly in sites such as the skin and nasal cavity. While this approach can effectively reduce the size of lesions, there are adverse effects associated with radiation therapy. These adverse outcomes are especially common when radiation therapy is used to treat tumors near mucosal tissues in dogs. Following treatment, the patient's mucosa often develops severe lesions including loss of mucosal epithelium resulting in chronic inflammation long after tumor regression. Treatments to minimize epithelial lesions from radiation therapy in companion animals are currently lacking. Toll-like receptor (TLR) protein agonists, such as the TLR5 agonist flagellin, have shown promise as radioprotectants to spare local mucosal tissues from the effects of radiation. Our lab has developed a liposomal-TLR agonist that is designed to stimulate the immune system and provide protection from respiratory infections. We found this same immune therapeutic has the potential to protect tissues from the adverse effects of radiation therapy. **Funding**: Center for Companion Animal Studies Young Investigator Award

DVM Student / Clinical Sciences

40. Approaches to study KDM1A in ovine placenta

Taylor K Hord, Ali Asghar, Gerrit J. Bouma, and Quinton A. Winger

The placenta is an organ that's developed during pregnancy that's required to regulate gas and nutrient exchange between the mom and the developing fetus, as well as endocrine signaling with secretion of progesterone, estrogens, and androgens. KDM1A is a histone lysine demethylase which plays a role in sex steroid signaling. The histone lysine demethylase KDM1A removes methyl groups from specific lysines on histone tails to activate or repress transcription. KDM1A specifically interacts with estrogen receptor 1 (ESR1) and androgen receptor (AR) to allow transcriptional activation in cancer cells. KDM1A also interacts with ESR1 and AR in placental trophoblast cells possibly regulating placental angiogenesis. The purpose of this study was to develop an approach to knock out KDM1A in vivo in sheep placenta to understand how its effects on ESR1 and AR expression and function. We chose utilize both a lentiviral-CRISPR/cas9 and a lentiviral-shRNA approach to knock out and knock down, respectively, KDM1A in placental cells in vivo. Placental tissue were collected at day 16 before attachment and implantation and snap frozen in liquid nitrogen for further analysis by western blot and qPCR analysis. Preliminary data indicates successful knockout of KDM1A protein using the lentiviral-CRISPR/cas9 construct in trophoblast cells, which also led to significant lowering of AR and ESR1. **Funding**: USDA grant 2017-67015-26460

Graduate Student / Biomedical Sciences

41. Effect of a single dose of oral trazodone on intraocular pressure in healthy horses

Rachel Hritz, Alexandra Moss, Rachel C Hector, Kathryn L Wotman

Trazodone has been shown to effectively elicit sedation when administered orally to horses. Therefore, it is becoming increasingly used to calm horses that show anxiety or excitement while on prolonged stall rest for medical treatments (e.g., ophthalmic disease). Literature in other species have identified alterations in intraocular pressure (IOP) after trazodone administration. This study evaluated the effect of oral trazodone on behavioral, physical, and selected ocular parameters in horses. Eight clinically normal horses of varying breeds (4 mares, 4 geldings, 9 ± 5 years) were acclimated to IOP and vertical pupil diameter (VPD) measurement over 48 hours before starting the study. Baseline heart rate, respiratory rate, rectal temperature, gastrointestinal borborygmi, level of sedation and IOP and VPD in both eyes were assessed prior to administration of 6 mg/kg oral trazodone the morning of the study. All parameters were measured again at 0.5, 1, 2, 4, 6, 8, 12, and 24 hours post administration. Jugular venous blood samples were also drawn at each time point for measurement of trazodone plasma concentrations. Mild sedation was evident in seven horses from 0.5 to 8 hours. Mean IOP was significantly decreased (-7 to -4 mmHg) in both eyes for the same time period. Mean VPD was significantly reduced (-1.2 mm) only at 0.5 hours. Rectal temperature was significantly reduced from 1 to 8 hours, and four horses exhibited moderate sweating. No other systemic effects were observed. Trazodone was detected in plasma in all horses up to 24 hours, with peak concentrations at 0.5 to 1 hour. Oral trazodone at 6 mg/kg produces sedation with minimal adverse systemic effects in healthy horses, but has a significant influence on IOP and VPD. Further investigation for its potential use in horses with ophthalmic disease is warranted.

DVM Student / Clinical Sciences

42. Osteoporosis Correlated to Degradation of Tendon Mechanical Properties in an Ovine Model

James W. Johnson, Jeremiah Easley, Brad Nelson, Eileen Hackett, Devin von Stade, Lauren Berens, Cecily Broomfield, Katie Bisazza, Erin McCready, Kirk McGilvray

Patients undergoing rotator cuff tear repair surgeries are currently experiencing failure rates between 20-94%, and osteoporotic patients are 4.6x more likely to experience failure. The purpose of this study was to determine if there was a correlation between osteoporosis and tendon degradation in a large animal model. Using a previously validated ovine model, osteoporosis was established in ten ewes. To generate differing levels of bone loss, the animals were given four weekly intravenous injections of either saline (PBS) or bisphosphonate (BP). Six shoulders obtained from unrelated studies were used as healthy controls. Non-destructive stress relaxation tests were performed followed by µCT analysis of the humeral head. The mechanical properties, cross-sectional areas, and trabecular morphometry of both tendon treatment groups were significantly and markedly changed (as measured by percent relaxation, maximum force, bone volume percent, and trabecular spacing). Furthermore, there were significant, moderate correlations between the deleterious mechanical properties of the tendons and trabecular morphometry. These data indicate that systemic osteoporosis in an ovine model effects not only bone quantity, but also the tendon's morphological and biomechanical viscoelastic properties, potentially explaining the clinical correlation seen between osteoporotic patients and increased clinical cases of rotator cuff tendon repair failures.

Graduate Student / Clinical Sciences

43. Myxoma ORFC virus detection in tissue

Mariah S Jordan, Laura V Ashton, and Amy L MacNeill.

Myxoma virus (MYXV) is non-pathogenic outside of its natural host but can cause apoptosis in cancer cells making MXYV an ideal oncolytic viral therapy. Oncolytic virus therapy uses a replicating virus to lyse cancer cells and not harm any of the cancer patient's heathy cells. The walleye dermal sarcoma virus (WDSV) has an orfC gene that encodes for a pro-apoptotic protein believed to induce seasonal regression of tumors. The orfC gene was isolated from the WDSV and inserted into MYXV to make a recombinant that expressed orfC and increased apoptosis in cell culture when compared to wild-type MYXV. When infecting rabbits with the MYXV-orfC recombinant, the virus was attenuated in the rabbits. We hypothesized that the MYXV-orfC virus DNA will be detected in the same organs as the wild-type virus because the MYXV-orfC virus should have similar tissue tropism as the wild-type virus. We extracted the DNA from tissues of rabbits that were infected with MYXV-orfC and rabbits that were infected with wild-type MYXV virus. Then a PCR protocol was followed using two primer sets (95/96 and 78/79) to amplify viral DNA from the heart, kidney, liver, lung, spleen, primary and secondary lesions. As we hypothesized, all the samples showed 800kb bands when using primers 78/79 which indicated the virus was present in those organs. The tissue samples of the organs from the rabbits infected with MYXV-orfC had a 1500kb band when using 95/96 primers for PCR while the wild-type virus had a 320 kb band. **Funding**: Clinical Pathology Research & Development Funds, The Animal Health Innovation Fund (CRC)

Undergraduate Student / Microbiology, Immunology and Pathology

44. Effect of vaccination with commercial distemper-adenovirus-2-parainfluenza-parvo vaccines on platelet numbers and development of anti-platelet antibodies in healthy dogs

Matt Khorsand, Maggie Williams, Michael Lappin, Nida Chornarm, Melissa Brewer, Jennifer Hawley, and Sarah Shropshire

Immune thrombocytopenia (ITP) occurs when platelets are destroyed as a result of antibody-mediated mechanisms. In humans, thrombocytopenia has been observed following vaccination for measles, mumps, and rubella. In dogs, it has been documented following vaccination for distemper virus and for a paramyxovirus similar to mumps. A causal relationship between vaccination and ITP, determined using anti-platelet antibodies has not been established. We previously demonstrated that in a small number of healthy research beagles, administration of commercial distemper-adenovirus-2-parainfluenza-parvovirus (DA2PP) vaccines did not result in clinically relevant changes to platelet number or the development of anti-platelet antibodies. This pilot study aims to expand on those results by evaluating whether administration of DA2PP vaccines to client-owned dogs causes a thrombocytopenia and if so, if it is associated with development of anti-platelet antibodies. Thirty client-owned dogs deemed healthy based on history, physical exam, complete blood count (CBC) and serum biochemistry were randomly divided into one of three commercially available DA2PP vaccine groups. On day 0, CBCs, biochemistries, and anti-platelet flow cytometry was performed and dogs were vaccinated with their respective vaccine. On day 10, repeat platelet count and anti-platelet flow cytometry were performed. Eighteen dogs have completed the study. To date, at day 0, all dogs had normal platelet counts, or platelet mass deemed adequate if clumps were present, and no dog was positive for anti-platelet antibodies. On day 10, 2/18 dogs had mild, clinically insignificant, decreases in platelet count that were not associated with development of anti-platelet antibodies. One dog became weakly positive for anti-platelet antibodies on day 10; this dog's platelet counts were below reference intervals but with adequate platelet mass on both days and count increased slightly from day 0 to 10. While preliminary, these results fail to show a link between vaccination with DA2PP and ITP. **Funding**: Center for Companion Animal Studies Young Investigator Award

DVM Student / Clinical Sciences

45. Use of a probiotic with supplemental fiber for treating antibiotic-induced diarrhea in cats

Jeremy A Kiene, Kelsey Dobesh, and Michael R Lappin

Diarrhea is a common and potentially significant complication in cats undergoing antibiotic therapy. Feeding a commercially-available probiotic containing *Enterococcus faecium* strain SF68 to cats prior to amoxicillin-clavulanate administration was shown to lessen diarrhea compared to a control group. However, data supporting the benefit of feeding probiotics to cats after antibiotic-induced diarrhea has started is lacking. This study examines effects of feeding a probiotic with SF68 and supplemental fiber on pre-existing feline antibiotic associated diarrhea.Sixteen healthy, young-adult research colony cats were administered amoxicillin-clavulanate at 62.5 mg/cat twice daily. The cats were fed a commercially available dry food for the duration of the study. Using a standardized fecal scoring system, trained, masked personnel scored feces every twelve hours, with scores >4 considered diarrhea. Cats with fecal scores >4 for 2 days had the antibiotic stopped and were randomized into 2 treatment groups. One group was fed the probiotic with fiber in 15g of canned food once daily and the other group was fed the palatability enhancer from the probiotic in 15g canned food once daily. Diarrhea was less severe overall in the probiotic group, with 24.5% of fecal samples scored >5, compared with 48.9% in the control group ($p = 0.0132$). Time to diarrhea resolution was 4.5 periods from initiation of treatment in the probiotic group, compared with 6.5 periods in the control group ($p = 0.472$). Results indicate that feeding this probiotic to cats with diarrhea provoked by administration of this antibiotic reduces diarrhea severity and may decrease time to diarrhea resolution. **Funding**: Nestle Purina PetCare and the Center for Companion Animal Studies

DVM Student / Biomedical Sciences

46. Effect of oral administration of trazodone on intraocular pressure in normal dogs

Kyle E. Kline, Sienna Drizin, Michael R. Lappin, Rebecca Ruch-Gallie, Kathryn L. Wotman

Trazodone has shown to be successful in reducing stress-related behaviors in hospitalized dogs, and to facilitate confinement and calming after surgical procedures. These effects are desired after ophthalmic surgeries to promote ocular healing and patient welfare. The potential for oral trazodone to influence intraocular pressure (IOP) should be evaluated in healthy dogs before considering its potential use in patients with ocular abnormalities. This study is a prospective randomized double-blinded crossover study used to evaluate the effect of oral trazodone on IOP in normal dogs. Eight research beagles were randomly assigned to treatment versus control groups. Control groups received placebo and treatment groups received 50 mg of trazodone by mouth (PO) once per day. The IOP was measured in both eyes via rebound tonometry prior to treatment (T_0), and again at designated hours post-treatment (T_1, T_4, T_8, and T_{12}) for a total of three days. A sedation score was recorded for each dog at every time-point based on a previously defined grading scale that ranged from 0 (most alert) to 9 (most sedate). Following a three-day washout period, the control and treatment groups were switched. Measurements and sedation scores were recorded for an additional three days following the same protocol. Statistical analysis was performed in SAS 9.4. A mixed model was fit to the data, and Dunnett's method was used to compare downstream time points versus baseline for each treatment. Wilcoxon signed-rank test was used to compare sedation scores between treatments at each time point. No statistical differences were detected between the baseline IOP mean values and post-treatment values in both the treatment and control groups. Based on the findings of this study, oral administration of clinically useful doses of trazodone in healthy dogs does not cause a significant change in intraocular pressure (IOP). **Funding**: Center for Companion Animal Studies Young Investigator Award

DVM Student / Clinical Sciences

47. Circadian Effects of Opioid Administration

Christian Korkis, Sierra Curdts, Shane Hentges, and Jozsef Vigh.

Purpose: The purpose of this study is to analyze the opioid effect on the circadian rhythm of sleep/wake cycle, locomotor activity, and body temperature of mice. Methods: DSI telemetry devices were implanted in all animals as a means of recording body temperature, activity, EEG, and EMG data in both the control and treatment groups. Experiments began after three days of recovery with five days of baseline recording. Then, osmotic mini pumps supplying morphine at 1 µL/hour for seven days were implanted. Sleep/wake was determined by EEG, EMG, and activity measures using Neuroscore software. The circadian oscillations of activity and body temperature data were analyzed using three paradigms: cosinor method (robustness), the Lomb-Scargle periodogram method, and via calculating intradaily variability. Results: The measure of robustness and the Lomb-Scargle periodogram method were directly related to each other, while intradaily variability was indirectly related to the other two measures. Robustness measures of the circadian rhythm of activity was much higher during the first few days of baseline recordings compared to the first few days following the implantation of morphine pumps. Following these first few days, the robustness of the circadian rhythm with opioids largely recovered and was similar to the first few days of baseline recordings. Morphine had a transient effect. The circadian oscillation of body temperature followed a very similar trend, again illustrating the transient opioid effect. This is consistent with the notion that changes in body temperature are secondary to changes of physical activity. This is also consistent with sleep/wake data showing that morphine induces wakefulness early in the experiment, while its effect on wakefulness is reduced later. **Funding**: NIH

Graduate Student / Biomedical Sciences

48. Incidence of septic arthritis following arthroscopy and joint injection was independent of antibiotic protocol

Danielle Krause, Lynn Pezzanite, and Dean Hendrickson

The increased incidence of multi-drug antimicrobial resistance in veterinary medicine, which increases healthcare costs and threatens patient outcomes, has prompted re-evaluation of prophylactic antibiotic usage in many cases. In equine veterinary surgery, systemic antibiotics are commonly administered prophylactically for elective procedures such as arthroscopy, and intra-articularly in joint injections. However, little evidence exists to justify prophylactic antibiotic dosing regimens in elective surgery or joint injections where postoperative and post-injection infection rates are reportedly low. The objective of this study was to evaluate and compare the incidence of septic arthritis and other complications following elective arthroscopy and joint injections to determine whether different prophylactic antimicrobial protocols affect the rate of septic arthritis or other complications. Records from the Colorado State University Veterinary Teaching Hospital were evaluated from 2010 to 2014 and equine patients undergoing elective arthroscopy or joint injection were identified. Patients undergoing arthroscopy or injection for septic arthritis were excluded from analysis. Patient signalment, attending clinician, joint involved, preoperative/prophylactic antibiotic regimen, and postoperative complications including incidence of joint infection were recorded. Data were examined for normality of distribution using a Shapiro–Wilks test. The effect of antibiotic usage on infection incidence and complication rate was examined by one-way ANOVA. Antimicrobial protocol did not affect incidence of postoperative complications. If prophylactic antibiotics are given preoperatively for elective arthroscopy, no additional benefit was observed with the addition of gram-negative coverage or with multiple doses of gram-positive and gram-negative coverage compared to a single dose of gram-positive antibiotic coverage alone. **Funding**: Center for Companion Animal Studies Young Investigator Award

DVM Student / Clinical Sciences

49. Are you counting correctly? Improving methods for categorical data analysis

Steven M Lakin and Zaid Abdo

Counting and categorizing data is fundamental to many analyses in the life sciences. For example, we count observations of microbial taxa when doing microbiome analysis. Counts that fall into various categories can be analyzed using a broad class of probability distributions called multinomial distributions. The standard multinomial distribution is simply the proportion (or probability) of observing a category; perhaps in a fruit basket we observe apples 50% of the time, while oranges and pears make up 25% of the observations. Note that these sum to 100%, which is an assumption of the multinomial distribution: if one category increases in probability, the others must decrease. This assumption of negative correlation between categories is often violated by biological data, which introduces error into the analysis. To improve count-based categorical analyses, we propose the use of two extended probability distributions called the Beta-Liouville Multinomial and Generalized Dirichlet Multinomial distributions. These distributions make fewer assumptions about the data, making them more flexible and often more accurate than the standard multinomial distribution. However, because they are more complicated, these advanced distributions must be solved using a statistical method called Maximum Likelihood Estimation (MLE). Here, we introduce a computationally efficient MLE method for the Beta-Liouville Multinomial and Generalized Dirichlet Multinomial distributions and show that these distributions perform as well or better on real-world datasets compared to the standard multinomial distribution. We also explain how it can be used in a variety of contexts, including for difficult problems like classifying DNA sequences against sparse databases. **Funding**: USDA National Scientist Training Program

Graduate Student / Microbiology, Immunology and Pathology

50. Preparation of platelet rich plasma in rats: a two-step centrifugation technique

Bethany E Liebig, Jennifer N Phillips, John D Kisiday, and Laurie R Goodrich

Almost 8 million bone fractures occur annually in the United States, of which 5-10% result in delayed or nonunion, causing increased pain and financial burden. These rates increase for the elderly or those with co-morbidities, such as diabetes and osteoporosis. Current strategies for promoting bone regeneration have not met this clinical challenge. Platelet-rich plasma (PRP) is plasma with a platelet concentration greater than whole blood. PRP is readily available, inexpensive, and has an abundance of growth factors associated with bone formation. For these reasons, PRP is an excellent candidate for adjunctive bone regeneration therapies. Our long-term goal is to study PRP's effect on bone healing using a rat segmental defect model. Our lab recently developed a rat PRP preparation protocol based on our experience making PRP for other species. We tested three two-step centrifugation protocols on 23 samples created by pooling blood from rats euthanized for an unrelated study. The average platelet concentration in whole blood was $917x10^3$ platelets/µl (SD=$176x10^3$ platelets/µl). Whole blood was centrifuged at 100xg for 30 minutes to separate the plasma and platelets from the leukocytes and erythrocytes. It was noted that platelets spun out of the plasma into a fluffy buffy coat on top of dense coats of leukocytes and erythrocytes. Therefore, the platelets could be collected with the plasma with gentle pipetting. The plasma and platelets were centrifuged a second time at 500xg for 20 minutes to pellet the platelets. Eighty percent of the platelet-poor plasma was removed, and the platelets were resuspended in the remaining plasma to obtain 5xPRP. The effect of concentrating platelets on the growth factor content was evaluated by measuring transforming growth factor beta (TGFb), which for 5xPRP was 5.8-fold higher than platelet-poor plasma. Our technique results in consistent PRP that will be used as a biotherapeutic for future rat studies. **Funding**: NRSA T32 pre-doctoral training award (T32OD012201)

DVM/PhD Student / Clinical Sciences

51. High-resolution tomography analysis of Drosophila vesicle priming

Morgan L Litchford, Jasmin A Hicks, and Noreen E Reist.

Neuromuscular junctions (NMJs) are specialized contact sites, synapses, where nerve cells communicate with muscles to provide the efficient and rapid signaling required for movement. This requires transmitter-laden synaptic vesicles within nerve terminals to contact with the plasma membrane in a process called docking. Calcium influx into the terminal then triggers docked vesicles to fuse with the nerve terminal plasma membrane. Upon vesicle fusion, chemical transmitter is released into the synaptic cleft between the nerve terminal and muscle fiber, activating receptors on the muscle cells. Vesicles fuse at specialized sites called active zones, which are regulated by a variety of proteins collectively called the active zone material. Synaptotagmin is the primary calcium sensor that triggers vesicle fusion, while SNARE proteins mediate the fusion event. Despite the significance of active zone material and vesicle fusion at NMJs, little is known about the physical structures mediating key molecular mechanisms within this system. My project investigates vesicle priming by utilizing the unprecedented spatial resolution (2-3 nm) of electron tomography in the fruit fly (*Drosophila melanogaster*) model system. I measured area of contact between docked vesicles and plasma membranes as well as the membrane thicknesses. Larger contact areas correlate to how "primed" a vesicle is, or how likely it is to fuse upon a calcium signal. By analyzing the frequency distribution of these contact areas, we are better able to understand the physical and molecular mechanisms mediating vesicle fusion and thus neurotransmitter release.

Undergraduate Student / Biomedical Sciences

52. Prevalence of *Cryptosporidium spp.* in scat samples from the Portland Watershed, Oregon.

Patricia Franco, Valeria Scorza, Michael R. Lappin.

Cryptosporidiumspp. parasites are a leading cause of waterborne outbreaks worldwide. There are more than 30 valid species of Cryptosporidium; most of them are host specific while others are zoonotic. The agents can cause diarrhea in some infected humans and other animals. The Bull Run watershed is the principal drinking water supply for Portland, Oregon. The watershed has no residential, commercial or agricultural development and due to its protected nature, wildlife is considered the most likely source of Cryptosporidium spp.. We have been performing detection of Cryptosporidium spp. from scat samples from the Bull Run Watershed since 2015. In this abstract, we report the prevalence of Cryptosporidium spp. in scat samples during 2019. We performed an 18S-hsp70 multiplex PCR and an 18S nested PCR assays as screening and confirmatory tests respectively followed by sequencing analysis. We tested a total of 220 fecal samples from rodents, bobcats, coyotes, bears, bats and mountain beaver among other wildlife during 2019. Samples were considered positives if Cryptosporidium spp. DNA was amplified by either PCR assay and confirmed by sequencing analysis. Thirteen of 220 samples (6.2%) amplified Cryptosporidium spp. DNA from one bat, two coyotes, four deer mice, four mountain beavers and two bobcats. Cryptosporidiumspp. was detected in a wide range of animal species and its prevalence is likely underestimated due to the low detection limit of these assays on scat samples. **Funding**: City of Portland and the Center for Companion Animal Studies

Staff / Clinical Sciences

53. Making Colorado State University more green: implementation of a laboratory sustainability program

Brooke A MacNeill, Rachel E Conway, Daphne L O'Grady, Shelby L Hanson, Misha J Harrison, Annie K Clift, Parisa Kariminejad, Courtney N McGinness, Lauren A Riggs, Tracy L Webb, Stacey Baumgarn, and Colleen G Duncan

Research laboratories are estimated to consume five times more energy than standard business offices, and laboratory plastics were estimated to account for 12 billion pounds of waste in 2014. Although laboratories, like hospitals, can have special challenges with recycling and other sustainable practices, programs are being developed to help decrease the negative environmental impacts of laboratory research. The objective of this project was to assess the feasibility of implementing a laboratory sustainability program to a research laboratory in a veterinary college. My Green Lab, a program recognized by the Association for the Advancement of Sustainability in Higher Education, American Energy Society, and International Institute for Sustainable Laboratories, has a multi-step laboratory certification program and was chosen to assess applicability and ease of implementation at CSU (Colorado State University). The team partnered with a research laboratory at the Veterinary Teaching Hospital to pilot the program. Comprehensive survey questions established by My Green Lab were used to ascertain a baseline of sustainability as well as action items in the areas of electricity, lighting, cold storage, fume hoods/ventilation, water, inventory management, recycling, waste reduction, hazardous waste, green chemistry, gloveboxes, travel, fieldwork, and community. The team assessed the process and survey outcome information and created resources for both specific and broad-based implementation of the recommendations. Once implemented, these resources will result in the first certified Green Lab at CSU and provide a template for similar research laboratories to use. A survey was then created to assess interest in laboratory sustainability by research personnel. The data and resources gathered during the certification process combined with data collected on the interest and impact of such changes in the college will help inform decisions on support for improved laboratory sustainability at CSU. **Funding**: CSU Facilities Management and the CSU Green Labs initiative

DVM Student / Microbiology, Immunology and Pathology

54. Cows as Canaries: Using a One-Health Approach to Investigate Air Pollution Effects

Heather E Martinez, Bonni L Beaupied, Colleen G Duncan, Craig S McConnel, Sheena E Martenies, and Sheryl L Magzamen

Livestock is a significant economic industry in 17 eastern Colorado counties where air pollution monitoring is severely limited. Companion and laboratory mammals have similar physiologic responses to air pollution exposure as humans, but few studies have investigated effects on livestock. The objective of this study is to investigate the effects of air pollution and temperature-humidity index (THI) on milk production and somatic cell count (SCC) in dairy cattle. We hypothesize that ozone and particulate matter (PM) are unmeasured confounders in the relationship between THI, milk production and SCC. We used milk production data from our partner dairy in Northern Colorado and PM, ozone, temperature, and humidity data from the local air quality and meteorological monitoring networks. We tested the associations between our daily environmental exposures and production parameters using a linear mixed model in SAS 9.4. In unadjusted models, a 1-unit increase in THI was associated with a 1432 cells/mL (95% CI: 1138, 1726) increase in SCC. The adjusted model with ozone and PM was associated with a 1294 cells/mL (95% CI: 962, 1628) increase in SCC. Similar trends in daily milk production were seen when adjusted for PM and ozone but did not yield statistically significant results. The broader objective of this work is to investigate the use of dairy cattle as a sentinel species for air pollution monitoring in Eastern Colorado. With preliminary evidence of an association between air pollutant levels and cow health, the immediate next step is to validate this relationship with additional partner dairies throughout the region. **Funding**: Vice President for Research/One Health Institute

Graduate Student / Environmental and Radiological Health Sciences

55. The Role of Osteosarcoma Cell-Derived Exosomes in the Promotion of Lung Metastasis

Alissa B. Mathias, Eric P. Palmer, Nicole Kruh-Garcia, and Daniel P. Regan

Osteosarcoma (OSA) is the most common primary tumor of bone and typically occurs in kids and young adults. About 30-40% of all OSA patients will develop lung metastases, on average 1.6 years after diagnosis. These metastatic lesions do not respond to previously effective first-line therapies and only 20% of these patients will survive 4 years post-relapse. Since the 1980's, there have been no improvements in the treatment of OSA, and more importantly, no diagnostic tools that help identify the 30-40% of patients at high risk for developing lung metastases. Recent literature has shown that in other tumor types, tumor cells secrete exosomes that deliver various proteins and mRNA to distant sites to help prime the area for metastatic development via influencing non-malignant host cells, such as fibroblasts and macrophages. Therefore, the aim of this project is to understand how OSA exosomes influence non-malignant cells of the lung, and specifically if lung fibroblasts (NHLFs) and alveolar macrophages (AM's) uptake and respond to these vesicles. Hypothesis: NHLFs are primary targets of OSA exosomes, which will increase NHLF IL-6 production and cause paracrine STAT3 activation of AM's into an M2 tumor-promoting phenotype. Results: Flow cytometry and confocal microscopy demonstrate *in vitro* uptake of OSA exosomes by NHLF cells. *In vitro* 'education' of NHLFs with OSA exosomes induces significant NHLF production of IL-6, IL-8, and CCL2, with the greatest mean difference between exosome-educated and naïve NHLF observed with IL-6 secretion. Conclusion: These data suggest that OSA exosomes efficiently prime NHLFs, and that NHLF IL-6 secretion in response to OSA exosomes may be associated with OSA metastasis. Future experiments to determine the role of IL-6 in OSA survival and metastasis, as well as RNA sequencing of AMs to characterize their phenotype and determine if these cells could serve as a biomarker to predict OSA metastasis. **Funding**: National Institute of Health

Graduate Student / Microbiology, Immunology and Pathology

56. Determining risk factors of puma predation of domestic cats in an expanding urban landscape

Veronica McCann, Erick Gagne, Mat Alldredge, and Sue VandeWoude

With increasing urban encroachment along wildlands, various wildlife species – including pumas - are faced with novel inter- and intraspecific interactions as they adjust their diet and behavior to the modified landscape. Wild felids' consumption of domestic cats has resulted in the spillover of lethal infectious diseases. The factors that influence puma predation on domestic cats, however, are not fully known. Using puma kill site data collected from 2009-2012 in the Colorado Front Range in combination with a literature review of Feline Leukemia Virus (FeLV) prevalence, risk factors for predation and the resultant potential for disease spillover were assessed. Although 34.3% of females and 15% of males tracked preyed on domestic cats, it was determined that pumas show vast individual variation in affinity for domestic cats, with just 3 individuals making up 41.8% of the kills studied. Seasonally, we found a concentration of cat kills in the summer months which was unexpected given the coinciding mule deer birth pulse. Compared with overall kills, domestic cat kills were associated with higher housing density, greater distance to the forest edge, shorter distance to primary or local road, and an increased encounter rate with mule deer. Despite reported FeLV prevalence varying across study regions and populations, sick, feral, shelter, and outdoor access groups consistently represented the highest prevalences. Roaming outdoors also likely makes these cats at greater risk of becoming prey to pumas and thus transmitting disease. This insight into puma predation on domestic cats in wildland-urban landscapes could inform city planning and conservation regulations to protect these felid populations from both predation and disease transmission.

DVM Student / Microbiology, Immunology and Pathology

57. Veterinarians' Perspectives on the Health Impacts of Climate Change: Educational Gaps and Opportunities

Katherine McCaw, Collin Kramer, Jill Zaresky, Colleen Duncan

Climate change is an emerging global health threat and there is a critical need for health care providers to be informed of the health consequences. Medical schools have initiated a number of research, education, and communication initiatives around this topic. However, the veterinary community has been slow to adopt similar practices despite the role veterinary practitioners play as stewards of animal, human, and environmental health. A student research group, Sustainability Advocacy in Veterinary Education (SAVE), conducted a national survey of veterinary students to determine their knowledge, interest, and access to educational opportunities about the impact of climate change on animal and human health; they determined that students believe climate change is real, has serious health consequences for their patients, and are interested in learning more. The goal of the current project is to determine how veterinarians feel about the topic, and if their perspectives on the role of veterinary medicine in climate change match those of students. An online survey was disseminated to practicing veterinarians through online platforms and listservs. Data collection is ongoing, but preliminary review of >500 responses suggests over half of veterinarians surveyed believe they should be knowledgeable about climate change related health topics and would seek out ancillary resources. This survey in conjunction with the SAVE survey suggests that current and prospective professionals in the veterinary field lack formal education on climate change as it pertains to human and animal health and would seek out educational materials. **Funding**: CSU One Health Institute and the Center for Companion Animal Studies

DVM/MPH Student / Microbiology, Immunology and Pathology

58. Localization of Nerve Growth Factor-β in the stallion reproductive tract

Alison Mickelson, Forgivemore Magunda, James Graham, and Patrick McCue

Nerve growth factor-β (NGF-β) is a protein produced in the reproductive tract of camelids (camelids, llamas, and alpacas) that has been identified as the ovulation induction factor in seminal plasma. NGF-β from seminal plasma deposited into the reproductive tract of the female camelid acts systemically to stimulate secretion of luteinizing hormone (LH) from the anterior pituitary, which in turn causes follicle maturation and ovulation. The objectives of the present study was to 1) determine if NGF-β is present in the reproductive tract of the stallion and 2) identify the specific site(s) of production. The hypothesis was that NGF-β would be present in the stallion reproductive tract and would primarily be localized in the Sertoli cells of the testis and in the prostate gland. Immunohistochemistry on paraffin embedded formalin fixed tissues was performed using a rabbit polyclonal anti- NGF-β antibody on a total of six male equine reproductive tracts; 1 one-day old colt, 1 one-year old colt and 4 adult stallion tracts. Strong apical labeling was present in the epithelial cells of the prostate, seminal vesicles and ampullae. NGF-β staining was also present in the interstitial cells of the testis. In conclusion, immunohistochemistry revealed that NGF-β is present in the stallion reproductive tract and the protein is primarily present in Leydig cells of the testes and the prostate, seminal vesicles, and ampullae. Future studies will focus on detection of NGF-β in stallion seminal plasma and determination of the amino acid sequence of equine NGF-β. **Funding**: Coyote Rock Ranch

Graduate Student / Clinical Sciences

59. Comparison of in vitro production of bison and cattle embryos and effect of L-carnitine during maturation of bison oocytes

Adel R Moawad, Hayley Benham, Jennifer P Barfield

Development of assisted reproductive technologies for bison are less advanced than for cattle yet have value for conservation and commercial applications in bison. The aim of the first experiment was to compare in vitro oocyte maturation, fertilization and embryo development rates in bison versus cattle. Cumulus oocyte complexes (COCs) were obtained from cattle and bison ovaries post mortem. Oocyte recovery rates were lower in bison than in cattle (4.3 v. 6.7, P<0.01). Nuclear maturation (23 h post-IVM) and fertilization rates (18 h post-insemination; p.i.) were lower (P<0.01) for bison (65.1% and 32.7%, respectively) than for cattle (88.3% and 70.9%, respectively). There were fewer 2-cell embryos at 24 h and 30 h p.i. in bison compared to cattle (24 h: 13.5% v. 25.0%; 30h: 33.7% v. 67.0%, P<0.01). Cleavage and blastocyst rates were lower for bison (58.2% and 14.6%; P<0.01, respectively) than for cattle (90.8% and 22.9%, respectively). Total cell number (74.9±4.8 v.114.2±5.8), trophectoderm cells (57.9±4.6 vs. 89.2±4.8) and ICM cells (16.9±2.3 vs. 25±1.9) were all lower in bison blastocysts (P<0.01). To improve oocyte competence in bison, we evaluated effects of L-carnitine (LC) supplementation during IVM on developmental potential of bison oocytes (experiment 2). COCs were matured in IVM media supplemented with 0, 0.15, 0.3, 0.6 or 1.2 mg/mL LC. No differences were observed in cleavage rates of control and LC treated groups. A dose dependent increase in blastocyst development was found with the lowest value recorded in control group (10.4%) and the highest value recorded in 1.2 mg/mL LC supplemented group (22.2%, P<0.01). Adding 1.2 mg/mL LC to the IVM medium improved hatching blastocyst rates compared with control. In conclusion, bison oocytes exhibit lower *in vitro* maturation, fertilization and developmental rates compared with cattle oocytes. L-carnitine supplementation during IVM of bison oocytes improved embryo development and hatching rates.

Post-doctoral Fellow / Biomedical Sciences

60. Pathogenesis and development of a rapid diagnostic test for Microsporum canis

Alex Moskaluk, Sally Kuhn, Mary Nehring, Sue VandeWoude

Microsporum canis is the primary pathogen in over 90% of feline dermatophytosis (ringworm) cases. Given that this fungus is highly contagious and can spread to humans, quickly diagnosing the disease is critical, especially in high density populations. However, a rapid, accurate, inexpensive point of care assay to assess active cases and discriminate between transient carriage and active infection is not currently available. Through this study, we usurped *M. canis* metabolic pathways that result in sulphite production to develop a novel colorimetric diagnostic assay. We have acquired approximately 160 domestic feline hair samples from shelter and dermatology veterinarians from four states to establish a robust dermatophyte sample archive. We have developed PCR assays for two *M. canis* genes (ITS-1 and SSU1) and have subjected approximately half of samples to PCR and Sanger sequencing. Results indicate high homology to an M. canis reference isolate and all but three dermatophyte positive samples have been identified as *M. canis*. We have preliminarily assessed a novel colorimetric assay that can detect dermatophyte sulphite metabolites from *M. canis* positive hair samples in 12-48 hours. This work expands the knowledge about *M. canis* molecular characteristics, provides a preliminary diagnostic tool for active dermatophytosis detection, and assesses potential therapeutic targets. **Funding**: NIH/NCATS Colorado CTSA Grant Number TL1 TR002533, CSU Office of the Vice President for Research Infectious Disease Research and Response Network Summer Fellowship

Graduate Student

Post-doctoral Fellow / Microbiology, Immunology and Pathology

61. Source Attribution of MRSA Exposure and Carriage Among Dairy Workers

Jessica Nunez, Stephen J. Reynolds, Bledar Bisha and Joshua Schaeffer

Dairy farmers experience a heavy burden of bioaerosol-related respiratory ailments. Bioaerosols are known to contain inflammagens (specifically endotoxin), and a diverse bacterial community that is associated with upper respiratory inflammation and pulmonary decrement among workers. However, identifying casual agents (beyond endotoxin) is still an area that warrants further research. Industrialization and modernization of the dairy industry has led to dramatic changes to production, work organization and tasks. Consequently, exposure patterns have been altered. Recently, we demonstrated that the mass of dairy bioaerosols is predominantly present in particle size ranges that span 10-100 µm in aerodynamic diameter; these are known to deposit in the upper respiratory system (i.e., the nasopharyngeal region). The nose contains complex bacterial communities and this microbiome may play a role in the inflammatory response to bioaerosols. Recently, the nasal microbiome in dairy farmers was shown to contain over two times the bacterial diversity (and abundance) as compared to non-farmers. It is believed that this diversity is protective against the colonization of methicillin- resistant *Staphylococcus aureus* (MRSA). In contrast, persistent nasal carriage of MRSA, specifically livestock-associated strain, has been demonstrated in swine production workers. Recent evidence shows an increase in soft tissue infections caused by LA-MRSA among at risk populations. The objective of this research was to characterize the presence and carriage of *Staphylococcus spp*. with a focus on livestock associated MRSA in the nose of dairy workers. We collected nasal lavage samples before and after each shift across five consecutive days. The presence of MRSA in each sample was evaluated using culture-based methods, specifically tryptic soy agar, mannitol salt agar, Chromagar *Staph Aureus*, Chromagar MRSA. To date, up to 7% of participants (n=10) were identified to be carriers of MRSA. Based on the results from selective media, the MRSA is attributed to mixed sources, including livestock. Confirmation of these isolates will be conducted using matrix-assisted laser desorption/ionization time-of-flight and polymerase chain reaction methods.

Graduate Student / Environmental and Radiological Health Sciences

62. Identifying spatial drivers of avian influenza virus using true prevalence estimations

Hazel P. O'Doherty, Erin E. Gorsich, Ryan S. Miller, Kim M. Pepin, Sarah N. Bevins, and Colleen T. Webb.

Spatial and temporal patterns of wildlife diseases can be used to investigate underlying drivers of disease spread and to inform management decisions and disease surveillance plans to prevent infection of domestic animals. However, uncertainty in diagnostic tests used on wildlife and clustering of sampling in space and time can create a biased view if only apparent prevalence, calculated strictly from observed values, is considered. Bayesian statistical frameworks serve as a solution to biased apparent prevalence calculations, by allowing uncertainty in diagnostics and sampling to be incorporated into estimates of true prevalence. Here, wild migratory waterfowl were sampled for the presence of avian influenza virus (AIV) and multiple Bayesian statistical models were used to make true prevalence estimates for all times and locations. The model with the best fit to the data is selected using deviance information criterion (DIC) procedure, with the inference that the selected model best captures the underlying drivers creating the observed pattern of AIV. Out-of-sample model validation is used to validate how well the selected model reproduces observed patterns. **Funding**: USDA-APHIS-10025-VSCEAH00-18-0017

DVM Student / Other : Biology

63. 3D co-culture of human breast cancer cells with primary lung fibroblasts promotes drug and molecular subtype-specific differences in chemoresistance

Daniel Regan, Eric Palmer, Elizabeth Brooks, and Daniel Gustafson

Increasing data demonstrate the molecular mechanisms underlying metastasis-promoting effects of stromal cells within the tumor microenvironment (TME) share significant overlap with those which mediate therapeutic resistance. Despite this, conventional drug screening assays typically fail to evaluate contributions of TME accessory cells on extrinsic mechanisms of chemoresistance. The lung is a common metastatic sites of human breast cancer, and lung fibroblasts (LFs) are one cell type of this microenvironment which are known to promote tumor cell colonization in mouse models of breast cancer metastasis. However, the role of LFs in mediating drug resistance in human breast cancer cells (BCCs) has not been evaluated. Therefore, we evaluated the effect of primary female human LFs on anti-cancer drug responses of BCCs utilizing a 3D organotypic co-culture model, in which tumor cells stably expressing luciferase are co-cultured 1:1 with LFs in a growth-factor reduced basement membrane extract. Drug response assays were performed for four currently utilized chemotherapeutic drugs: doxorubicin (DOX), paclitaxel (PTX), the autophagy inhibitor chloroquine (CQ), and the pan PI3K inhibitor buparlisib (BUP), in seven human breast cancer cell lines representing all molecular subtypes of the disease. We identified 4 cell lines and 4 compounds associated with lung fibroblast specific induction of chemoresistance. Among them, the MB468 cell line, lung fibroblast-mediated resistance to CQ was associated with increased IL-6 secretion, and phosphorylation of GSK-3a/b, CREB, and ERK1/2. Our results demonstrate a feasible and high-throughput model for screening stromal cell effects on cancer cell chemosensitivity. Additionally, we have identified lung fibroblast-mediated induction of chemoresistance for multiple combinations of human breast cancer molecular subtypes and clinically relevant compounds. Studies are ongoing to fully define the paracrine signaling factors involved in lung fibroblast mediated drug resistance in these cells, in order to test therapeutic combinations, we predict would have increased efficacy in the lung metastatic setting. **Funding**: NIH 5K01OD022982

Staff / Microbiology, Immunology and Pathology

64. Inhaled tigecycline is highly effective against chronic pulmonary *M. abscessus* infection with promising clinical implications

Camron Pearce, Amanda Walz, Zohaib Ali, Jakko van Ingen, Mercedes Gonzalez-Juarrero

Mycobacterium abscessus (*M. abscessus*) is a nontuberculous mycobacterium that causes chronic pulmonary infections. Due to *M. abscessus's* intrinsic antibiotic resistance, treatment is often complex with low cure rates. All of the currently existing drug-regimes against this pulmonary infection require multidrug treatment and are very poorly tolerated by the patient. Tigecycline, a glycylcycline class antibiotic, demonstrates bactericidal effects against *M. abscessus* without eliciting bacterial resistance mechanisms, however, this antibiotic requires intravenous administration and causes significant side effects that limit its use. Here, we tested the hypothesis that tigecycline administered via inhalation has the potential to maximize the bactericidal effect while reducing side effects. GM-CSF knockout mice with pulmonary *M. abscessus* infection were treated by intrapulmonary tigecycline aerosols in 0.25 mg, 1.25 mg, and 2.50 mg doses for 28 days. Assessment of pulmonary bacterial burden after full treatment duration shows that inhaled tigecycline is highly effective, dose-dependent, and well tolerated in mice. Utilizing fluorescent staining techniques, the bacteria was found to be primarily intracellular within foamy macrophages. We concluded that inhaled tigecycline represents a viable treatment option for *M. abscessus* pulmonary disease. Future studies should address the pharmacokinetics, and ultimately, translation into clinical trials. **Funding**: NIH R01AI120670

Graduate Student / Microbiology, Immunology and Pathology

65. Measuring tumor oxygenation in veterinary radiotherapy patients using a novel optical spectroscopy device

David Perry, Lauren Harrison, Susan LaRue, Mark Dewhirst, Greg Palmer, Mary-Keara Boss

Tumor hypoxia, or low oxygenation, is associated with both resistance to radiotherapy and aggressive biological behavior. Tumor oxygenation has been measured via insertion of intratumoral electrodes; invasive for patients and time consuming for researchers. In this study, we explored the use of a non-invasive optical spectroscopy device to measure tissue oxygenation for veterinary patients undergoing radiotherapy. We hypothesized that tumor oxygenation would differ 1) from normal tissue, 2) pre- to post-radiation exposure, and 3) over the course of the radiotherapy protocol. Three to five measurements were taken before and after each fraction of radiotherapy at the tumor site and a corresponding normal tissue site. Patient characteristics, anesthesia data, and protocol details were recorded. 15 cases were enrolled. By two-way ANOVA, there were significant differences found between normal and tumor tissue oxygenation in soft tissue sarcomas, squamous cell carcinomas and oral melanomas, and no difference found in osteosarcomas. Soft tissue sarcomas had significantly higher tumor oxygenation compared to normal tissue, while the oxygenation of squamous cell carcinomas was significantly lower than normal tissues. A significant difference in tumor oxygenation pre- and post-radiation treatment was only seen in one case. Subjectively, tumor oxygenation did not change over course of treatment for most cases. The results of this pilot study provide preliminary data supporting the use of this device in comparative oncology trials. This non-invasive, rapid tissue oxygenation measurement method can easily be integrated into trials, allowing our team to tie serial tumor oxygenation analysis into evaluation of treatment outcomes. **Funding**: Riley Anderson Memorial Research Fund, NIH 5T35OD015130-07

DVM Student / Environmental and Radiological Health Sciences

66. *In vivo* dose titration of amikacin in equine joints reveals sustained synovial fluid concentrations and dose-dependent cartilage toxicity

Lynn Pezzanite, Gabriella Piquini, Lyndah Chow, Nikki Phillips, Paul Longhofer, Daniel Gustafson, Russell Moore, Steven Dow, Laurie Goodrich

Antibiotics exert direct cytotoxicity against mammalian cells. Equine veterinarians administer aminoglycosides intra-articularly (IA) to treat septic arthritis or prophylactically when injecting joints with corticosteroids as a treatment for osteoarthritis. No previous studies have evaluated the potential toxicity of aminoglycosides (e.g. amikacin) against normal joint cells *in vivo*. Antibiotics used IA are off-label and previous *in vivo* IA dose titrations have not been performed. The objectives were to determine the length of time that amikacin remains above MIC for common equine pathogens (^{3}4ug/mL) when injected at a range of concentrations and to evaluate the effect of amikacin on synovial fluid (SF) parameters and biomarkers of inflammation, collagen degradation, and cartilage matrix synthesis. The hypotheses were that 1) amikacin would remain above MIC for ^{3}24h at all doses and 2) would display dose dependent cytotoxicity. Amikacin (500, 125, or 31.25mg) or control (lactated ringers solution) was injected into the tarsocrural joint of 3 horses/group following aseptic preparation. Synovial fluid was sampled at 0,0.5,1,2,4,8,24h. SF amikacin concentrations were determined by high-pressure liquid chromatography/mass spectrometry. SF was evaluated for clinicopathological parameters (TNCC, TP, leukocyte differential) and biomarkers (CRP, CPII, C2C, C12C). SF amikacin concentrations remained ^{3}MIC for all treatments for 24h. Biomarkers of cartilage damage and inflammation were significantly increased (CRP 500mg vs. control 8,24h; CPII 125mg vs. control 8,24h, 125mg 0vs.24h, 500mg 0vs.8h, 0vs.24h, 8vs24h; C2C, 31.25mg vs control 8h, 125mg 0vs8h, 500mg 0vs24h and 8vs24h). Limitations include small sample size, individual variability in baseline biomarker values, and lack of sampling at later time points. Sustained concentrations of amikacin in SF above MIC for most equine bacterial pathogens for all doses of amikacin injected suggest amikacin doses used by equine practitioners to treat septic arthritis may be significantly reduced and remain effective. The effects on equine cartilage in situ warrant further investigation. **Funding**: USDA Animal Health and Disease Grant No. NI18AHDR1019785G010, Shipley Foundation, and CCTSI NIH/NCATS CTSA TL1TR002533.

Post-doctoral Fellow / Clinical Sciences

67. Antimicrobial selection for intra-articular administration may minimize cytotoxicity to equine chondrocytes and synovial cells

Gabriella Piquini, Lynn Pezzanite, Lyndah Chow, Steven Dow, and Laurie Goodrich

Intra-articular (IA) antibiotic usage is prevalent in equine practice. However, recent emergence of antimicrobial resistance prompts re-evaluation of antibiotic prophylaxis. Furthermore, many commonly used antibiotics exert direct cytotoxicity to equine cells, and appropriate IA doses have not been defined. The objective was to screen antibiotics for cytotoxicity against equine joint cells and provide dosing recommendations to practitioners for intra-articular use. Based on previous work, we hypothesized that aminoglycosides compared to other antibiotics would cause the highest toxicity to chondrocytes and synovial cells in both monolayer and in situ (explants). Chondrocytes and synovial cells were harvested from 3 horses and plated on 24-well plates (100,000 cells/wells in triplicate) for 48 hours. Joint cells were exposed to antibiotics (15) at various doses (25 to 0.39mg/ml in complete DMEM media) for 24h and viability was assessed by trypan blue dye exclusion. The half maximal inhibitory concentration (IC50) was determined for each antibiotic by normalizing dose response for each concentration to control and transforming data to estimate IC50 by nonlinear regression in GraphPad Prism8. Cartilage and synovial explants were obtained from 3 horses, minced and exposed to antibiotics (6) for 48h (synovium) or 72h (cartilage). Live/dead staining was performed, and fluorescence was visualized using Olympus IX83 spinning disk confocal microscope. Percentage of live versus dead cells was quantified. Antibiotics from different classes expressed dose-dependent but variable cytotoxicity to joint cells. Aminoglycosides and doxycycline had the lowest IC50 (most toxic). Ampicillin sulbactam, imipenem, tobramycin, ceftiofur sodium and amoxicillin had IC50>25mg/ml for ≥1 cell line, representing potentially less cytotoxic IA alternatives. Further studies are necessary to extrapolate these *in vitro* data results to the *in vivo* joint environment. Appropriate antimicrobial selection based on culture and sensitivity and evidence-based dosing may minimize damage to native articular cartilage and synovial cells. **Funding**: USDA Animal Health and Disease Grant No. NI18AHDR1019785G010, Center for Companion Animal Studies Young Investigator Award

DVM Student / Clinical Sciences

68. Monkeypox virus transmission between small mammal species in an artificial ecosystem

Stephanie Porter, Angela Bosco-Lauth, Airn Hartwig, Jeff Root, Richard Bowen

Following the eradication of smallpox, monkeypox virus is the orthopoxvirus that causes the most severe clinical disease in humans. Monkeypox is a zoonosis that results in similar clinical signs to smallpox, with a case fatality rate of up to 10%. The virus is endemic to Africa, but was imported to the United States in 2003 through the exotic pet trade, and has recently been diagnosed in international travelers and a health care worker in Israel and the United Kingdom. There is concern that prevalence of monkeypox cases may be increasing due to climate change, the lack of smallpox vaccination among the general public, and globalization. Little is known about transmission of monkeypox virus, but rodents are implicated as hosts. In order to better understand how monkeypox may spread in the future, our objective is to improve understanding of how the virus is maintained and transmitted by small mammals. We first characterized viral shedding and disease course in four species (deer mice, fox squirrels, Wyoming ground squirrels and cottontail rabbits) following experimental infection with monkeypox virus. These initial studies revealed a spectrum of disease: deer mice did not shed virus or develop clinical disease, ground squirrels did not show signs of disease but shed virus orally, cottontails displayed mild symptoms and shed virus at low levels, while fox squirrels developed severe disease, shed at high levels, and had virus disseminated throughout their organs. Inter- and intra-species transmission were then studied by inoculating three ground squirrels, and introducing them to a room containing conspecifics, fox squirrels, and cottontails. We expect the ground squirrels to serve as reservoir hosts, infecting the animals they are housed with, without themselves developing disease. This work will contribute to our knowledge of how monkeypox virus is maintained in nature, and inform future control strategies. **Funding**: Animal Models Core

Graduate Student / Microbiology, Immunology and Pathology

69. Evaluation of a Phantom-less Computed Tomography Protocol for Measurement of Bone Mineral Density

Megan Posukonis and Christopher Kawcak

Bone mineral density (BMD) measurement is an emerging diagnostic tool for fracture risk assessment in horses and humans alike. However, current research opportunities are limited by case and data availability. BMD is frequently calculated from dual x-ray absorptiometry or quantitative computed tomography (CT) image sets, though in the clinical realm images are often acquired without the use of a radiographic phantom necessary to convert image characteristics to a quantitative BMD. The ability to measure BMD from clinical image sets acquired without a radiographic phantom would facilitate large scale retrospective BMD research studies from the wealth of spontaneous injury clinical cases. We hypothesize that modern fan-beam CT scanners have high positional precision, such that BMD can be accurately calculated from a phantom calibration curve generated in a separate image series. To address this hypothesis, we are performing serial CT scans of equine cadaver limbs and analyzing the difference between scans in Hounsfield units (HU) and calculated BMD via a de novo Matlab program. We will then assess the difference in BMD calculated from a traditional in-image phantom calibration curve against a curve obtained from a different image series. This study should allow us to determine whether phantom-less CT image sets acquired in the clinical setting can be used for future quantitative BMD research studies. **Funding**: Boehringer Ingelheim Veterinary Scholars Program

DVM Student / Clinical Sciences

70. Coinfection of Epizootic Hemorrhagic Disease Virus and Bluetongue Virus in Culicoides Cell Culture

Lauren Riggs, Jennifer Kopanke, Molly Carpenter, and Christie Mayo

Epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) are two segmented orbiviruses transmitted in North America by the same vector, *Culicoides sonorensis*. The primary objective of this work is to investigate how EHDV-BTV coinfection affects the evolution and replication of each of these viruses. Because the two viruses will be competing for resources, we hypothesize that one virus will replicate to higher titers during coinfection. Previous studies have determined that reassortment does not occur between EHDV and BTV, but we will characterize the replication kinetics of each virus when co-infected on C. sonorensis cells (CuVaW3) as compared to cells infected with only a single virus (EHDV-1 or BTV-10). Infections with EHDV-1 or BTV-10 alone, as well as coinfection EHDV-1 and BTV-10 will be performed on confluent monolayers of CuVaW3 cells. Supernatant from each condition will be collected during a 96 hour growth curve. Viral copy numbers will be evaluated using qRT-PCR and infectious titer will be determined using TCID50. These results could alter our understanding of coinfection in insect vectors and may be useful in predicting disease in areas where BTV and EHDV co-circulate. **Funding**: Foundation For Food and Agriculture Research

DVM Student / Microbiology, Immunology and Pathology

71. Prospective evaluation of orthoses and prostheses in canine patients

Sydney B Rosen and Felix M Duerr

The use of orthoses (braces) and prostheses have expanded in veterinary medicine as treatments for orthopedic injuries, congenital defects and amputated limbs. Currently available research is limited to retrospective studies and surveys. All studies relied solely on subjective outcome measures and few studies recorded data on complications, such as skin sores that dogs may develop from wearing an orthosis or prosthesis. The goal of this study is to determine the outcomes associated with the application of an orthosis or prosthesis in dogs. We hypothesize that gait analysis data will show a significant improvement at the 3-month recheck in comparison with baseline data. Consecutive cases presented to the CSU-VTH are currently being enrolled in a prospective study using objective outcome measures to determine the outcome and complications associated with this technology. We are tracking patients for 12 months to analyze the number of skin complications and objective gait analysis data to determine change in lameness severity. To date, 29 patients of various breeds with mean body weight of 34 kg have been enrolled. Preliminary survey results indicate that 45% (n=10/22) of patients experienced sores during the first month of wearing the device. Of those sores, 70% (n=7/10) were evaluated by a veterinarian. Preliminary gait analysis of 10 dogs showed an average improvement of 17% in percent body weight distribution after 3 months. 10% of patients (n=3/29) enrolled in the study have discontinued wearing the device due to either issues with fit or lack of perceived benefit. These preliminary results suggest that orthoses and prostheses result in improvement of function in certain cases, yet skin complications are relatively common. **Funding**: Morris Animal Foundation, Sebastian's Love, Center for Companion Animal Studies Young Investigator Award

DVM Student / Clinical Sciences

72. An evaluation and comparison of phosphorus, calcium, magnesium, sodium, potassium, protein and fat in commercially available adult and senior cat diets

Anais Sanchez Rodriguez, Stacie Summers, Lei Zhang, Jonathan Stockman

Nutritional requirements for cats change according to age. While there are guidelines by the Association of American Feed Control Officials (AAFCO) and the National Research Council for nutritional requirements for adult cats, growing cats, and cats during reproduction, there are no established requirements for senior pets. Therefore, all senior diets adhere to the adult guidelines and each company may interpret the senior requirements differently. The objective of this cross-sectionalstudy was to measure the caloric distribution of protein, fat, and select electrolytes (phosphorus, calcium, sodium, potassium) in commercially available senior cat diets and compare results to adult cat diets. Canned and dry foods were randomly chosen from local pet food stores. Thirty-one foods labeled for senior cats and 59 diets labeled for adult cats were included in the study. All senior cat foods were formulated to meet AAFCO nutrient profile for adult maintenance. For the adult cat foods, 31 diets were reported to meet the AAFCO nutrient profile for adult maintenance and 28 diets for all life stages. Descriptive statistics were performed for select electrolytes (g/1,000 kcal ME), calcium to phosphorus (Ca:P) ratio, protein and fat concentrations (g/1,000 kcal ME), and caloric density (kcal/100 grams). Nutrients were compared between senior cat foods and adult cat foods using Mann Whitney U test and Kruskal Wallis with Dunn's multiple comparisons test. No significant difference was found between adult diets formulated for adult maintenance, adult diets formulated for all life stages, and senior diets in phosphorus, calcium, potassium, sodium, protein and fat concentrations and Ca:P ratio. In conclusion, adult and senior cat had similar nutrient profiles. Further research is required to establish senior cat nutritional guidelines, and caution should be taken when making broad recommendations for the use of commercially available senior diets in cats. **Funding**: Center for Companion Animal Studies Young Investigator Award

DVM Student / Clinical Sciences

73. Sexually-divergent effects of infralimbic cortex stimulation on endocrine and cardiovascular stress reactivity

Derek Schaeuble, Tyler Wallace, Sebastian A. Pace, Morgan K. Schackmuth, Adam J. Chicco, Brent Myers

Stress, defined as a real or perceived threat to homeostasis, promotes adaptive physiological responses. Unfortunately, the burden of prolonged stress contributes to a variety of pathologies including cardiometabolic disorders. While the impact of stress on cardiovascular disease is well-documented, mechanisms have yet to be defined. Our previous studies found that inhibition of glutamate release from the infralimbic cortex (IL), a subregion of prefrontal cortex, leads to impaired vascular function after chronic stress. This led to the hypothesis that IL activity may be sufficient to restrain cardiovascular and endocrine responses to stress. In the current study, we used optogenetics to stimulate glutamatergic IL neurons in male and female rats exposed to stress. During optic stimulation, rats were exposed to restraint stress with blood collection to examine hormonal stress reactivity. Animals were also implanted with radiotelemeters to measure cardiovascular stress reactivity during exposure a novel environment. Furthermore, all animals underwent echocardiographic assessment of cardiac structure and function before and after chronic variable stress. In males, activation of IL glutamate outflow reduced corticosterone and glucose responses to restraint. In contrast, females did not have altered corticosterone secretion but exhibited enhanced glucose responses. When placed in a novel environment, IL activation reduced male heart rate and blood pressure reactivity, while increasing female heart rate. Analysis of the autonomic components of heart rate variability indicated that IL stimulation reduced net sympathetic drive in males but not females. Furthermore, IL activation in males prevented cardiac structural and functional changes after chronic stress. Together, these data suggest IL glutamate neurons are critical for integrating physiological responses to stress. These findings also provide a sex-specific mechanism linking stress and cardiovascular health outcomes. **Funding**: NIH

Graduate Student / Biomedical Sciences

74. Environmental Sustainability: The Role of Veterinary Teaching Hospitals in Global Health Education

Stephanie Schiavone, Sage Smith, Mallory Marschall, Shannon Doherty-Garces, Michelle Salomon, Sera Lee, Stephanie Cruz-Castro, Isabella Mazariegos, Tracy Webb, Molly Carpenter, Stacey Baumgarn, Colleen Duncan

Climate change is one of the greatest public health threats of the 21st century. While less is known about the effects of climate change on animals, the health and welfare impacts can be inferred from those of humans. A recent survey of veterinary students highlighted the perceived paucity of climate change education within their curriculum despite the fact that respondents overwhelmingly felt that veterinarians and students should be knowledgeable about the practice and promotion of environmental sustainability within clinical practice. A majority of the American Veterinary Medical Association (AVMA) accredited veterinary schools host a teaching hospital where veterinary students receive their core clinical education. We hypothesized that teaching hospitals may provide a good opportunity for students to learn, by example, how veterinary clinics may be able to decrease their environmental footprint. To determine the role that teaching hospitals play in the area of sustainability, we designed an anonymous online survey for distribution to all AVMA-accredited veterinary schools with an associated veterinary teaching hospital. Our inquiries to subjects included where they feel the most waste is accumulating in their hospital (e.g. water, electricity, etc), and how high of a priority hospital sustainability is to them. Results from this survey can be utilized to inform and incorporate decisions regarding the most appropriate environmentally sustainable practices for each hospital. In addition, the results can identify areas for improvement that emerge across multiple hospitals, thus affording opportunities for collaborative and synergistic activities.

DVM Student / Microbiology, Immunology and Pathology

75. Parthenolide: a promising phytomedicine for deadly cancers in people and dogs

Lisa J Schlein, Barbara J Rose, Douglas H Thamm

The purpose of this research is to explore the therapeutic potential of parthenolide (PTL) to treat various hematopoietic neoplasms in dogs; additionally, some dog breeds are predisposed to development of mast cell neoplasia and histiocytic sarcoma, providing translational study populations for rare and deadly human diseases. Growth inhibition assays were performed using a panel of canine mast cell, histiocytic sarcoma, lymphoma, and leukemia cell lines, with PTL alone or in combination with redox-perturbing standard-of-care therapeutics. Cell death was assessed using flow cytometry. Immunofluorescence and immunoblotting were used to assess NFkB localization and phosphorylation, respectively. All immortalized canine cell lines evaluated are sensitive to PTL therapy and undergo dose-dependent apoptosis following exposure to drug. PTL exposure leads to inhibition of NFkB, generates reactive oxygen species, and depletes intercellular glutathione in canine cell lines. Preliminary studies indicate that some standard-of-care therapeutics synergize with PTL. These initial studies show that PTL is a promising therapeutic for hematopoietic neoplasms in dogs. Murine modeling and evaluation of spontaneous tumors in dogs are underway and will futher investigate PTL's potential in the clinical setting. **Funding**: NIH/NCATS Colorado CTSA Grant Number TL1 TR002533

Resident / Clinical Sciences

76. *In vitro* cytotoxic effects of cannabidiol on canine melanoma cells

Reed H Schultz, Chase Gross, Katie Stone, Dominique Ramirez, Daniel L Gustafson, Stephanie McGrath

In both human and canine patients, melanoma carries a poor prognosis, however many cancer therapies are ineffective against melanoma. Recently, targeted therapies have been the subject of novel treatments for these cancers. Cannabinoids have been shown to activate several cell pathways and found to be effective against breast cancer, prostate cancer, lung cancer, and cervical cancer. Synthetic cannabinoids (WIN-55,212-2 and JWH-133) have been shown to activate apoptotic pathways in melanoma cells. We hypothesize that cannabidiol (CBD) is cytotoxic to melanoma cells. We tested this using image-based cytotoxicity assays, migration assays, western blot and flow cytometry in 2 different melanoma cell lines, Jones and CML6M. We have found that CBD is cytotoxic with an IC50 over 96 hours of 5.99 μM and 5.30 μM, for Jones and CML6M cell lines, respectively. We also found that migration is inhibited at concentrations below cytotoxic doses. These data provide promising results for the use of CBD as a melanoma treatment, but continued research is needed into the mechanism of action and pharmacokinetics. **Funding**: NIH 5T35OD015130-07

DVM Student / Clinical Sciences

77. Vasoactive Intestinal Peptide Regulates Goblet Cell Production in the Small Intestine

Luke A Schwerdtfeger, Stuart A Tobet

Innervation of the intestinal epithelial barrier has been known for over 100 years, however, the role(s) these neuronal fibers play in maintaining the epithelial and mucus barriers are still poorly understood. Goblet cells are a subset of epithelial cells that are responsible for the gut wall mucus barrier, an early stage line of pathogen defense. The present study was conducted to demonstrate both the proximity of mouse ileal goblet cells to submucosal neuronal fibers, and to test the hypothesis that goblet cell production could be regulated by neuronally generated vasoactive intestinal peptide (VIP). Glycosaminoglycans were labeled ex vivo via a copper-free chemical labeling process, and goblet cells were counted and shown to have at least one peripherin immunoreactive fiber within 3µm of the cell, 51% of the time. Treatment with a VIP receptor antagonist (VPACa) resulted in an increase in the percentage of goblet cells in close proximity to fibers containing immunoreactive peripherin in ileal crypts. Treatment with VPACa or tetrodotoxin substantially decreased goblet cell counts in both the intestinal crypts and villi. Numerous small intestinal crypt epithelial cells showed signs of proliferation as marked by incorporation of 5-Ethynyl-2'-deoxyuridine (EdU), with no difference in EdU+ cell counts across all treatments. However, the number of ex vivo labelled goblet cells that also incorporated EdU in ileal crypts was decreased by 77% when treated with VPACa compared to controls. The present study demonstrates a close relationship of goblet cells to neuronal fibers. The results show potential VIP receptor regulation of gut wall goblet cell production. They are consistent with the hypothesis that the intestinal barrier depends on the interaction of multiple cell types in the gut wall, including enteric neurons. **Funding**: National Science Foundation NRT Grant No. 1450032

Graduate Student / Biomedical Sciences

78. The Value of Sustainability in Veterinary Practices: A Client Perspective

Danielle Scott, Sarah Deluty, Veronica Martin, Katherine McCaw, Jessica Rupert, Sabrina Waugh, Colleen Duncan

Health and environment are inextricable, and consequently medical professionals play an integral role in responding to climate change. While veterinary students believe that environmentally sustainable practices are important, it is unknown how pet owners perceive the role of veterinary medicine in addressing climate change from both a health and sustainability standpoint. An online questionnaire was disseminated via Amazon MTurk to pet owners who had used veterinary services within the last three years. The overwhelming majority of pet owners responding believe climate change is occurring, and two-thirds of pet owners would value knowing their veterinarian received training on the health impacts of climate change on animals. Over half of the respondents would pay more for veterinary services at a clinic with a reduced environmental impact. Additionally, clients would like practices with a reduced impact to display a certification to aid in identification of sustainable practices. This evidence suggests there is an economic incentive for veterinary practices to actively seek out education around the health impacts of climate change, and to implement and market sustainable initiatives. By prioritizing sustainability in veterinary practices, it would mutually benefit both practitioner and client through shared health and financial incentives.

DVM Student / Microbiology, Immunology and Pathology

79. Contribution of intra-abdominal immune dysfunction to musculoskeletal inflammaging

Cassie Seebart, Alexa R Personett, Ariel Timkovich, Karyn L Hamilton, Christian Puttlitz, Katie J Sikes, Michelle T Foster, and Kelly S Santangelo

Aging is associated with the development of several musculoskeletal diseases, including osteoarthritis (OA) and sarcopenia. Age-related chronic inflammation, referred to as "inflammaging," may be associated with the development of both of these disorders. As the gastrointestinal (GI) tract has a large surface area and greatly influences systemic immune regulation, it is possible that the GI immune system plays a key role in the development of musculoskeletal inflammaging. We predict that surgically compromising mesenteric lymph nodes will alter GI immune function and create a pro-inflammatory environment, leading to higher incidence of OA and sarcopenia. Eight female and fifteen male, three-month-old Dunkin Hartley guinea pigs, a strain that we are actively characterizing as a model of musculoskeletal inflammaging, were randomly assigned to receive lymph node ablation surgery via cauterization (n=11) or sham surgery (n=12). Using ANY-maze behavioral tracking software, guinea pig movement (speed, time spent mobile, distance traveled, etc.) was monitored at baseline and once weekly after surgery. Animals were harvested eight weeks after lymph node ablation. Musculoskeletal and immune tissue samples were collected for histology and immunohistochemistry, and blood was collected for complete blood counts, serum biochemistries, and inflammatory biomarker testing. Statistical analyses were performed with unpaired t-tests. In females, the ablation group had a significant increase in movement parameters at 3 weeks post-surgery compared to the control group. In contrast, the male ablation group demonstrated a significant decrease in movement parameters 2-4 weeks post-surgery compared to the control group. Other outcomes are currently being evaluated. Results from this study will provide further insight into the pathogenic factors and mechanisms that facilitate the process of inflammaging and its connection to age-related musculoskeletal diseases. Funding was provided by the Columbine Health Systems Center for Healthy Aging, the Office of the Vice President for Research, and the Research and Scholarly Success Initiative. **Funding**: Columbine Health Systems Center for Healthy Aging, the Office of the Vice President for Research, and the Research and Scholarly Success Initiative

DVM Student / Microbiology, Immunology and Pathology

80. Developing an environmental sustainability assessment tool for United States veterinary hospitals

Rowan Seabolt, Kelsey Kafesjian, Rachel Jacobson, Lyn Davis, Stacey Baumgarn, Molly Carpenter, Tracy Webb, Colleen Duncan

As veterinarians, our role is to serve our patients, our community, and the local environment. Given the profound health impacts associated with climate change, the healthcare sector is motivated to identify ways to minimize their environmental impacts. The objective of this project is to develop a self-assessment tool for United States veterinary clinics to analyze their environmental footprint so they can identify the appropriate steps to improve it. We conducted a scoping literature and internet review to identify assessment tools from other countries, companies, veterinary clinics, and hospitals. These were compared for common themes and gaps then modified to form an assessment tool suitable for private veterinary clinics in the U.S. The self-assessment tool contains a list of questions evaluating the impact of particular clinic actions, the feasibility of a more sustainable action, and the willingness of the interested parties to implement improvements. We are piloting the draft survey at several veterinary clinics to assess feasibility and applicability. Feedback from the pilot study will be used to refine the assessment tool. Our long-term goal is to make the assessment tool electronically available to interested veterinary clinics around the U.S. Data and feedback collected from participating clinics could be used to identify ways to support veterinary clinics electing to adopt sustainability practices without compromising patient care or economic viability.

DVM Student / Microbiology, Immunology and Pathology

81. Determination of feline hepadnavirus infections in cats in the US

Cassidy Stone, Erick Gagne, Sue VandeWoude

Hepadnaviruses are DNA viruses that infect a variety of species. The most common virus in this class is human hepatitis B virus which causes hepatitis in millions of people worldwide. A domestic cat hepadnavirus was recently isolated and found in 6 – 11% of pet cats in Australia and Europe. The virus has since been shown to be associated with certain types of feline hepatitis. This virus is the first hepadnavirus isolated from carnivores, and is closely related to human hepatitis B, which is known to cause hepatocellular carcinoma in people. Because of possible disease association and lack of studies evaluating this agent in the US, we have tested samples from a large bioarchive for the presence of this agent. This included 286 samples from shelter animals in California, Colorado, and Florida, 57 samples from a multi-cat household with FeLV infection, and 28 clinical samples from cats with hepatic lymphoma. Using both traditional PCR and a recently developed qPCR, we have identified one positive sample from a cat from California after screening more than 300 samples. Preliminary studies indicate the virus is similar in sequence to other reported viruses. Our studies document that hepadnavirus infection in US cats may be relatively rare. **Funding**: Boehringer Ingelheim

DVM Student / Microbiology, Immunology and Pathology

82. Topically applied liposomal TLR ligand complexes to treat equine corneolimbal squamous cell carcinoma.

Alena Strnadová, Lyndah Chow, Lynn Pezzanite, Franco Maranon, Brittany Martabano, Steven Dow, and Kathryn Wotman.

Corneolimbal squamous cell carcinoma (SCC), the most common cancer affecting the equine eye, represents a potentially vision-threatening disease in horses. Current treatments (surgery, chemotherapy) are expensive, may require general anesthesia, and do not offer reliable outcomes. A new option for treatment of equine SCC is a liposomal toll-like receptor ligand complex (LTR) immunotherapy, which has been shown to elicit antitumor activity in multiple tumor models, including dogs. We hypothesized that topical and/or intralesional administration of LTR would induce tumor regression or stasis in horses with SCC by activating local innate immune responses. The objectives of this study were to investigate the *in vitro* activity of LTR using horse lymphocytes and SCC tumor cell lines, and to assess safety and efficacy in horses with SCC in a clinical study. The ability of LTR to activate equine immune cells will be assessed by incubating blood leukocytes with LTR and measuring cytokine concentrations by RT-PCR and ELISA. The impact of LTR immune activation on equine SCC proliferation and survival will be assessed by culture of tumor cells with conditioned medium from LTR activated leukocytes. Efficacy of topical and intralesional LTR treatment in controlling or reducing tumor size will be evaluated in equine SCC patients (n=8). Serial ophthalmic exams, tumor cytology and cytokine analysis of tears will be performed. Tumor measurements will be recorded via photograph and reduction in tumor size will determine response to treatment. Local and/or systemic side effects will be recorded. Liposomal toll-like receptor complexes may offer an alternative treatment in equine corneolimbal SCC, leading to earlier intervention and improved outcomes. **Funding**: Center for Companion Animal Studies Young Investigator Award, USDA-NIFA-10207-AHDRXXXX-19-0001

DVM Student / Clinical Sciences

83. SPS1: A novel resistance factor to prion disease

Julianna Sun, Julie Moreno, Jifeng Bian, and Glenn Telling

Prions are infectious proteins which cause fatal neurodegenerative disease in humans and animals. Of concern, the prevalence of chronic wasting disease (CWD), a prion disease affecting deer, elk, and other cervids, is rapidly growing, with outbreaks now in 24 of the 50 United States and additional presence in Canada, Europe and Asia. It is significant to note then that there is no effective treatment which neither stops nor slows down the course of prion disease. Prions differ from other pathogens in that they do not contain any functional DNA or RNA. Their infectious property stems from the conversion of the normal prion protein (PrP^{C}) into the aberrant disease-causing form (PrP^{Sc}). However, the mechanism by which PrP^{Sc} causes PrP^{C} to misfold is largely unknown. In addition to PrP, other host factors have been suggested to modulate pathogenesis, species barriers, and prion strain diversification, but none have been proved. Our preliminary data using an *in vitro* model of CWD in which rabbit kidney epithelial cells have been genetically modified to express deer or elk PrP, has potentially revealed the first resistance factor to prion disease, selenophosphate synthetase 1 (SPS1). Via RNA sequencing and RT-PCR, we have shown elevated mRNA levels of SPS1 in cells resistant to prion infection as opposed to those susceptible to infection. Little is known of SPS1, barring that it has a putative function involving the use of selenocysteine, the 21st amino acid, to synthesize selenoproteins. In general, selenium deficiency in humans has implications in heart disease, neuromuscular disorders, cancer, and inflammatory disorders. Most notably, selenium compounds have recently been the focus of treating Alzheimer's disease, which is often characterized as a prion-like disease. This work thus proposes to evaluate the relationship between SPS1 and PrP and predicts that treatment with selenium compounds could have neuroprotective effects in CWD. **Funding**: College Research Council, R21 NS09192709, and the Office of the VPR PRSE funds.

Graduate Student / Microbiology, Immunology and Pathology

84. Manipulation of Toll-like receptor 4 ameliorates injury-induced osteoarthritis progression

Ariel E Timkovich, Sara M Wist, G Aaron Holling, Maryam F Afzali, John D Kisiday, Kelly S Santangelo

Post-traumatic osteoarthritis (PTOA) is a debilitating condition affecting millions of people worldwide. As there is currently no cure for this ailment, novel therapies are needed to suppress the progression of pathology after joint injury. *Our central premise is that sterile inflammation mediated via damage associated molecular patterns (DAMPs) and, subsequently, the Toll-like receptor 4 (TLR4) pathway is a principal driver in the progression of PTOA*. We postulate that TLR4 activation is initiated/perpetuated by damage associated molecular patterns released following joint damage. Thus, knockout of the TLR4 pathway or treatment with a TLR4 antagonist immediately after injury may suppress the development of PTOA. To test this theory, four groups were utilized: (1) 8-week old, wild type mice; (2) TLR4 knockout mice; (3) wild type mice treated with a known TLR4 antagonist; (4) and mice injected with a vehicle control. The right knee of each mouse was scraped with an 18g needle on the midline of the patellofemoral groove to produce a full-depth cartilage lesion. The left knee was used as a sham surgery control. Using a quantitative gait analysis system, we evaluated gait changes over time. Following study completion, animals were harvested, and knee joints were processed for Nanostring® gene expression and pathologic assessment. Statistical analyses were performed with Prism 8.0 (GraphPad) or R with statistical significance set to P £ 0.05. We found that knockout and systemic antagonism of TLR4 significantly improved relevant gait parameters, implying modification of clinical signs. Additionally, gene analyses showed reduced expression of inflammatory genes in animals treated with the TLR4 antagonist. Structural characterization of knees using histology is underway and will determine if structural changes occurred in our treatment groups of interest. This research was funded by the Colorado Clinical and Translational Sciences Institute (University of Colorado – Denver). **Funding**: Colorado Clinical and Translational Sciences Institute (University of Colorado – Denver)

Graduate Student / Microbiology, Immunology and Pathology

85. Investigation of an educational veterinary resource on climate change that encourages positive action

Bradleigh Vahl, Lindsay Adelmeyer, Christina McBroom, Sam Neal, Marie Simon, Tracy Webb, Molly Carpenter, and Colleen Duncan

As leaders of the One Health initiative, veterinary professionals play a key role in promoting public, animal, and environmental health. Despite their involvement, however, the impacts of global climate change have been inadequately addressed in veterinary medicine relative to the efforts in human health. A recent study synthesized the opinions of veterinary students on the issue and found that the majority believe climate change is an imminent threat to public and animal health and that educational opportunities on the topic should be available. The purpose of this study was to research existing climate change materials to justify and develop a framework for an animal health focused educational resource. The methods were to collect and integrate data through systematic literature reviews, interviews with experts, and broad internet searches. This research identified potential markets, target audiences, platforms, and course content. Preliminary analysis of this data validates the need for an organized veterinary educational resource addressing the relationship between animal health and climate change. We propose the use of an online platform that includes both core and elective content in addition to a forum through which animal health professionals can interact. This resource could bridge the educational gap in veterinary medicine and provide animal health professionals with the tools they need to mitigate the negative animal health impacts associated with climate change.

DVM Student / Microbiology, Immunology and Pathology

86. Sexual divergence in prefrontal regulation of depression-relevant behaviors

Tyler Wallace, Derek Schaeuble, Sebastian Pace, Morgan Schackmuth, Brent Myers

Depression, a mental illness characterized by decreased motivation and social behavior, accounts for the most years lived with disability worldwide according to the World Health Organization. Depression rates are heavily sexually-dependent with females being nearly twice as likely to be diagnosed. Historically, explanations for this gender gap have been largely cultural while neurobiological explanations are lacking. Previous research has demonstrated that the medial prefrontal cortex (mPFC) has altered activity and metabolism in depression patients. We sought to investigate the direct contribution of mPFC neurons to motivation and social behavior. We utilized optogenetics to alter the activity specifically in glutamate-releasing neurons of the mPFC in rodents during behavioral tests. First, animals were placed in a two-chamber arena where they received stimulation to increase mPFC activity in one room and no stimulation in the other. Males showed a strong preference for the room where mPFC activity was increased but females did not. This demonstrates that overall mPFC activity in males contributes to a positive motivational state but not in females. Animals then received mPFC stimulation while in an arena with a novel object and social interactor. In males, mPFC stimulation increased time interacting socially compared to controls, while in females it had no effect. These results suggest that mPFC glutamate release counters depression-like symptoms in males but not in females. Our future work will further dissect sexually dimorphic neural circuitry to assist in developing novel depression treatments with higher success rates and lower off-target effects. **Funding**: R00 HL122454, College Research Council

Graduate Student / Biomedical Sciences

87. Cardiac and inflammatory biomarkers in bovine pulmonary hypertension and congestive heart failure

Shayna Warner, Greta Krasfur, Steve Dow, Bill Wheat, Tim Holt and Frank Garry

Bovine congestive heart failure (BCHF), historically known as "brisket disease" remains a significant problem in cattle herds residing at high altitudes. BCHF also occurs with increasing prevalence in feedlot cattle at lower elevations. Evidence suggests that BCHF in these different settings has different underlying mechanisms. High altitude BCHF is known to be induced by hypoxia while feedlot BCHF may be incited by obesity driven inflammation. Identifying individual cattle at risk for BCHF relies on traditional pulmonary arterial pressure testing (PAP test) which indirectly measures the workload placed on the right side of the heart. This procedure is invasive and requires skill, technique and expensive equipment. In other species, including humans, serum concentrations of several cardiac and inflammatory biomarkers are diagnostic and prognostic for cardiovascular disease. Similar adjunctive diagnostic tools in the management of bovine health would be highly beneficial. We are studying cattle at high altitudes and cattle in lower altitude feedlots to measure inflammatory and cardiac biomarkers and compare these markers with corresponding PAP scores. Enzyme linked immunoassay (ELISA) will be used to screen serum samples from groups of cattle residing in high altitude and feedlot (lower altitude) environments. Serum amyloid A, IL-6 and a generalized multiplex ELISA will be used to screen for markers of inflammation. Cardiac troponin and pro-brain natriuretic peptide (pro-BNP) will be used to screen for markers of cardiac myocyte injury. A pilot study on feedlot cattle showed significant elevation of IL-6 in cattle with high PAP scores compared to low PAP score cattle. No significant differences were found in similar screening of cattle at high altitudes. Further validation studies with larger sample sizes will investigate whether elevations in these biomarkers are related to PAP scores along with postmortem exams and histopathology of cardiac tissue to confirm BCHF. **Funding**: Eldred Foundation and Integrated Livestock Medicine

Graduate Student / Clinical Sciences

88. The relationship between black carbon and polycyclic aromatic hydrocarbon exposures and mortality in Allegheny County, Pennsylvania

Rebecca Warren, Sheena E Martenies, Jennifer L Peel, Tan Yi, Allen Robinson, Albert Presto, Sheryl Magzamen

Black carbon (BC) and polycyclic aromatic hydrocarbons (PAHs) are major components of PM2.5 that are associated with adverse health outcomes. However, little work has examined the effects of PM constituents on mortality risk. Our multiple regression analysis estimated the effect of neighborhood-level ambient PM2.5, BC, and PAHs exposure on mortality in Allegheny County, PA. Utilizing local-scale land use regression models of these pollutant exposures, we estimated the potential effects on five-year census tract-level age-adjusted non-accidental, cardiopulmonary, cancer, and other mortality rates. Models were adjusted for age, percent of non-White residents, percent of residents ages ≥ 25 with less than a high school diploma, and percent of residents ages ≥ 18 with health insurance. Pollutant exposures were not consistently related to all types of mortality in the adjusted models. Only one adjusted model had an effect estimate that did not span the null, although the relationship was opposite our hypothesis. An interquartile range (0.25 µg/m3) increase in BC concentration was associated with a 5.9% (95% CI: -11.07 to -0.36%) decrease in log-transformed cancer mortality. However, in all mortality categories, education and health insurance covariates had a robust relationship with outcomes. In the adjusted BC model, a 1-point increase in the percentage of the population with less than a high school diploma was associated with a 2.3% (95% CI: 1.50, 3.01%) increase in log-transformed cancer mortality; a 1-point increase in the percentage of the population with health insurance was associated with a 2.1% (95% CI: -2.95, -1.26%) decrease in log-transformed cancer mortality. We did not find a consistent relationship between PM2.5, BC, and PAH and age-adjusted mortality rates in Allegheny County, PA. However, lacking health insurance and having less than a high school education continue to be considerable risk factors for these diseases. **Funding**: Heinz Endowments Breathe Project

Staff / Environmental and Radiological Health Sciences

89. Inactivation of Mycobacterium tuberculosis for Safe Use Outside of the BSL-3 Laboratory

Jackson Watkins, Phillip Knabenbauer, Raymond Goodrich, Karen Dobos

Bacteria that cause fatal disease through aerosol transmission pose a difficult challenge to the safety of researchers who work with those pathogens. Specifically, this work requires high-containment laboratories, which generate both physical and financial barriers to research. Inactivation of pathogens through techniques like caesium-source gamma-irradiation, heat inactivation, and UV-radiation have historically been employed to both broaden research efforts and reduce worker risk. These techniques do not come without their challenges, and recent short-comings in cost and reliable calibration of our current methods have lead us to look into alternative methods of inactivation. Mirasol® Pathogen Reduction Technology (MPRT) has been demonstrated to completely inactivate pathogens in human blood [1][2][9]. The MPRT has been a useful tool in hospitals for quality control during blood transfusions [1][2]. The process of inactivation involves a photosensitizing reaction between ultraviolet (UV) light and riboflavin, which causes irreversible oxidation of guanine bases in cellular DNA [3][5]. Because of its clinical efficacy, we predicted that MPRT could be a useful tool for inactivating large pellets of pathogenic bacteria, and as a replacement for the caesium-source gamma-irradiation method we currently use. By measuring reduction in colony forming units (CFU) at increasing UV doses, we characterized the damage done to Mycobacterium smegmatis (M. smegmatis), as a model organism, by MPRT. We found that while a significant reduction in replicating M. smegmatis cells could be consistently achieved over multiple replicates, a surviving population of cells remained, even after high doses of UV light. Further characterization of the damage through flow-cytometric assays and mutant analysis indicated that M. smegmatis may not readily uptake riboflavin from exogenous sources as efficiently as its pathogenic near-neighbor. Due to large log reduction, and emerging physiological discrepancies in our model organism, we conclude that MPRT has potential as a technique for large-scale pathogenic inactivation.

Graduate Student / Microbiology, Immunology and Pathology

90. Developing a predictive model for indoor black carbon for the Denver, CO metropolitan area

Sherry D. WeMott, Sheena E. Martenies, Grace Kuiper, Kacy Lorber, Cody Dawson, Kevin Andresen, William B. Allshouse, Anne P. Starling, John L. Adgate, Dana Dabelea, Sheryl Magzamen

Background: Though individuals spend ~90% of their time indoors, ambient air pollution data are frequently used in exposure assessment for epidemiologic studies. We sought to understand the relation between indoor and outdoor concentrations of black carbon (BC) and additional housing characteristics associated with indoor BC concentrations among cohort study participants in Denver, CO. Methods**:** Households from the Healthy Start cohort were selected for monitoring based on diversity in housing type and built environmental characteristics. For one week in spring and summer 2018, households were provided two low-cost air samplers, one for outdoor and one for indoor measurement. Participants completed questionnaires addressing housing specifics such as building type, flooring, and use of heating and cooling systems. BC was measured using transmissometry and weekly mean concentrations were log transformed prior to model fitting. A linear model was fit using all available predictors obtained from surveys as well as outdoor BC concentrations. Model selection was performed using stepwise backwards elimination; model fit was evaluated using AIC. Results**:** A total of 25 household participated in the study: 11 filters had BC measurements below LOD, and data were available for 39 filters. The average (SD) indoor and outdoor concentrations were 0.99 (0.73) and 1.09 (0.62) $\mu g/m^3$, respectively. Outdoor BC concentrations were significant predictors of log(indoor BC) in a single-predictor model ($\beta = 0.49$, $p = 0.02$, $R^2=0.12$). The final model included outdoor BC, single family home, tile flooring, and use of electric heater and had and adjusted R^2 of 0.62. **Conclusion**: Outdoor BC and housing characteristics were able to account for ~60% of the variability in indoor BC concentrations measured in Denver, CO homes. In the absence of personal monitoring, household characteristics and time-activity patterns may be used in calibrating ambient air pollution concentrations for personal exposure estimation. **Funding**: NIH

Graduate Student
Staff / Environmental and Radiological Health Sciences

91. The role of *Streptococcus equi* subspecies *zooepidemicus* and influenza viruses in upper respiratory infections in shelter cats.

Anna S Winner, Hayley Clark, Jennifer Hawley, Michael R Lappin

Feline respiratory disease complex (FRDC) is characterized by clinical signs of respiratory and/or ocular disease in susceptible cats and is a prevalent problem in overcrowded settings like animal shelters. Included in the complex are a variety of pathogens, particularly feline herpesvirus-1 (FHV-1), feline caliciviruses (FCV), *Bordetella brochiseptica*, Mycoplasma spp., and *Chlamydia felis*. Recently, a number of cats have been shown to be infected by influenza A viruses or *Streptococcus equi* subspecies *zooepidemicus*, but little is known about the incidence of these potential pathogens in cats in shelters in Colorado. This study aims to determine the estimated prevalence rates for influenza A viruses and *S. zooepidemicus* to determine whether these are additional major contributing pathogens to FRDC. Archived DNA and RNA extracts from 2 previous shelter studies have been stored at -80°C. Samples from cats housed in shelters in north-central Colorado that developed acute clinical signs of upper respiratory tract disease in 2008 (52 cats) and in 2019 (24 cats) were analyzed and genotyped using PCR assays for influenza A viruses and for *S. zooepidemicus*. In the experiment completed in 2019, while none of the samples from the 21 cats were positive for RNA of influenza viruses or DNA of *S. zooepidemicus*, nucleic acids of C. felis (4.2%), FHV-1 (8.3%), *Mycoplasma* spp. (20.8%), or FCV (37.5%) were amplified from many cats. In the experiment completed in 2008, while none of the samples from the cats tested were positive for RNA of influenza viruses (n = 48 cats), DNA of *S. zooepidemicus* (n = 42 cats), or *C. felis* (n = 31), nucleic acids of FCV (2.1%), *Mycoplasma* spp. (58.1%), or FHV-1 (74%) were amplified from many cats. Both single and co-infections were detected in both sample sets. Understanding what infectious agents contribute to FRDC is crucial to its treatment and prevention. **Funding**: Petsmart Charities

DVM Student / Clinical Sciences

92. Development of a Cerenkov imaging procedure as a lower-cost alternative for radiopharmaceutical imaging of positron emitters

Paige K. Witter, Justin Bell, and Alexander Brandl

Cerenkov luminescence imaging (CLI) is an emerging means of in vivo characterization of radiopharmaceutical biodistribution in small animals. Traditional radiopharmaceutical imaging involving Position Emission Tomography (PET) imaging is limited in the number of subjects which can be scanned in a given time and by the high associated cost of the imaging system. CLI offers a less expensive alternative that can scan up to five small animal subjects simultaneously. Cerenkov radiation is produced when a charged particle passes through a dielectric medium faster than the speed of light traveling in that medium. Radionuclides ^{18}F (half-life, 109.8 min) and ^{64}Cu (half-life, 12.7 hours) decay via positron emissions which satisfies the conditions for Cerenkov radiation production in water and tissue. This study will investigate the use of the IVIS® Spectrum (in vivo imaging system) at the Colorado State University (CSU) Veterinary Teaching Hospital (VTH) for detecting Cerenkov radiation associated with the decay of ^{18}F and ^{64}Cu in small animal phantoms. We will also investigate the relationship between the measured intensity of Cerenkov emission to radionuclide activity and the associated radiation dose the small animal phantom receives. The development of radiation detection and quantification processes used in this study will aid in the development of a procedure for cheaper and quicker biodistribution analysis and dose estimation at target sites for radionuclides used for PET imaging. Cerenkov imaging using the IVIS® Spectrum has great potential in becoming a cost-effective imaging modality for radiopharmaceuticals used in small animal translational research. **Funding**: U.S. Nuclear Regulatory Commission, Grant Award Number NRC-HQ-84-14-G-0034.

Graduate Student / Environmental and Radiological Health Sciences

93. Retrospective study of 240 dogs receiving gabapentin for chronic pain relief

Lily Davis, Lori Kogan, Robin Downing, Peter Hellyer

Chronic, maladaptive pain is recognized as a significant problem for people and animals with treatment often involving both pharmacologic and non-pharmacologic therapies. Gabapentin, due to its analgesic effects and efficacy, has been used to treat maladaptive pain for several years. Although gabapentin has proven helpful in the treatment of canine chronic pain, there is minimal data on specific efficacy, dosage, and side effects. We hypothesized that the dose of gabapentin for dogs can be significantly increased to provide clinically meaningful analgesia and improve quality of life with minimal side effects. To test this hypothesis, we conducted a retrospective study involving the systematic review of 240 medical records of dogs that received gabapentin for chronic pain. We found a wide range of tolerated doses (6 - 500 mg/kg/day) with only 10% of patients experiencing sedation - the most common side effect. We concluded that gabapentin appears to be tolerated at a wider variety of doses than has been previously described. These results suggest that gabapentin dosage can be increased as needed to improve pain management and quality of life with minimal fear of sedation and therefore, could be a more effective pain management pharmacologic therapy.

DVM Student / Clinical Sciences

94. Evaluation of select nutrients and vitamin D3 in senior cat foods

Lei Zhang, Stacie Summers, Anais Sanchez Rodriguez, Jonathan Stockman

Dietary management with reduced protein (65-95 g/1,000 kcal metabolizable energy [ME]) and low phosphorus (0.9-1.3 g/1,000 kcal ME) prescription diets is recommended in cats with chronic kidney disease (CKD) to slow progression of disease and reduce episodes of uremia. Diets labeled for senior cats, generally thought to be restricted in protein and phosphate, are suggested to be a suitable alternative to renal diets in cats with CKD when the owner or patient refuse prescription diets. However, there is no data currently available regarding the nutritional value of commercially available canned and dry senior cat foods. The objective of this descriptive study was to quantify protein, phosphorus, calcium, magnesium, sodium, potassium, and vitamin D3 in commercial senior cat foods and to compare findings to the nutrient content of feline renal prescription diets. A total of 31 senior cat foods were purchased from pet food stores. All diets had analyzed concentrations of select nutrients above the AAFCO Cat Food Nutrient Profile minimum value for adult maintenance. Of particular interest was the protein concentration, phosphorus concentration, and calcium to phosphorus (Ca:P) ratio in the senior diets. The median phosphorus concentration was 3.2 (range, 1.4-4.4 g/1,000 kcal ME) and the median protein concentration was 93 (range, 74-142 g/1,000 kcal ME). Eight of 31 foods (26%) contained phosphorus concentrations (≥ 3.6 g/1,000 kcal ME) documented to cause renal damage in healthy adult cats. Three (10%) foods had low Ca:P ratio (≤ 1.0). Of the 31 foods, 17 (54%) had a protein concentration less than 95 g/1,000 kcal ME. In conclusion, diets marketed for senior cats are highly variable in their nutrient content, and concentrations of phosphorus and calcium are generally higher compared to therapeutic renal diets. Therefore, making broad recommendations for the use of commercially available senior diets in cats is not advised. **Funding**: Center for Companion Animal Studies Young Investigator Award

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