# CVMBS Research Conference 2021

https://vetmedbiosci.colostate.edu/research-day/

## Schedule of Events

### Poster Presentations

| | | |
|---|---|---|
| Wednesday, January 27 | 4 pm- 7 pm | Zoom Rooms 1-3 |
| Thursday, January 28 | 10 am- 4 pm | Zoom Rooms 1-3 |

### Keynote Presentation- Zoom Room 1

**Dr. Stephanie McGrath**, recipient of the Zoetis Research Excellence Award

"Cannabis, Seizures, Brain Tumors, Pain, and Alzheimer's – a day in the life of a clinical researcher"

For the complete article on Dr. McGrath please click here

Dr. McGrath will be presenting in Zoom Room 1 on Saturday, January 30 at 11:00 am

### Zoom Room 1

https://zoom.us/j/93545836620?pwd=c3Q4UnZldjgwWmRhQ25ZUWNZaUZOdz09

### Oral Presentations

| | | |
|---|---|---|
| Saturday, January 30 | 12 pm- 3:45 pm | Zoom Rooms 1-3 |

## iPoster

Posters will be available for passive viewing from Monday, January 25th-April 30th, 2021, to view live presentations of posters, view the schedule to locate presentation times.

https://csu.ipostersessions.com/Default.aspx?s=csu_cvmbsresearchday_2020_gallery

## Zoom Presentations

Please check the schedule to find presentations assigned to each room.

1. CVMBS Research Day Room One
   https://zoom.us/j/93545836620?pwd=c3Q4UnZldjgwWmRhQ25ZUWNZaUZOdz09
2. CVMBS Research Day Room Two
   https://zoom.us/j/94014030399?pwd=SklOdk1DZUk4aEl0aGZpTWJVSWhVZz09
3. CVMBS Research Day Room Three
   https://zoom.us/j/94720010331?pwd=ak1Ub3pBcnE4M2F2S1h3SXQ1UldYQT09

# Congratulations Again to 2020 CVMBS Research Day Winners:

**Oral Presentations**

| | |
|---|---|
| First Basic | Bridget Eklund, Graduate Student, MIP, "Microbes in the Mucosa: A Probiotic-Based Vaccine and the Gut Microbiome." Mentor: Zaid Abdo and Gregg Dean |
| Second Basic | Amy Fox, Graduate Student, MIP, "Cyto-feature engineering: a flow cytometry analysis pipeline to uncover immune populations and association with disease." Mentor: Henao-Tamayo |
| First Clinical | Chase Gross, Graduate Student, CS, "Cannabis For The Cure: Cannabidiol Induces Apoptosis and Perturbs Mitochondrial Function in both Human and Canine Glioma Cells." Mentor: Stephanie McGrath |
| Second Clinical | Jayne Ellis, DVM Student, CS, "Comparison of a single versus staged two dart anesthesia induction protocol in Przewalski's horses." Mentor: Mama Khursheed |

**Poster Presentations**

| | |
|---|---|
| First | Lynn Pezzanite, Post Doctoral Fellow, CS," *In vivo* dose titration of amikacin in equine joints reveals sustained synovial fluid concentrations and dose-dependent cartilage toxicity." Mentor: Steven Dow |
| Second | Gabi Piquini, DVM Student, CS, "Antimicrobial selection for intra-articular administration may minimize cytotoxicity to equine chondrocytes and synovial cells." Mentor: Laurie Goodrich |
| Third | Molly Butler, Graduate Student, MIP, "Cyclin-Dependent Kinase 8 is a Transcriptional Regulator During Dengue Infection." Mentor: Joel Rovnak |

**Version 2.0**

| | |
|---|---|
| First | Sherry WeMott, Graduate Student, ERHS, "Developing a predictive model for indoor black carbon for the Denver, CO metropolitan area." Mentor: Sheryl Magzamen |

Golden Pipette Award – Biomedical Sciences

## 2021 CVMBS Research Day Organizing Committee

Kelly Santangelo - Faculty Co-Chair - Microbiology, Immunology and Pathology
Adam Chicco - Faculty Co-Chair - Biomedical Sciences
John Kisiday – Faculty Co-Chair – Clinical Science
Aimee Oke - Committee Coordinator - CVMBS Dean's Office
Theresa Rulon - Committee Coordinator - CVMBS Dean's Office

# Saturday, January 30 | 12-3:45 p.m.

## Zoom Room 1: Oral Presentations

https://zoom.us/j/93545836620?pwd=c3Q4UnZldjgwWmRhQ25ZUWNZaUZOdz09

| Time | Presenter | Topic | Dept. |
|---|---|---|---|
| 12:00 | Parkinson, Samantha | The effect of deep tissue heating on cervical pain and dysfunction in horses \| Haussler | CS |
| 12:15 | Davey, Emma | Clinical findings in dogs treated with oral cannabidiol (CBD) versus topical prednisolone acetate 1% ophthalmic suspension for experimentally induced uveitis \| Henriksen | CS |
| 12:30 | Diaz, Devon | Systemic tissue plasminogen activator for thrombolysis in 15 dogs and 5 cats \| Guillaumin | CS |
| 12:45 | Frank, Jade | Identifying risk factors and determining incidence of clinical signs in feline cognitive dysfunction syndrome \| McGrath | CS |
| 1:00 | Fukushima, Ken | Effect of a commercially available synbiotic on mycophenolate associated diarrhea \| Lappin | CS |
| 1:15 | Gregory, Carly | Evaluation of outcome associated with feline trauma: a Veterinary Committee on Trauma (VetCOT) registry study \| Hall | CS |
| 1:30 | Maldonado, Mikaela | The effect of chiropractic treatment on lameness and concurrent axial skeleton pain and stiffness in horses \| Haussler | CS |
| 1:45 | BREAK | | |
| 2:00 | Pfluger, Brigitte | Dried blood spot-based metabolomics reveals rice bran supplementation modulates weaning infant nutrition and growth in Mali \| Ryan | ERHS |
| 2:15 | Walczak, Raelyn | Evaluation of 18F-fluorodeoxyglucose positron emission tomography-computed tomography for staging of canine insulinoma \| Griffin | ERHS |
| 2:30 | Williams, Kate | Exercised myoblast-derived exosomes enhance myogenesis: a promising cell-free therapy for healthy muscle aging \| Ehrhart | CS |
| 2:45 | Wilson, Jessica | Economic impacts of Restricted antimicrobial use implemented under Veterinary Feed Directive in the United States: A Systematic Review on Swine production systems \| Rao | CS |
| 3:00 | Worthington, Delaney | Mosquito Bloodmeals as an Alternative Source for Pathogen Surveillance in Central America \| Ebel | MIP |
| 3:15 | Vick, Zaria | Antibiotic neurotoxicity and links to early neurodegeneration \| Moreno | ERHS |
| 3:30 | Geldert, Christina | Dietary supplementation with phytochemicals improves diversity and abundance of honey bee gut microbiota \| Seshadri | Other |

# Zoom Room 2: Oral Presentations

https://zoom.us/j/94014030399?pwd=SklOdk1DZUk4aEl0aGZpTWJVSWhVZz09

| Time | Presenter | Topic | Dept. |
|---|---|---|---|
| 12:00 | Auerbach, Jeremy | Relocating to opportunity: An analysis of the Sun Valley neighborhood redevelopment \| Magzamen | ERHS |
| 12:15 | Curtis, Benjamin | Development of coronavirus vaccines targeting the mucosal immune system by using genetically modified *Lactobacillus acidophilus* bacteria \| Dean | MIP |
| 12:30 | Henry, Mikaela | Development of a novel vaccine for feline enteric coronavirus using recombinant *Lactobacillus acidophilus* \| Dean | MIP |
| 12:45 | Labb, Samantha | Separation of americium in higher oxidation states from curium for nuclear waste recycling \| Sudowe | ERHS |
| 1:00 | Moskaluk, Alexandra | Validation of a novel, rapid DNA extraction method on clinical ringworm samples in domestic felines \| VandeWoude | MIP |
| 1:15 | Omar, Asma | Maternal high-fat diet increases fetal muscle fat metabolism and fatty acid transporter expression in an ovine model \| Chicco | BMS |
| 1:30 | Waugh, Sabrina | Characterization of Antigen and Adjuvant Expression by Recombinant *Lactobacillus acidophilus* Strains for SARS-CoV-2 Vaccine Development \| Vilander | MIP |
| 1:45 | BREAK | | |
| 2:00 | Alfino, Lauren | The FAK of the Matter: Improving Osteosarcoma Survival \| Regan | MIP |
| 2:15 | Baxter, Bridget | Beans/Bran Enriching Nutritional Eating For Intestinal health & Cancer Including Activity for Longevity (BENEFICIAL) \| Ryan | ERHS |
| 2:30 | Cooper, Sarah | Utilizing High-dimensional Quantitative Pathology to Characterize Granuloma Heterogeneity in Tuberculosis \| Podell | MIP |
| 2:45 | Doser, Rachel | Reactive Oxygen Species Modulate Activity-Dependent Glutamate Receptor Transport \| Hoerndli | BMS |
| 3:00 | Doster, Enrique | Validation of remote sensing and simulation methods for animal infectious disease modeling \| Noyes | Other |
| 3:15 | Dutt, Taru Shikha | Single cell RNA sequencing to dissect the immune landscape of lung in response to SolaVAX™ vaccine \| Henao-Tamayo | MIP |
| 3:30 | Flores, Alexis | Geographic Highest Risk Regions for Emerging Zoonotic Diseases a Result of Extreme and Sudden Climate Change \| Salman | CS |

# Zoom Room 3: Oral Presentations

https://zoom.us/j/94720010331?pwd=ak1Ub3pBcnE4M2F2S1h3SXQ1UldYQT09

| Time | Presenter | Topic | Dept. |
|---|---|---|---|
| 12:00 | Georges, Hanah | BVDV and epigenetics; an example of immune deficiencies caused by maternal viral infections \| Hansen | BMS |
| 12:15 | Harris, Macallister | Investigating the role of mycobacterial lipid antigens and CD1-restricted T cells in host-protective tuberculosis immunity using a guinea pig model \| Podell | MIP |
| 12:30 | Hayes, Joshua | The Pseudo Pelger-Huët Anomaly as A Potential Biomarker for Acute Radiation Dose in Rhesus Macaques (*Macaca mulatta*) \| Johnson | ERHS |
| 12:45 | Logsdon, Deirdre | Single-cell multi-omics reveals similarities between early trophoblast and neurons \| Yuan | BMS |
| 1:00 | Manchester, Alison | Role of bile acids in modulating innate immune responses in dogs \| Dow | CS |
| 1:15 | Moore, Joshua | BREATHE: Better Racing and Exercise in Air That Horses Enjoy \| Magzamen | ERHS |
| 1:30 | Nealon, Nora Jean | Association of virulence factor genes with class I integron-encoded antimicrobial resistance in *Salmonella* Typhimurium \| Rao | CS |
| 1:45 | BREAK | | |
| 2:00 | Rutten, Jessica | Analysis of the 2017-2019 ACVECC VetCOT Trauma Initiative Annual Report \| Hall | BMS |
| 2:15 | Sauerwein, Leah and Maria Koycheva | A Systematic Review of Environmental Sustainability In Veterinary Practice \| Duncan | MIP |
| 2:30 | Schlein, Lisa | Feverfew: cheerful foliage and source of an anti-cancer compound \| Thamm | CS |
| 2:45 | Schlemmer, Samantha | HER2/*erbB2* mutation, expression, and activation status in canine thyroid carcinoma \| Thamm | CS |
| 3:00 | St Clair, Laura | Inhibition of human sialidases disrupts dengue virus replication \| Perera | MIP |
| 3:15 | Stewart, Holly | Development of an experimental model of bone marrow lesions using the rat femoral condyle \| Kawcak | CS |
| 3:30 | Timkovich, Ariel | Development of Full and Partial Models of Mid-Substance ACL Rupture \| Santangelo | MIP |

# Wednesday, January 27 | 4-7:00 p.m.

## Zoom Room 1: Poster Presentations

https://zoom.us/j/93545836620?pwd=c3Q4UnZldjgwWmRhQ25ZUWNZaUZOdz09

| Time | Presenter | Topic | Dept. |
|---|---|---|---|
| 4:00 | Fennel, Abigail | Characterization of the microbiome associated with *Culicoides sonorensis* \| Borlee | MIP |
| 4:30 | Foley, Caroline | Viability of equine embryos produced *in vitro* as assessed with zona pellucida measurements \| Carnevale | BMS |
| 5:00 | Pezzanite, Lynn | Use of activated mesenchymal stromal cells (MSC) to treat drug-resistant septic arthritis in horses \| Dow | CS |
| 5:30 | MacNeill, Brooke | Evaluation of the cattle fever tick eradication program in the Texas/Mexico border region \| Salman | CS |
| 6:00 | McCoy, Lia | Urine and renal cultures from middle aged to older cats with and without chronic kidney disease \| Lappin | CS |
| 6:30 | Bonilla, Andres | Anti-Nucleus Pulposus vaccine for the development of Degenerative Disc Disease \| Easley | CS |

## Zoom Room 2: Poster Presentations

https://zoom.us/j/94014030399?pwd=SklOdk1DZUk4aEl0aGZpTWJVSWhVZz09

| Time | Presenter | Topic | Dept. |
|---|---|---|---|
| 4:00 | Woyda, Reed | Human Clinic vs Animal Agriculture: Comparison of *Escherichia coli* Antimicrobial Resistance \| Abdo | MIP |
| 4:30 | Chornarm, Nida | Detection of anti-erythrocyte and anti-platelet antibodies using flow cytometry in experimental *Babesia gibsoni* and *Candidatus* Mycoplasma haematoparvum co-infected dogs \| Lappin | CS |
| 5:00 | Flynn, Grace | Treatment of ocular feline herpesvirus using a novel topical immunotherapy \| Dow | CS |
| 5:30 | Guilbert, Lauren | Susceptibility of peridomestic rodents and other small mammal wildlife to infection with SARS-CoV-2 and their potential role in interspecies transmission \| Bowen | BMS |
| 6:00 | Petch, Raegan | High rate of feline leukemia virus spillover in North American pumas \| VandeWoude | MIP |
| 6:30 | Merriman, Sean | Regionally biased CNVs accompany large-scale chromosomal rearrangements in *S. cerevisiae* \| Argueso | ERHS |

## Zoom Room 3: Poster Presentations

https://zoom.us/j/94720010331?pwd=ak1Ub3pBcnE4M2F2S1h3SXQ1UldYQT09

| Time | Presenter | Topic | Dept. |
|---|---|---|---|
| 4:00 | Cao, Jenna | CAR T cells targeting the checkpoint molecule B7-H3 for treatment of osteosarcoma in dogs \| Dow | CS |
| 4:30 | Whitcomb, Luke | Polyunsaturated fatty acid metabolism contributes to age-related impairment of cardiac mitochondrial calcium tolerance \| Chicco | BMS |
| 5:00 | Davis, Hailey | Effect of *Bifidobacterium longum* 999 supplementation on stress associated findings in cats with feline herpesvirus 1 infection \| Lappin | CS |
| 5:30 | Cerna, Petra | Comparison of two TLR activating immune stimulants for induction of interferon responses in cats \| Lappin | CS |
| 6:00 | Eklund, Bridget | NOD2 expression by mucosal CD11c+ cells is required for a humoral immune response against the *Lactobacillus acidophilus* vaccine platform \| Dean | MIP |
| 6:30 | Fox, Amy | Bactcountr: a tool for calculating colony forming units \| Henao-Tamayo and Anderson | MIP |

# Thursday, January 28 | 10-4:00 p.m.

## Zoom Room 1: Poster Presentations

https://zoom.us/j/93545836620?pwd=c3Q4UnZldjgwWmRhQ25ZUWNZaUZOdz09

| Time | Presenter | Topic | Dept. |
|---|---|---|---|
| 10:00 | McCaw, Katherine | Impacts of the COVID-19 Pandemic on Veterinary Education \| Duncan | MIP |
| 10:30 | George, Zack | Vulnerable Populations, Veterinary Telemedicine, and COVID-19: Access to care and telemedicine for vulnerable pet owners during the pandemic era \| Frey | Other |
| 11:00 | Deluty, Sarah | The Benefits and Challenges of Experiencing Homelessness with a Pet in Fort Collins \|Frey | BMS |
| 11:30 | Haines, Laurel | Osteosarcoma-derived exosomes selectively home to the lung and induce changes in the cytokine profile of the pulmonary microenvironment \| Regan | MIP |
| 12:00 | Walker, Audrey | Evaluation of SARS-CoV-2 infection and transmission in domestic livestock \| Bosco-Lauth | BMS |
| 12:30 | Mathias, Alissa | Investigating the impact of osteosarcoma exosomes on the lung microenvironment \| Regan | MIP |
| 1:00 | Maxwell, Jon | The impacts of climate change on animal health: a gap analysis \| Hollmen | Other |
| 1:30 | Lake, Alexandra | Could neurobasal-CTS + B27 be a universal culture media? \| Tobet | BMS |
| 2:00 | Lederman, Jessica | The use of altrenogest releasing intravaginal rings in the mare for the purpose of estrus suppression \| Hatzel | CS |

| | | | |
|---|---|---|---|
| 2:30 | Marquez-DiPaulo, Patricia | Predisposing Factors of Nasal Fistulas and Osteonecrosis Development Following Stereotactic Radiation Therapy: 91 cases (2010 - 2020) \| Griffin | ERHS |
| 3:00 | Crowdis, Katt | Immunohistochemistry characterization of the immune response to LPS in the equine gastrointestinal tract \| Regan | MIP |
| 3:30 | Starkey, Julie | Elucidating the regulation and function of *Mycobacterium tuberculosis* RelBE toxin-antitoxin systems \| Slayden | MIP |

## Zoom Room 2: Poster Presentations

https://zoom.us/j/94014030399?pwd=SklOdk1DZUk4aEl0aGZpTWJVSWhVZz09

| Time | Presenter | Topic | Dept. |
|---|---|---|---|
| 10:00 | Mazariegos, Isabella | Pet ownership as a barrier for finding housing: a review of pet policies across homeless shelters in the United States \| Geller | Other |
| 10:30 | Wertheimer, Rachel | Impacts of the COVID-19 Pandemic on Access to Veterinary Care Within Animal Shelters, Rescues and Humane Societies \| Frey | Other |
| 11:00 | Heise, Natascha | A large-scale VR deployment: A novel approach to distance education \| Clapp | BMS |
| 11:30 | Bashor, Laura | Molecular adaptation of SARS-CoV-2 in nonhuman mammalian hosts \| VandeWoude | MIP |
| 12:00 | Bergum, Nicolas | Novel route of morphine-induced sleep disorders \| Vigh | BMS |
| 12:30 | Stewart, Joseph | Measuring Systemic Instability in Saccharomyces cerevisiae \| Argueso | ERHS |
| 1:00 | DeFillipo, Brian | Relationship between climate change and waterborne diseases in companion animals: a reconsideration of veterinary surveillance \| Duncan | MIP |
| 1:30 | Stalnaker, Justine | A randomized dose escalation, safety, tolerability, and drug interaction study of cannabidiol administration in dogs with intractable epilepsy \|McGrath | CS |
| 2:00 | Latham, Amanda | Neuropathogenesis in Guinea Pigs Exposed to Mycobacterium tuberculosis \| Moreno | ERHS |
| 2:30 | Harrison, Misha | Mesenchymal stromal cells from load-bearing connective tissues: a review of cellular properties and laboratory expansion methods for manufacturing cell therapies \| Kisiday | CS |
| 3:00 | Gilberto, Vincenzo | Neuronal treatment of models of Alzheimer's disease using CBD and Trazodone \| Moreno | ERHS |

# Zoom Room 3: Poster Presentations

https://zoom.us/j/94720010331?pwd=ak1Ub3pBcnE4M2F2S1h3SXQ1UldYQT09

| Time | Presenter | Topic | Dept. |
|---|---|---|---|
| 10:00 | Smith, Sage | The expanding problem of pet owner vulnerability in the era of COVID-19 \| Frey | Other |
| 10:30 | Liebig, Bethany | Adult equine chondrocytes are capable of extensive *in vitro* expansion and express CD146 with time in culture \| Kisiday | CS |
| 11:00 | Trageser, Erin | Treatment of thymoma with intensity-modulated stereotactic body radiation therapy or non-modulated hypofractionated radiation therapy: retrospective study of fifteen canines \| Boss | ERHS |
| 11:30 | Berezin, Casey-Tyler | Elucidating the Endogenous Opioid Circuit in the Retina \| Vigh | BMS |
| 12:00 | Towner, Nicole | Ecology of Vesicular Stomatitis Virus in North America \| Mayo | MIP |
| 12:30 | Pearce, Camron | Characterizing nanoparticle localization in *M. tuberculosis* infected lungs \| Gonzalez-Juarrero | MIP |
| 1:00 | Buglewicz, Dylan | Carbon-ion Cancer Radiotherapy: Double-Strand DNA Break Distribution and Repair \| Kato | ERHS |
| 2:00 | Hicks, Jasmin | Synaptic Ultrastructure at the Neuromuscular Junction \| Reist | BMS |
| 2:30 | Richards, McKenzie | Testing the Validity of Using a Biochemical Analytical Assay for Diagnosing Canine Cognitive Dysfunction, as a Translational Model for Alzheimer's Disease \| Moreno | ERHS |
| 3:00 | Watts, Remy | Microenvironmental immune effects of stereotactic radiotherapy and immunotherapy in canine solid tumors \| Boss | ERHS |
| 3:30 | Townsend, Stephanie | Neurotoxic signaling due to air pollutant exposure \| Moreno | ERHS |

# VETERINARY SUMMER SCHOLARS PROGRAM

DVM STUDENTS DIVE INTO RESEARCH WITH PROJECTS AND FIELD TRIPS
APPLY BY FEB. 8, 2021!

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. 8 for the summer 2021 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, 22 veterinary students from CSU and abroad participated in a modified remote CSU Veterinary Summer Scholar Program. Students spent the summer working remotely on research and a few on-campus projects related to COVID-19. Many of the projects conducted by CSU students last summer are being presented today at the CVMBS Research Conference.

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 past years, or to apply for the summer 2021 program, please visit the website at: https://vetmedbiosci.colostate.edu/dvm/veterinary-summer-scholars-program.

## BY THE NUMBERS

- 22 scholars in the 2020 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.
- 399 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 2020 PROGRAM:

National Institutes of Health
Boehringer Ingelheim
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 students and early-career researchers like Dr. Stacie Summers 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 2020, corporate and non-corporate sponsors donated more than $30,000 to the program. This funding was deferred to 2021 because of COVID-19 but will be distributed to 15 research projects involving students in our DVM Program.

The Young Investigator Grant Program began in 2006 with a donation of $20,000 from HESKA Corp. In its 15 years, the program has grown to support five times the number of research projects that it supported in its first year – a credit to sponsors who understand the importance of bolstering young scientists, and a credit to our DVM students for the impressive quality of their research efforts.

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 2020 or to make a donation, please visit the Center for Companion Animal Studies website at *companionanimals.colostate.edu*

YOUNG INVESTIGATOR AWARDS

| | |
|---|---|
| 15 – 25 research grants funded per year | Student, intern, resident and faculty |
| Over 200 grants funded | Over 50 faculty have participated |
| Over 200 PVM students on publications | Several research awards for students |

## 2020 YOUNG INVESTIGATOR GRANT PROGRAM SPONSORS

### platinum sponsor

Boehringer-Ingelheim -Merial

### gold sponsors

Bayer Animal Health
IDEXX Laboratories
Merck Animal Health
Nestle Purina Petcare
Veterinary Centers of America

### silver sponsors

Elanco
Royal Canin
Zoetis Animal Health

### bronze sponsors

Hill's Pet Nutrition and SCAVMA
International Veterinary Seminars
Virbac

A big thank you to our Research Day sponsors

Boehringer Ingelheim

zoetis

CSUVENTURES

INFECTIOUS DISEASE RESEARCH CENTER
COLORADO STATE UNIVERSITY

## O-1. Relocating to opportunity: An analysis of the Sun Valley neighborhood redevelopment

*Jeremy Auerbach, Rebecca Warren, and Sheryl Magzamen*

The Denver Housing Authority is transforming Sun Valley, a neighborhood of public housing townhomes constructed in the 1950's, into a new community of apartments with modern design features (e.g., central A/C) and amenities (e.g., food market) by 2025. Through community-engaged research focused on pathways to reduce or eliminate environmentally driven health disparities we initiated a larger research project to investigate the relation between housing and health for low income families. Four waves of annual resident survey data were analyzed to discover trajectories of health, determinants of health status, and lay the foundation for a natural experiment to determine how physical and mental health outcomes are associated with the transition to new housing. A longitudinal survey analysis was conducted to establish a baseline of mental health and test changes in these outcomes during construction of the new housing. This study established the baseline health of the residents in order to evaluate the expected improvements associated with the move to newer housing stock. These results were shared with our community partner, Choice Neighborhoods Initiative, a U.S. Housing and Urban Development program, to inform development of housing and programs for mental and physical health, identify at-risk groups for negative health outcomes, and provide new research directions to follow the health impacts of housing change. The results are ultimately intended to provide an understanding of the health benefits of moving to new housing as well as best practices for public housing redevelopment and, ultimately, for the design of all housing.

Post-doctoral Fellow / Environmental and Radiological Health Sciences

## O-2. Development of coronavirus vaccines targeting the mucosal immune system by using genetically modified *Lactobacillus acidophilus* bacteria

*Benjamin Curtis, Allison Vilander, Yong Jun Goh, Kimberly Shelton, Maxwell Drummond, Mikaela Henry, Rodolphe Barrangou, Gregg Dean*

In early 2020 the world was thrown into chaos when a new coronavirus, Severe Acute Respiratory Syndrome 2 (SARS CoV2), initiated the most significant pandemic in a century. As a laboratory already researching coronaviruses, we recognized the challenges to effectively vaccinating against this novel coronavirus. Vaccines requiring cold-chain transportation and storage will limit the availability and distribution of leading vaccine candidates worldwide. The probiotic bacteria, *Lactobacillus acidophilus*, is an attractive vaccine platform because it does not require cold-chain storage and is administered orally to deliver antigens directly to the mucosal immune system, creating immunity where coronaviruses enter the body. Using homologous recombination and CRISPR-SpyCas9n, we have engineered *L. acidophilus* to express coronavirus antigens and adjuvant molecules. Most coronavirus vaccines have featured the entire or a large portion of the viral spike protein which is responsible for binding host target cells. In some cases, this approach has resulted in antibody dependent enhancement leading to more severe disease. To avoid this, we have inserted two key peptides of the spike protein, S1/S2 and S2', which are involved in the conformational changes required for the binding and entry of the virus. Screening of serum samples from humans, hamsters, and cats infected with SARS CoV2, and serum and fecal samples from cats infected with feline enteric coronavirus have shown that the S1/S2 and S2' sites illicit IgA and IgG antibody responses. Furthermore, we have detected antigen specific IgA within vaginal washes, showing that our *Lactobacillus* vaccine system successfully incites immune responses at distant mucosal sites. This suggests that vaccinating by the oral route can induce immune responses at other mucosal sites. Development of these vaccines will permit safe and effective vaccination globally. **Funding:** The Morris Animal Foundation –D20FE-013, The Morris Animal Foundation – D20FE-401 (Fellowship), The Alvin Hoerlein Fellowship, CSU Intramural Funding

Graduate Student Resident / Microbiology, Immunology and Pathology

## O-3. Development of a novel vaccine for feline enteric coronavirus using recombinant *Lactobacillus acidophilus*

*Mikaela E Henry, Benjamin Curtis, and Gregg A Dean*

Coronaviruses are present throughout the world and affect a wide variety of species. In cats, feline infectious peritonitis virus (FIP) has a nearly 100% mortality rate and to date no effective vaccine has been produced. Because FIP is a mutant variant of the common and innocuous enteric virus, feline enteric coronavirus (FECV), we hypothesize that vaccination against FECV will in turn protect cats from FIP, an approach that has never been attempted. To achieve this, we aim to use *Lactobacillus acidophilus* bacteria to deliver FECV antigens to the mucosal immune system. The viral peptides S1/S2 and S2' are involved with virus binding and entry into the target cells. We designed primers which could be used to integrate these viral peptides into the gene encoding a surface layer protein (SlpA) on the bacteria. Using polymerase chain reaction (PCR), we amplified the N and C terminals of each peptide and then ligated them using overlap PCR. The peptide sequences were gel extracted and inserted into a cloning vector plasmid (pJET1.2) and then transfected into chemically competent *E. coli* (EC101) for amplification. PCR was performed to confirm the presence of the insert sequence after each round of cloning. The plasmid was then extracted from the *E. coli* and the insert was moved to our expression vector, pTRK1053, via restriction digestion and ligation. After transfection and growth in EC101s, the plasmid was extracted, and the successful development of our vaccine plasmid was confirmed via Sanger sequencing prior to transfection into our *Lactobacillus* vector via electroporation. Next, we aim to integrate the SlpA gene containing our viral peptides into the *L. acidophilus* genome via homologous recombination and then vaccinate SPF cats followed by challenge using CSU colony cats that are naturally infected with FECV. The Morris Animal Foundation is funding this study.

DVM Student / Microbiology, Immunology and Pathology

## O-4. Separation of americium in higher oxidation states from curium for nuclear waste recycling

*Samantha A. Labb, Ralf Sudowe*

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

Graduate Student / Environmental and Radiological Health Sciences

## O-5. Validation of a novel, rapid DNA extraction method on clinical ringworm samples in domestic felines

*Alex E Moskaluk, Sally L Kuhn, Sue VandeWoude*

*Microsporum canis* is the primary pathogen in approximately >90% of cases of feline dermatophytosis (ringworm), the most common infectious skin disease in cats. While infection is self-limiting in immunocompetent individuals, infection is of concern due to its contagious nature, particularly in high density animal setting like shelters and rescue facilities. Feline dermatophytosis can have various clinical presentations depending on the cat's overall health. Furthermore, cats can be carriers without presenting with lesions, spreading spores to the environment, other cats and people. Currently, extended quarantine of suspect dermatophyte cases is the standard protocol in many shelters, or euthanasia is used as a management option. Fungal culture is perhaps the most commonly used diagnostic approach, but false negatives and positives occur, and up to 21 days may be required to complete the analysis. This study aimed to evaluate the sensitivity and specificity of a rapid 10-minute DNA extraction method for clinical samples by comparing the resulting PCR to standard fungal cultures. Clinical hair samples from 120 domestic cats suspected of ringworm infections were collected from shelters and private practices across the United States and were subjected to fungal culturing and DNA extraction for PCR. Importantly, the rapid extraction method had a sensitivity of 85% and specificity of 80% when compared to fungal cultures. Collectively, our data concluded that this rapid extraction method could be beneficial for initial screening of suspected ringworm cases. **Funding:** CSU Office of Vice President for Research: IDRRN Summer Fellowship, CSU/NIH T32 Post-doctoral Fellowship Denver/NIH CCSTI TL-1 Post-doctoral Fellowship,

Graduate Student / Microbiology, Immunology and Pathology

## O-6. Maternal high-fat diet increases fetal muscle fat metabolism and fatty acid transporter expression in an ovine model

*Asma K Omar, Gerrit J Bouma, Quinton A Winger and Adam J Chicco*

Excessive maternal dietary fat consumption during pregnancy may be linked to adverse effects on offspring health, including greater risk of developing metabolic syndrome later in life. Metabolic syndrome is generally considered to be a preventable condition, but the extent to which it is "programmed" during fetal development remains unclear. The aim of this study was to determine the effect of a maternal high-fat diet (MHFD) during pregnancy on fetal muscle oxidative metabolism and related protein and mRNA expressions in an ovine model. **Methods:** White-faced ewes were fed either a control diet (Show-rite NewCo Lamb Feed-17% protein, 5% Fat) or a high-fat diet (Show-rite NewCo Lamb Feed+ 6% Rumen-protected Fat) from 2-3 weeks before pregnancy until mid-gestation (75 days), when a C-section was performed to collect the placenta and fetal tissues for analysis. **Results**: MHFD tended to increase fetal body and organ weights, but only significantly increased fetal body length and liver mass ($P < 0.05$). MHFD increased mRNA expression of placental (cotyledon) fatty acid transport protein-1 (FATP-1) and peroxisome proliferator activated receptor gamma, suggesting an upregulation of placental fatty acid metabolism and transport. Fetal muscle fatty acid oxidation capacity was greater in animals from MHFD pregnancies, with no effect on pyruvate oxidation. This was associated with greater fetal muscle mRNA and protein expression of FATP4, while mRNA expression glucose transporters (GLUT1 and GLUT3) decreased. Muscle expression of insulin signaling enzymes reflected a mild decreases in insulin sensitivity, but these did not reach statistical significance. **Conclusions**: These studies indicate that MHFD induces an increase in placental and fetal muscle fatty acid transport and oxidation capacity, and favors lower blood glucose uptake compared to controls. Whether these shifts in fetal metabolism predispose offspring from MHFD pregnancies to elevated blood sugar and Type 2 diabetes later in life merits further investigation.

Graduate Student / Biomedical Sciences

## O-7. Characterization of Antigen and Adjuvant Expression by Recombinant *Lactobacillus acidophilus* Strains for SARS-CoV-2 Vaccine Development

*Sabrina C Waugh, Gregg A Dean, and Allison C Vilander*

*Lactobacillus acidophilus* strains genetically modified to express viral epitopes and the adjuvants FimH and FliC on their surfaces can act as an applicable oral vaccine platform against human Rotavirus and SARS-CoV2. To create these vaccines, viral epitopes known to induce protective antibodies are incorporated within the bacteria's surface layer protein A (slpA). The adjuvants FimH and FliC are anchored to the bacteria's surface and stimulate pathogen-associated molecular patterns (PAMPs) to strengthen the immune response against the viral epitopes. It is known that these epitopes and adjuvants can alter the physical characteristics and behavior of *L. acidophilius*. However, the changes that occur, and their effects on interactions with the recipient's immune system, have not been extensively studied. To study these characteristics, we are using scanning and transmission electron microscopy to evaluate changes in the size, shape, surface thickness, and aggregation patterns of several *L. acidophilus* modified to express certain antigens. The strains will be imaged at both exponential and log phases of growth to most thoroughly describe the antigen presentation and bacterial surface alterations inducted by the modifications to the surface layer proteins. Viewing and describing these changes will help more definitively identify viable vaccine candidates and suboptimal characteristics that can be targeted and screened for in subsequent work. This electron microscopy evaluation will facilitate the development of the *L. acidophilus* platform as an effective oral vaccine platform.

DVM Student / Microbiology, Immunology and Pathology

## O-8. The FAK of the Matter: Improving Osteosarcoma Survival

*Lauren N Alfino, Kai C Wilczewski-Shirai, Chris G Andretsos, Sophi J Schofield, Laurel Haines, Eric P Palmer, Dan P Regan*

Osteosarcoma (OS) is the most common primary malignant bone tumor. New therapeutic strategies for OS are desperately needed, as overall survival rates of OS patients have remained unchanged for 30 years. Spontaneous OS 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 . Preliminary data demonstrates integrin signaling pathways are enriched in canine and human OS. 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 OS cell lines via cell survival/proliferation, migration and combination drug synergy assays. FAKi14 demonstrated significant dose dependent inhibition of proliferation/survival in all cell lines at pharmacologically achievable concentrations. Cilengitide demonstrated significantly less anti-proliferative effects than FAKi14, with clinically relevant IC50 values not reached in most cell lines; however, Cilengitide mediated significant dose dependent inhibition of OS cell migration in the majority of evaluated cell lines. Together, FAKi14 and Cilengitide demonstrated synergistic antiproliferative effects, as evidenced by Chou-Talalay combination indices < X. 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 OS.

DVM Student / Microbiology, Immunology and Pathology

## O-9. Beans/Bran Enriching Nutritional Eating For Intestinal health & Cancer Including Activity for Longevity (BENEFICIAL)

*Bridget A Baxter, Melanie Beale, Hillary Ford, Hannah Haberecht, Sangeeta Rao, Sarah Hipp-Ships, Heather Leach, & Elizabeth P Ryan*

BENEFICIAL is a pilot randomized, placebo-controlled trial to examine increased fiber intake with rice bran and navy beans, compared to a fiber supplement, while accounting for physical activity levels to reduce colorectal cancer risk. There were 20 participants enrolled with stage 0-I colon cancer and randomized according to body mass index and sex. Participants were allocated to placebo (Fibersol-2) (10 g corn soluble fiber supplement per day) or intervention (rice bran 30g + navy bean 30g) for 3-months. Fiber intake was measured through study foods and normal diet using Nutritionist Pro via the analysis of 3-day food records and the ASA 24 nutritional composition database generated the healthy eating index (HEI). Non-targeted metabolomics of blood, urine, and stool samples was completed to identify biomarkers of dietary intake and to measure impacts on host and gut metabolic pathways of importance to reducing colonic inflammation and cancer risk. Physical activity was measured using ActivePal accelerometers worn by participants at baseline and end of study for 7 consecutive days. All participants received a physical activity education session aligned with the American Cancer Society. Increased daily fiber intake through consumption of study foods and powders for all participants' was associated with improved HEI scores. All participants exceeded physical activity guidelines for moderate to vigorous activity (min/week), however average steps per day were not met (steps/day). Participants' consuming rice/navy bean showed changes in B vitamins, short chain fatty acids, secondary bile acids and phytochemicals. Significantly decreased serum triglycerides, cholesterol and elevated HDL in the intervention group occurred after 3 months when compared to the control. This study demonstrated a practical and affordable means of adhering to national guidelines for colorectal cancer control and prevention in a high-risk population.

Staff / Environmental and Radiological Health Sciences

## O-10. Utilizing High-dimensional Quantitative Pathology to Characterize Granuloma Heterogeneity in Tuberculosis

*Sarah Cooper, Hadley Gary, Mac Harris, James DiLisio, Amy Fox, Burton Karger, Marcela Henao-Tamayo, 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 and their role in granuloma heterogeneity in situ remain poorly understood. Understanding the contextual and spatial relationships of the unique populations in and around granulomas during lesion formation can give insight into the immune correlates of protection afforded by vaccination. Here, we utilize multiplex fluorescent immunohistochemistry (IHC) and in situ hybridization (ISH) on fixed mouse lung tissue to identify immune cell populations and *M. tuberculosis* organism and physiologic activity. We use spatial image analysis to detect and quantify these targets within *M. tuberculosis* infected lungs of Bacillus Calmette–Guérin (BCG) vaccinated and unvaccinated mice. Our data show an overall increase in mean CD4 and CD8 T cells in lesions of vaccinated and unvaccinated mice over time, with unvaccinated mice having slightly higher populations of both. Additionally, B cell populations decreased slightly over time with vaccinated mice having higher populations of B cells at both timepoints. Interestingly, when broken down by mouse and lesion, heterogeneity of cell populations exists not only between vaccinated and unvaccinated groups, but within these groups, and even within the same mice. In conclusion, we can specifically identify and quantify *M. tuberculosis* and immune cell populations present in granulomas in situ in order to better understand the role of granuloma heterogeneity in tuberculosis disease.
**Funding:** NIH-BAA-AAI2001700104

Graduate Student / Microbiology, Immunology and Pathology

## O-11. Reactive Oxygen Species Modulate Activity-Dependent Glutamate Receptor Transport

*Rachel L Doser, Gregory C Amberg and Frederic J Hoerndli*

Cognition, learning and memory depend on regulatory changes in the number and function of the glutamate receptors (GluRs) at synapses, the connections between neurons. This regulation requires long-distance transport of GluRs from the cell body, where they are made, to synapses. Neuronal signaling as well as this transport are metabolically demanding processes in which energy consumption and production are tightly coupled and regulated. The majority of energy production unavoidably produces a class of chemically reactive molecules called reactive oxygen species (ROS), which are modulators of calcium signaling. Although a role for calcium signaling in GluR transport has been described, it is unclear what mechanisms are involved and if it is linked to physiological ROS signaling. To investigate whether an interplay between calcium and ROS signaling regulates GluR transport, we use the transparent genetic model C. elegans to visualize GluRs *in vivo* in real-time. This approach revealed that small changes in ROS levels decrease GluR transport out of the cell body, as well as decrease delivery and exocytosis at synapses. Furthermore, it revealed that this change in GluR transport is due to ROS acting on or directly downstream of calcium channels. For future experiments, we aim to determine how ROS and calcium regulate the delivery of GluRs to individual synapses to ultimately impact neuronal excitation. These studies demonstrate a physiological signaling role for ROS and provide insight as to why unnaturally high ROS levels are correlated with abnormal cognitive function, learning and memory in the aged and diseased brain. **Funding:** College Research Council

Graduate Student / Biomedical Sciences

## O-12. Pathogens, Pipelines and Phylogenomics: Evaluating the computational protocols used to identify foodborne outbreaks from WGS data

*E. Doster, E. Seabolt, J. Kaufman, and N. Noyes*

The genotyping of bacterial pathogens is critical for outbreak investigations and whole-genome sequencing (WGS) is increasingly employed as a novel tool for characterizing bacterial genomes due to unprecedented levels of precision now possible. However, the best approach for determining "sequence relatedness" between WGS samples is still unclear, with many options available and new tools being consistently developed. The overall goal of this project is to support an accurate, reproducible, transparent, and uniform approach to WGS analysis for purposes of outbreak detection and pathogen surveillance.

To achieve our overarching objective, we plan to utilize a systematic analytic experiment to generate 15 different datasets grouped by sample type, sequence quality, geographical source, host species, and a random selection of genomes for 3 bacterial pathogens in NCBI's pathogen database; Salmonella spp., Escherichia/Shigella, and *Listeria monocytogenes*. Each dataset will be analyzed using both the core- and pan-genomes, in addition to each of the following four comparative approaches; based on single nucleotide polymorphisms (SNP-based); k-mer-based; gene-by-gene allelic comparison (also termed a core genome or whole-genome MLST comparison), and finally a novel comparison based on functional domains.

We highlight ongoing computing challenges in analyzing large genomic datasets and present examples highlighting how the analytic approach can impact the results garnered from WGS analysis. Results from this study will serve as a guideline to help inform other research teams and public health officials in decision-making around using WGS for outbreak investigations. **Funding:** Foundation for Meat and Poultry Research and Education (FMPRE)

DVM/PhD Student / Other

## O-13. Single cell RNA sequencing to dissect the immune landscape of lung in response to SolaVAX™ vaccine

*Taru S. Dutt, Amy Fox, Burton Karger, Andres Obregon-Henao, Marylee Layton, Izabela Ragan, Lindsay Hartson, Richard Bowen, Mark Stenglein, Raymond Goodrich and Marcela Henao-Tamayo*

SARS-CoV-2, the causative agent of COVID-19, is a global pandemic and to date, has caused more than 64 million infections and over one million deaths. Therefore, the urgent need for vaccines prompted an international response, with more than 200 candidate anti-SARS-CoV-2 vaccines in development. However, no vaccine is licensed till date for human use. Our team at CSU developed a candidate vaccine, 'SolaVAX™', by using Riboflavin and UV light to selectively inactivate SARS-CoV-2 while preserving the integrity and antigenicity of its proteins. The vaccine proved to effectively reduce viral loads and immunopathology in the lungs of vaccinated hamsters. Here, we applied single-cell RNA sequencing (scRNA-seq) to characterize immune cell populations and transcriptional changes in lungs of Syrian Hamsters infected with SARS-CoV-2 after vaccination or not with SolaVAX™ (with and without adjuvant). Our results dissected different immune cell populations, e.g., B cells, effector T cells, Dendritic cells, inflammatory Monocytes, NK cells, Macrophages, and neutrophils in the lungs of different groups. Compared to non-vaccinated hamsters, SolaVAX™ vaccinated groups showed significant differences in the distribution of leukocyte populations infiltrating the lungs. Importantly, inflammatory genes were decreased in SolaVAX™ vaccinated groups along with the SARS-CoV-2 viral reads. Vaccines work by targeting and preparing the immune system to rapidly respond upon exposure to similar antigens. However in the case of coronavirus infections like SARS-CoV-2, vaccines can also result in vaccine-induced immunopathology. Thus, a better understanding of vaccine-elicited protective vs deleterious immunological responses is warranted and can be accomplished through the approach described herein. **Funding:** CSU, NIH

Post-doctoral Fellow / Microbiology, Immunology and Pathology

## O-14. Geographic Highest Risk Regions for Emerging Zoonotic Diseases a Result of Extreme and Sudden Climate Change

*Alexis M Flores, Colleen Duncan, Sadie Skeels, Mo Salman*

Burdens of global climate change have become scientifically relevant in the past three decades and increasingly relevant with the COVID-19 pandemic. Zoonotic disease emergence can act as a sentinel for regions in which climate change has become most severe. A systematic review was conducted to investigate the association between climate change factors and zoonotic disease emergence and reemergence. We performed a comprehensive literature search of three databases for relevant literature over the past 10 years- 665 abstract were identified resulting in 52 full papers meeting inclusion criteria. Meta-analysis and spatial data analysis were used to determine which region had the greatest numbers of climate change factors that influenced zoonotic emergence. Results of the full paper characterization and spatial analysis identified 48 regions, 9 climate factors, and 39 zoonoses spanning four disease classes. Regions with highest climate factors identified included China, the United States, Canada, Brazil, Kenya, Australia, and Russia. Relevant climate factors associated with disease emergence included: land surface temperature, rainfall, El Nino Southern Oscillation (ENSO), land-use change, flood, and drought. Strong evidence showed regions with increases in land surface temperature and rainfall resulted in increased vector borne and bacterial zoonotic emergence. In addition, relevant literature was used to identify knowledge gaps to determine which regions may lack evidence of climate change factors and zoonotic disease emergence and includes recommendations for animal, human, and environment health agencies to limit zoonotic disease risk based on relevant findings.

Graduate Student / Clinical Sciences

## O-15. Dietary supplementation with phytochemicals improves diversity and abundance of honey bee gut microbiota

*Christina D. Geldert, Zaid Abdo, Jane E. Stewart, Arathi Seshadri*

The purpose of this study is to determine the impact of beneficial phytochemicals on the diversity and abundance of the gut microbiome in the honey bee (*Apis mellifera*). Eight-day-old honey bee workers were fed 25 ppm of phytochemical (caffeine, gallic acid, p-coumaric acid or kaempferol) in 20% sucrose. Guts of bees collected at 3 and 6 days were excised and subjected to next-generation sequencing for bacterial 16S and fungal ITS regions. Although phytochemical supplementation fostered gut microbial diversity and abundance, the patterns differed between phytochemicals and there was a temporal stabilization of the bacterial community. While bacterial and fungal communities responded differently, all phytochemical treatments displayed an increased abundance of the most represented bacterial genera, *Snodgrassella* sp. and *Lactobacillus* sp. Phytochemical supplementation improves gut microbial diversity and abundance, reiterating the need for diverse habitats that provide bees with access to pollen and nectar-rich in these micronutrients. Diverse gut microbiota can provide a strong line of defense for bees against biotic stressors while improving worker bee lifespan. This is the first report on the impact of phytochemical supplementation on gut microbiota in honey bees and these findings have implications for strategic hive management through standardization of effective phytochemical and probiotic feed supplements. **Funding:** Project Apis m and USDA Animal Health and Disease program

DVM Student / Other

## O-16. BVDV and epigenetics; an example of immune deficiencies caused by maternal viral infections

*Hanah M Georges, Hana Van Campen, Thomas R Hansen*

Maternal infection with Bovine Viral Diarrhea Virus (BVDV) has life-long negative effects on progeny. Despite current preventative measures, BVDV continues to plague the cattle industry, costing $1.5 billion annually and producing persistently infected (PI) calves that remain the primary reservoirs of the virus. Previous *in vivo* studies concluded that attenuation of the PI fetal immune system was caused by a lack of T-cell response in the fetus, resulting in an inability for T-cells and B-cells to mature properly. In this study, it was hypothesized that T-cell activation and signaling genes were epigenetically altered after fetal infection. Splenic tissue from PI and control fetuses were collected on day 245 of gestation, 170 days post-maternal infection. DNA was isolated and subjected to reduced representation bisulfite sequencing. Methylation sequencing files were bioinformatically analyzed using the methylKit R package. Within set parameters, 2,641 regions were differentially methylated: 1,951 hypermethylated and 691 hypomethylated regions. Results revealed hypermethylation of nuclear factor of activated T cells (NFAT) 1 and 4, which is likely to shift the Th cell differentiation from Th1 to Th2 cells. An increase in NFAT2 and signal transducer guanine nucleotide exchange factor VAV1 expression due to hypomethylation would promote anergy of T-cells, further exacerbating the shift from Th1 to Th2 cells. This shift of Th cells is associated with T-cell receptor hyper-reactivity and lymphoproliferative disorder. Additionally, the hypomethylation of ORAI and calmodulin may contribute to the Th2 hyper-reactivity by increasing the amount of calcium transported into a cell upon T-cell activation. The observed epigenetic modification of critical T-cell genes may help explain inability of postnatal PI calves to fight secondary infections efficiently, contributing to performance loss and continued BVDV viral shedding. This work is supported by: USDA AFRI NIFA Predoctoral Fellowship 2019-67011-29539/1019321, 2016-38420-25289 and W3112 Project. **Funding:** USDA AFRI NIFA Predoctoral Fellowship 2019-67011-29539/1019321, 2016-38420-25289 and W3112 Project

Graduate Student / Biomedical Sciences

## O-17. Investigating the role of mycobacterial lipid antigens and CD1-restricted T cells in host-protective tuberculosis immunity using a guinea pig model

*Macallister C Harris, James Dilisio, Hadley Gary, Edward Chang, D. Branch Moody, Brendan K Podell*

CD1 is a group of glycoproteins on antigen-presenting cells (APCs) that present lipid antigens to T cells. *Mycobacterium tuberculosis* (Mtb) has a lipid-rich cell wall which is essential for the pathogenesis of tuberculosis. Guinea pigs serve as the best translational model for CD1 immunology as they have both group 1 and group 2 CD1 complexes, comparable to human CD1. Our goal is to determine the frequency, phenotypes, and functionality of CD1 T cells against Mtb using the guinea pig model. We performed ex-vivo and in-vivo experiments to analyze lipid antigen-specific CD1 T cell responses with Mtb infection. Assays to detect lipid-specific CD1 T cell activation include cellular proliferation, cytotoxicity assays, and interferon-gamma (IFNγ) release assay (Elispot) using both synthetic and Mtb-derived lipids. The cytotoxicity assay demonstrated that the CD1b1 and CD1b3 complexes play roles in the presentation of Mtb lipids as noted by T cell killing of fibroblasts that express specific CD1 complexes that can present Mtb lipids. Similarly, cellular proliferation exhibited lipid specific T cell proliferation. IFNγ production by the stimulated CD1-restricted T cells (Elispot) was weak indicating CD1 T cells may not extensively produce IFNγ. Immunohistochemistry successfully showed CD1 APCs in lungs and spleens of infected guinea pigs. We characterized CD1 T cells in infected guinea pigs at the tissue level, demonstrating Mtb lipid immunology. As a result, we laid the groundwork for investigating whether augmenting lipid immunity in the guinea pig model will enhance immunity against tuberculosis. Fruition of such work may lead to the development of effective tuberculosis vaccines. **Funding:** R21AI144662, U19AI111224

Graduate Student

Post-doctoral Fellow / Microbiology, Immunology and Pathology

## O-18. The Pseudo Pelger-Huët Anomaly as A Potential Biomarker for Acute Radiation Dose in Rhesus Macaques (*Macaca mulatta*)

*Joshua M. Hayes, Ronald Goans, J Mark. Cline, John D. Olson, Susan M. Bailey, & Thomas E. Johnson*

The potential for malicious use of radiation, or radiation accidents could potentially lead to acute, high radiation doses to members of the public. Following an acute accidental exposure to high doses of radiation, medical intervention is pivotal to the survivability of the patient and the sooner the appropriate measures are taken the better the odds for survival. Early estimates of acute accidental radiation doses can be determined via biomarkers such as dicentric chromosome analysis or scenario reconstruction using computer software. However, both take valuable time, and can be expensive. Here, potentially faster, and cheaper quantitative biomarkers for radiation exposure were evaluated in acutely exposed Rhesus Macaques from the Wake Forest School of Medicine, Department of Comparative Medicine. Increased frequencies of abnormal neutrophils in peripheral blood, referred to as pseudo Pelger-Huët anomalies (PPHAs), have been shown to be potential biomarkers of radiation exposure in several scenarios, including the 1958 Y-12 criticality accident and the radium dial painters (Goans et al, 2017 & Goans et al, 2018). We have confirmed the PPHA morphology to be present in Rhesus Macaques and a dose response curve, a biokinetics model, and determination of background prevalence of the morphology has been constructed utilizing peripheral blood smears. The dose response curve consists of macaques that received doses ranging from 0 Gy to 8.5 Gy (LD90/30) exposures and a blood smear at a common time point post-irradiation, and the biokinetics model utilized only 4 Gy exposures and blood smears taken periodically over 3.1 years post-irradiation. Results have shown a linear correlation between PPHA concentration and acute radiation dose and the PPHA morphology appears stable over 3.1 years post-irradiation.

Graduate Student / Environmental and Radiological Health Sciences

## O-19. Single-cell multi-omics reveals similarities between early trophoblast and neurons

*Deirdre Logsdon, Hao Ming, Jiangwen Sun, William B. Schoolcraft, Rebecca L. Krisher, Zongliang (Carl) Jiang, Ye Yuan*

Molecular events associated with human implantation are poorly understood. Here, we performed single-cell whole genome bisulfite sequencing (scWGBS) on human trophoblast cells (TB; cytoTB, syncytioTB, and migratory TB) obtained from peri-implantation stage human embryos cultured to embryonic day (D) 8, D10, and D12 as described earlier for single cell RNA sequencing (scRNA-seq) experiments (PMID: 31636193). Ninety-six samples were sequenced and approximately 25 million 150 bp paired-end reads per sample were obtained. We captured approximately 20 million CpG sites with 66% total coverage of all CpG sites in the human genome. Global DNA methylation of cytoTB increased from D8 to D10 and maintained relatively constant to D12. SyncytioTB had a lower, and migratory TB a similar global methylation level compared to cytoTB. Global DNA hypomethylation in syncytioTB may be correlated with the significantly reduced ($p < 0.001$) DNMT3A mRNA expression compared to migratory TB and reduced ($p < 0.0001$) DNMT3b mRNA expression compared to cytoTB. We then identified differentially methylated regions within each cell type and noted a large number of significantly hypomethylated pathways ($p < 0.0001$) linked to neuronal behavior and used these pathways to look for genes differentially expressed at the transcription level. By applying a multiomics approach, our data suggest that DNA methylation is an important driving force for directing TB lineage emergence during implantation and that there are analogies between early trophoblast differentiation and neuronal behavior. This research was funded by Colorado Center for Reproductive Medicine and approved by Western Institutional Review Board (Study no: 1179872). **Funding:** Colorado Center for Reproductive Medicine

Graduate Student / Biomedical Sciences

## O-20. Role of bile acids in modulating innate immune responses in dogs

*Alison C Manchester, William H Wheat, and Steven Dow*

Bile acids (BAs) act as signaling molecules in the intestines, impacting gut bacterial communities and host inflammatory responses. Macrophages regulate intestinal mucosal inflammatory reactions. We hypothesize that BAs direct pro- and anti-inflammatory processes via differential cytokine release from canine macrophages. A canine macrophage cell line (MH588, a malignant histiocytoma) was cultured under standard conditions (5% $CO_2$, 37°C in DMEM with 10% FBS). Fifty thousand cells were plated in a 24 well plate and pre-treated for 2 hours with 5, 50, or 100 µM unconjugated cholic acid (CA), chenodeoxycholic acid (CDCA) or lithocholic acid (LCA). Cells were then treated with lipopolysaccharide (LPS, 300 ng/mL) for 48 hours, when supernatants were removed for ELISA quantification of IL-10 and TNF-α. Experiments were performed in triplicate. Control wells were untreated (negative) or treated with LPS (positive). Cytokine concentrations were compared using one-way ANOVA. Interleukin-10 and TNF-α concentrations were low to undectable in untreated cells. In cells treated with CA or CDCA, IL-10 and TNF-α concentrations were comparable to LPS treatment. Conversely, pre-incubation with LCA at 50 µM was associated with reduced TNF-α concentrations (mean 500 ± 26 pg/mL; $P < .0001$) compared to LPS treatment (mean 1039 ± 10 pg/mL), as well as elevated IL-10 concentrations (mean 1236 ± 178 pg/mL; $P < .0001$) compared to LPS treatment (mean 519 ± 14 pg/mL) without or with CA (mean 502 ± 52 pg/mL) or CDCA (mean 685 ± 80 pg/mL). LCA, but not CA or CDCA, promotes anti-inflammatory cytokine release from canine macrophages treated with a proinflammatory stimulus (LPS), implicating this BA as a mediator of intestinal inflammation. Further work is needed to better characterize the interactions between intestinal BAs, microbiota and inflammation.

Graduate Student, Post-doctoral Fellow / Clinical Sciences

## O-21. BREATHE: Better Racing and Exercise in Air That Horses Enjoy

*Josh K Moore, Colleen G. Duncan, Katie A. Seabaugh, Sheryl L. Magzamen*

The link between air pollution and human health is well established; public advisories are issued when pollutant concentrations exceed acceptable levels set by the United States Environmental Protection Agency (EPA). Air pollution is an unquestionable threat to public health. However, the veterinary community has only recently investigated its influence on animal health. Equine athletes have exceptional performance abilities and respiratory requirements. They consume up to 1,800 liters of air per minute at peak performance, thus making them uniquely exposed to hazardous air pollutants. In this study, we aim to identify the magnitude of effect of inhaled hazardous air pollutants – fine particulate matter ($PM_{2.5}$), ozone ($O_3$), and nitrogen dioxide ($NO_2$) – on thoroughbred performance. We acquired race-day, two-, and four-day prior pollutant air quality indices (AQI) for 119 staked races from Santa Anita track, from 2000-2019. We collected performance and air pollution data from Equibase and the EPA, respectively. Multiple regression analysis of the data displayed a statistically significant effect of two-day prior $NO_2$ AQI on thoroughbred performance. Within the selected sample population, a 10-point increase in the two-day prior $NO_2$ AQI resulted in a 1.20 point decrease in the Equibase Speed Figure (ESF). We believe this preliminary study will demonstrate the utility in monitoring performance and environmental data, such that informed strategies can be implemented to mitigate occupational and welfare hazards to equine athletes. **Funding:** Veterinary Summer Scholars Program

DVM Student / Environmental and Radiological Health Sciences

## O-22. Association of virulence factor genes with class I integron-encoded antimicrobial resistance in *Salmonella* Typhimurium

*Nora Jean Nealon, Roberta Magnuson, Joy Scaria, Sangeeta Rao*

Antimicrobial resistance (AMR) is an escalating global public health concern in *Salmonella enterica* serovar Typhimurium, an enteric pathogen readily transmitted between livestock and people. *Salmonella* Typhimurium AMR is especially dangerous when isolates possess mammalian virulence factors, which are genes that contribute to pathogenicity. Previous studies have suggested that the presence of integron-encoded AMR genes is associated with differences in virulence factor presence elsewhere in the genome. However, the association of virulence factor genes with class I integron-containing *Salmonella* is still unknown. The objective of the current project was to screen the whole genome sequences of *Salmonella* Typhimurium isolates with AMR-containing class I integrons for virulence factors, and to compare virulence factor presence between isolates with and without integrons. Thirty-three *Salmonella* Typhimurium isolates (bovine source) were donated from laboratory repositories across the United States and confirmed to be serovar Typhimurium via traditional slide agglutination. PCR and gel electrophoresis with established protocols were used to identify integrons. DNA was isolated for whole genome sequencing on an Illumina MiSeq using a commercial extraction kit. Denovo assembly algorithms were used to reconstruct each genome in SPAdes through a Geneious Prime interface. PCR and gel electrophoresis identified class I integrons in 16 isolates. Preliminary genome analysis revealed that *Salmonella* Typhimurium with class I integrons contained unique variants of type III secretion system genes (*spv*) and fimbriae genes (*pef*) that were not present in isolates lacking integrons. Type III secretion systems aid *Salmonella* in sensing and invading into lymphocytes and fimbriae facilitate attachment to host cells. The results from this study can be applied to predict the pathogenicity of *Salmonella* Typhimurium isolates based on the presence of integron-associated AMR genes, and identify antimicrobial resistant isolates that may pose a disease risk to cattle and people.

DVM/PhD Student / Clinical Sciences

## O-23. Analysis of the 2017-2019 ACVECC VetCOT Trauma Initiative Annual Report

*Jessica I Rutten, Kelly E Hall*

The ACVECC-Veterinary Committee on Trauma (VetCOT) Registry is a multi-institutional data collection effort, geared towards standardizing and collating clinically useful data describing small animal trauma. Few multi-center data registries exist in veterinary medicine due to the challenge of data consolidation among hospitals, yet their importance is monumental and their uses are expansive. Multi-institutional data registries like the VetCOT Registry can serve to enhance trauma patient care, promote research collaborations, expand and formalize education on veterinary trauma, and enhance the visibility of veterinary specialty colleges. Trauma is a leading cause of death in dogs across age groups. Novel, epidemiological information may be clinically useful in improving mortality rates, as access to collated data on veterinary patient trauma provides useful information for a multitude of prospective epidemiological studies. Utilizing the web-based data capture system, REDCap, data were recorded by 30 veterinary trauma centers across the world, capturing over 40,000 cases since its establishment in 2013. The registry has since been revised and was summarized in 2017. This report summarizes cases presenting to their trauma centers between April 2017 and December 2019. **Funding:** Summer Scholars Award, NIH

DVM Student / Biomedical Sciences

## O-24. A Systematic Review of Environmental Sustainability In Veterinary Practice

*Maria K Koytcheva, Leah K Sauerwein, Tracy L Webb, Stacey A Baumgarn, Sadie A Skeels, Colleen G Duncan*

Upon acceptance to the veterinary profession, one recites an oath swearing to protect animal health and welfare, conserve animal resources, and promote public health. Consequently, veterinary professionals must responsibly implement sustainable changes in their daily practice to mitigate climate change, an indisputable source of negative impacts on both animal and human health. Since the medical field has proactively taken the first steps in conducting a literature review regarding environmental sustainability in the context of human hospitals, the objective of this literature search is to replicate the methods of this review through the lens of veterinary medicine. The goal of a review of this magnitude is to outline concise, achievable, eco-friendly changes to the way veterinarians practice medicine. The results of this search illustrate the drastic void in experimentally derived, evidence-based clinical guidelines pertaining to environmental sustainability in all forms of veterinary practice. Given this absence, comparative recommendations were drawn from empirical research in engineering, human hospitals, and behavior change and integrated into an online continuing education platform for veterinarians and associated staff. Critical next steps to fill this literature gap include integrating climate change impacts on animal health in the veterinary education curriculum, establishing an evidence-based sustainability certification for clinics, and expanding the scope of research on the veterinarian's role in mitigating climate change. As crucial pillar of one health professionals, veterinarians declare to serve animals, humans, and their environment; thus, this pillar cannot fall behind the curve driven by the force of climate change. **Funding:** Colorado State University One Health Institute

DVM Student / Microbiology, Immunology and Pathology

## O-25. Feverfew: cheerful foliage and source of an anti-cancer compound

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

Parthenolide (PTL) is a secondary metabolite of the feverfew plant with anti-cancer properties. In screening 31 cancer cell lines of various types in dogs, we have identified many that are sensitive to PTL. Two deadly cancer types—histiocytic sarcoma and hemangiosarcoma—are relatively more common in some breeds of dogs than they are in humans, and therefore, dogs can be used as a naturally occurring large animal model of these rare cancers to improve treatment outcomes in both dogs and humans. In our work so far, we have demonstrated that PTL-mediated NF-kB inhibition and alteration of cellular redox balance contribute to selective cell death in canine cancer cells, while sparing normal peripheral blood mononuclear cells (PBMCs) from harmful effects of the drug. Pre-treatment with the antioxidant N-acetylcysteine (NAC) mitigates PTL-mediated cell death to varying degrees in different cell lines. Preliminary data from patients with diffuse B cell lymphoma (DLBCL) show that primary cells exhibit reactive oxygen species (ROS) generation and experience significant cell death following PTL therapy, consistent with our observations in cell lines. Work in progress will determine whether NF-kB activation is increased in preserved tissues from canine patients, which would demonstrate a selective target for PTL therapy in >150 tumor samples. Additionally, we are working on an assay that will help identify ideal combination therapeutics with PTL and are creating a mouse model of disseminated HS to study PTL's effects in a living animal. **Funding:** Morris Animal Foundation Postdoctoral Fellowship

Post-doctoral Fellow / Clinical Sciences

## O-26. HER2/*erbB2* mutation, expression, and activation status in canine thyroid carcinoma

*Samantha Schlemmer, Rupa Idate, Dan Regan, Dawn Duval, and Douglas Thamm*

Human epidermal growth factor receptor 2 (HER/*erbB2*) mutation and/or overexpression has been reported in several human carcinomas and can be associated with a poor prognosis. Recently, a missense mutation (V659E) was detected in *erbB2* on whole exome sequencing of a canine thyroid carcinoma (CTC) cell line. If this mutation can be detected in dogs with thyroid carcinoma, they may benefit from HER2-tageted therapy based on the high incidence of metastatic disease and equivocal response to chemotherapy for this disease. To determine the mutation rate and receptor expression in canines, we are performing Sanger sequencing and immunohistochemistry (IHC) for HER2 and phospho-HER2 on 24 primary CTCs and 1 cell line. Fisher's exact test and analysis of variance will be used to assess associations of clinicopathologic features and Kaplan-Meier curves with log-rank test will be prepared for overall survival and disease-free interval. Preliminary results confirm the presence of V695E mutation and phospho-HER2 immunolabeling in the cell line. Additionally, HER2 inhibitor drug dose-response on the cell line suggests sensitivity to lapatinib. While patient tumor specimens are still pending, the initial findings with the cell line implies that a subset of dogs with CTC may benefit from treatment with HER2 inhibitors.

Post-doctoral Fellow / Clinical Sciences

## O-27. Inhibition of human sialidases disrupts dengue virus replication

*Laura A. St Clair, Elena Lian, Rebekah C. Gullberg, and Rushika Perera*

The human sialidase enzymes (or neuraminidases) catalyze the removal of α-glycosidically linked sialic acid residues from glycoconjugates, including glycoproteins and glycolipids. Sialic acid residues are thought to play vital roles in cellular signaling. Through their physiochemical effect on glycoconjugates, sialic acid residues are involved in conformational changes that determine the active/inactive state of glycoproteins. Sialic acid residues are also important binding recognition and masking sites for cellular processes. There are four known human neuraminidases (NEU1-4), each having specific subcellular localizations and substrate preferences. In previous studies, NEU1-4 activity has been shown to be increased during infection with dengue virus serotype 2 (DENV2). In both *in vitro* and *in vivo* models, it was shown that the dengue NS1 protein increased NEU1-4 activity, resulting in an increase in free sialic acid. This increased activity was also shown to be linked to the events resulting in endothelial hyperpermeability/vascular leakage, a hallmark of severe dengue disease. However, the role for this increase in NEU1-4 activity was not understood. In an siRNA screen of enzymes involved in the sphingolipid metabolic pathway, we uncovered that NEU1-4 are vital for DENV2 infectious virus release and replication. Here we will present data that sheds light on the roles of NEU1-4 during the DENV lifecycle.

Graduate Student / Microbiology, Immunology and Pathology

## O-28. Development of an experimental model of bone marrow lesions using the rat femoral condyle

*Holly L. Stewart, Katie J. Sikes, Natalie Serkova, Jeremiah T. Easley, Christopher E. Kawcak*

Bone marrow lesions (BMLs) are well-reported to cause pain and result in morbidity across species; and appear to be early indicators of inflammation that predispose to degenerative changes within the joint. The investigators have previously developed a reproducible experimental model for BMLs using the ovine stifle (knee) joint, but have not evaluated the applicability of this model across other species. The purpose of this study was to develop an experimental model for BMLs using the rodent stifle and to understand the clinical manifestations associated with BML generation. Four skeletally-mature, male Sprague Dawley rats were used for this pilot study. BMLs were bilaterally induced using a 22-gauge needle penetrating through the articular cartilage and into trabecular bone. Magnetic resonance imaging (9.4 T) was performed pre-operatively, and at 3, 14, 45 and 90 days post-operatively. Gait analysis was performed weekly. Animals were humanely euthanized at 14 (N = 1), 45 (N = 1) or 90 (N = 2) days post-operatively. BMLs were reliably generated in the medial femoral condyle of all rats within 14 days of surgery and became a more defined are of signal intensity over time. Gait analysis revealed a significant (P = 0.01) difference in the stride velocity in the forelimbs compared to baseline and compared to the hind limbs over time. Taken together, this study confirms an experimental model for BMLs in the rodent stifle is possible, and BMLs may result in compensation in non-affected limbs.

Post-doctoral Fellow / Clinical Sciences

## O-29. Development of Full and Partial Models of Mid-Substance ACL Rupture

*Ariel Timkovich, Joseph Sanford, Kimberli Fernandez, David Joseph Burnett, Emma Hurley, Maryam Afzali, Tammy Haut Donahue, Katie Sikes, Kelly Santangelo*

The anterior cruciate ligament (ACL) is the most commonly injured knee ligament. Surgical reconstruction is the gold standard for treatment of ACL ruptures but results in 20-50% of patients developing post-traumatic osteoarthritis (PTOA). Given the relationship between these two etiologies, there is need to understand the development of PTOA post-ACL rupture to produce novel treatment strategies. *The goal of this project was to develop both partial and full models of mid-substance ACL rupture in male and female mice using non-invasive mechanical methods (tibial displacement) and characterize early PTOA changes following full ACL rupture with our model*. Mice were anesthetized and placed in a custom bioreactor and received tibial displacement at either 1.6 or 2.0mm (partial or full rupture, respectively). Mice were either euthanized immediately post-injury to determine success rates or 14 days post-injury to evaluate early PTOA progression. Mobility and behavior changes were longitudinally tracked using AnyMaze cage monitoring software. Following injury, mice exhibited altered mobility and potential pain response 1-day post; they returned to pre-surgery movement levels by 14 days. Our model demonstrated high efficacy inciting both full and partial ACL ruptures in both male and female mice. Loads obtained during these protocols (1-2.5N) were less than previously published reports (3-12N), which may contribute to the mid-substance ACL ruptures achieved with our model (versus avulsion fractures in other models). Our developed partial and full ACL rupture models will be utilized as a clinically relevant model for testing potential therapeutics and to further our understanding of PTOA following ACL rupture.

Graduate Student / Microbiology, Immunology and Pathology

## O-30. Antibiotic neurotoxicity and links to early neurodegeneration

*Zaria D. Vick, Timothy E. Hoffman, Tanner Murphy, Marie E. Legare, 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, equine, rabbit, and mouse schemes via physiologically based pharmacokinetic models. Further, to aid in our understanding of this neurotoxicity, we have assessed a murine primary cell model with the treatment of metronidazole. In a cell viability assay, our results concluded metronidazole does not inherently cause neuronal cell death. However, with the establishment of a vulnerable model, we then see decreases in cholesterol markers resulting in glial dysfunction. Using these exposed cells in combination with our computational models, liquid chromatography and mass spectrometry (LC/MS), reverse transcription-polymerase chain reaction (RT-PCR), and cholesterol assay works, we have a well-rounded means of investigation. These results will add to human and veterinary medicine by enabling us to better understand this proposed mechanism of neurotoxicity for metronidazole. The ultimate goal of our work is to understand metronidazole as a neurotoxin and its conceivable link to neurodegenerative diseases.

Graduate Student / Environmental and Radiological Health Sciences

## O-31. Exercised myoblast-derived exosomes enhance myogenesis: a promising cell-free therapy for healthy muscle aging

*Katherine Williams, Laura Chubb, Ruth Rose, Karyn Hamilton, Nicole Ehrhart*

Aging is characterized by an accumulation of cellular damage and impaired physiological function over time, which manifests as chronic age-related diseases including cancer, cardiovascular disease, and osteoporosis. Sarcopenia, a progressive decline in muscle mass and function, is one such age-related disease that is linked to frailty, increased hospitalizations, and increased mortality. While there are currently no therapeutics targeting sarcopenia, resistance exercise has been demonstrated as most the effective strategy to slow the progression of this disease. However, the numerous chronic diseases that accompany aging make it difficult for many people to achieve minimum recommended levels of exercise. It is therefore critical to develop therapeutics that promote healthy muscle maintenance later into life. We are investigating the potential of exercised C2C12 myoblast-derived exosomes (EMDEs) to promote myogenesis and reduce signs of myoblast aging, with the long-term goal of attenuating age-related muscle dysfunction. C2C12 myoblasts were mechanically stimulated using a FlexCell bioreactor to simulate exercise *ex vivo*, and the number and function of exosomes derived from these cells was investigated. *Ex vivo* exercise of myoblasts resulted in increased exosome production, and treatment of myoblasts with EMDEs resulted in increased proliferation and increased myotube formation compared to treatment with static exosomes. We conclude that EMDEs enhance myogenesis *in vitro*. Future work aims to investigate the functional cargo of EMDEs, with the hypothesis that microRNAs are responsible for the impact of EMDEs on myogenesis. Additionally, we will evaluate the ability of EMDEs to promote healthy muscle function in an aged mouse model. **Funding:** NIH T32 Training Grant, CVMBS, Lab of Comparative Musculoskeletal Oncology and Traumatology

DVM/PhD Student / Clinical Sciences

## O-32. Economic impacts of Restricted antimicrobial use implemented under Veterinary Feed Directive in the United States: A Systematic Review on Swine production systems

*Jessica Wilson, Rebecca Hill, Sadie Skeels, Sangeeta Rao*

Antimicrobials are commonly used as feed additives to improve growth, performance and reduce morbidity and mortality in livestock including swine. However, long-term antimicrobial use, even at sub-therapeutic levels, raises concern for increased resistance that may decrease the efficacy of clinically necessary future treatments for both animals and humans. In 2017, the US Food and Drug Administration implemented the Veterinary Feed Directive (VFD) requiring medicated feeds to be distributed and regulated by veterinarians, thus prohibiting the use of antibiotics for production efficiency. The goal of this study is to estimate potential economic impacts of the VFD on the swine industry in the United States by identifying changes to production processes, clinical outcomes, and environmental factors associated with restricted antimicrobial use in swine production.

A systematic review was conducted using the four databases: PubMed, CAB, Medline, and AgEconSearch, as well as searches conducted through google scholar. There were 3,098 papers identified for screening, of which 64 papers met study criteria and were included for full-text evaluation. Common clinical outcomes obtained from those papers included morbidity and mortality, while economic outcomes included cost of production and healthcare, increased market prices, use of antimicrobials and overall weight gain or loss. At this time, a majority of the results are underway and being analyzed. Overall, there are economic gains and losses of implementing the VFD on hog farms that extend beyond the direct effects to the swine industry, and it is important to understand the public health significance of restricting antimicrobial use in food animals.

Graduate Student / Clinical Sciences

## O-33. Mosquito Bloodmeals as an Alternative Source for Pathogen Surveillance in Central America

*Delaney Worthington, Bekah McMinn, and Gregory Ebel*

Xenosurveillance is a method that utilizes mosquito bloodmeals to sample for pathogens in the place of traditional sampling techniques which can be expensive, invasive, and require additional resources that are not easily accessible in resource-poor areas. This study was performed to determine if *Aedes aegypti* and *Culex quinquefasciatus* mosquitos are viable candidates for xenosurveillance in order to detect non-vector borne pathogens in bloodmeals collected from mosquitos in Central America. Mosquitos were fed serial dilutions of a bloodmeal containing HIV, SARS-CoV-2, POWV, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Individual bloodmeals from each mosquito were then collected onto Whatman FTA cards at 6, 12, and 24 hours post-feeding. Then, nucleic acids were extracted and real-time reverse transcription PCR was performed to determine whether RNA from each of the five non-vector borne pathogens could be detected. Both *Aedes aegypti* and *Culex quinquefasciatus* mosquitos are viable candidates for xenosurveillance. This study provides clear evidence that pathogens can be detected at clinically relevant levels in bloodmeals from these species of mosquitos. Our results show this technique can be utilized to detect pathogens in a non-invasive manner in low-resource areas. This technique will be utilized as part of a greater xenosurveillance project in the Trifinio Region of Guatemala as a detection method for non-vector borne pathogens affecting populations in the area. **Funding:** OneHealth Institute

Graduate Student / Microbiology, Immunology and Pathology

## O-34. The effect of deep tissue heating on cervical pain and dysfunction in horses

*Samantha D Parkinson, Gustavo M Zanotto, Mikaela D Maldonado, Melissa R King, and Kevin K Haussler*

Neck pain and stiffness is becoming more commonly recognized in horses and is often treated using multimodal pharmaceutical and rehabilitation approaches. In humans, deep tissue heating has been shown to reduce pain and increase flexibility. The objective of this project was to determine the effects of diathermy as a deep tissue heating modality on neck pain and stiffness and associated forelimb function in horses. Twenty-two horses with neck pain and variable degrees of undefined forelimb lameness were randomly assigned to treatment and control groups. Cervical pain, stiffness, and muscle hypertonicity were assessed by manual palpation. Forelimb lameness was assessed using an inertial sensor system. Forelimb postural stability was evaluated using a portable media device with built-in inertial sensing components. All outcome parameters were evaluated once weekly for 3 weeks. The treatment group received active diathermy application to the lower cervical region (C3-C6), twice weekly for a total of 6 treatments, while the control group received a sham (inactive) treatment. Data was analyzed using a mixed model that was fit separately for each response variable. Using manufacturer recommended treatment parameters, there were no significant differences noted over time or between treatment groups for any outcome parameter evaluated. While cervical pain and stiffness decreased by week 3 in both treatment and control groups, the improvement was not significant. Chronic forelimb lameness could have prevented resolution of compensatory mechanisms within the lower cervical region. It is likely that diathermy may need to be combined with other treatment modalities such as stretching or chiropractic to significantly reduce neck pain and stiffness. Diathermy can be used safely in horses as a deep tissue heating modality; however, specific clinical applications and effective treatment parameters need further evaluation. **Funding:** INDIBA Animal Health, Barcelona, Spain

Resident / Clinical Sciences

## O-35. Clinical findings in dogs treated with oral cannabidiol (CBD) versus topical prednisolone acetate 1% ophthalmic suspension for experimentally induced uveitis

*Emma L Davey, Hannah Terhaar, Hannah Patterson, Ann Hess, Stephanie McGrath, Michael R Lappin, and Michala de Linde Henriksen*

The purpose of this study was to determine if administration of cannabidiol (CBD) oil can be used as an anti-inflammatory drug for anterior uveitis by evaluating clinical signs in dogs with experimentally induced uveitis. Sixteen research beagles underwent complete ophthalmic examinations including measurement of intraocular pressure (IOP – measured in mmHg), and aqueous flare (scale from 0-3+), as well as fluorescein stain for corneal ulceration (positive versus negative). Experimentally induced uveitis was achieved by performing aqueous paracentesis. Treatment was given and clinical signs were evaluated for three days. Dogs were randomly assigned to four different treatment groups: Group 1 - CBD oil 10mg/kg PO BID, Group 2 - topical prednisolone acetate 1% ophthalmic suspension OU, Group 3 - CBD oil 10mg/kg PO BID + topical prednisolone acetate 1% ophthalmic suspension OU, Group 4 - no treatment. Statistical analysis was performed using two-way repeated measures ANOVA. Preliminary results for this study were as follows: Mean IOP Day 3: Group 1:11.25mmHg, Group 2:14.63mmHg, Group 3:14.25mmHg, Group 4:13.75mmHg. Mean aqueous flare at Day 3 was as follows: Group 1:0.375, Group 2:0.25, Group 3:0.125, and Group 4:0.6875. No dogs (0%) developed corneal ulcerations after aqueous paracentesis in Group 1, 50% (4/8 eyes) in Group 2, 37.5% (3/8 eyes) in Group 3, and 25% (2/8 eyes) in Group 4. CBD as an anti-inflammatory treatment for experimentally induced uveitis seems to have a positive effect on clinical signs such as aqueous flare and no effect on IOP. Additionally, dogs on topical prednisolone acetate 1% appear to be predisposed to corneal ulcerations whereas CBD-treated eyes do not have the same predisposition. No funding to declare.

DVM Student / Clinical Sciences

## O-36. Systemic tissue plasminogen activator for thrombolysis in 15 dogs and 5 cats

*Devon Diaz, Brian Scansen, Zachary Lake, Sarah Shropshire, Christopher Orton, and Julien Guillaumin*

Purpose: To describe systemic administration of tissue plasminogen activator (TPA) in dogs and cats.

Materials/methods: Single academic center retrospective study. Electronic medical records at Colorado State University were searched for TPA use between 2010 and 2020. Dogs or cats were included if TPA was injected into an intravenous catheter in an attempt to thrombolyse a distant known/suspected thrombus. Data collected included demographics, final diagnosis, TPA dose, location of known/suspected thrombus, clinicopathologic and imaging data, response to TPA injection, complications and outcome. Descriptive statistics were used. Normality was assessed by the Shapiro-Wilk test. Data are reported as mean (standard deviation) or median (range) as appropriate.

Results: Twenty cases (15 dogs and 5 cats), representing 32 injections were included. Mean age was 5.8 years (5.9). Median body weight was 11 kg (4-43). Known/suspected thrombus included pulmonary thromboembolism (n=6), thrombus after tricuspid valve surgery (n=5), canine aortic thrombus (n=3), cranial vena cava thrombus (n=2), feline aortic thromboembolism (n=2), renal artery thrombus (n=1) and femoral and iliac veins thrombus (n=1). Various primary diseases were represented. Median time between diagnosis/suspicion of thrombus and TPA injection was 11.7 hours (2-150). Fifteen dogs and 4 cats received a single dose. Total dose was 1.1 mg/kg (0.2-3.2). Individual dose was 0.6 mg/kg (0.1-1.5). Clinical improvement and/or clot resolution occurred in 75% of cases. Complications included acute kidney injury in 4 cats and 1 episode of bruising. Discharge rate was 70%.

Conclusion: Systemic TPA injection seems to be effective and safe in dogs and requires more investigation in cats.

Resident / Clinical Sciences

## O-37. Identifying risk factors and determining incidence of clinical signs in feline cognitive dysfunction syndrome

*Jade Frank, Brittany MacQuiddy, Doreen Martinez, Colleen Duncan, Julie Moreno, Stephanie McGrath*

The Senior Pet Studies Team was developed to identify prevalence and associated risk factors of feline cognitive dysfunction (FCD) in aging cats. FCD is similar to human Alzheimer's disease, causing clinical signs such as behavioral changes, restlessness and cognitive decline. Little research has been performed assessing potential risk factors that may contribute to cognitive decline and diagnosis is based on exclusion of comorbidities. To assess prevalence of FCD in the aging cat population, a medical record search from 2002-2019, including cats that presented to the Veterinary Teaching Hospital (VTH) at CSU aged 7- 20 years, was performed. Cats with underlying conditions were considered negative for FCD. Cats with signs of FCD, in the absence of comorbidities, were considered positive for FCD. The goal was to identify cats with a presumptive diagnosis of FCD; 86 cats were diagnosed with FCD, making the prevalence in this population approximately 15%. To further characterize FCD, a survey was distributed to owners of cats examined at the VTH. The goal of the survey was to identify the incidence of clinical signs in patients with FCD and associated environmental and social risk factors. Preliminary results yielded 870 responses and 81 of these cats were presumed positive (PP) based on clinical signs and age. Risk factor analysis concluded that 12.3% of PP cats were overweight, 83.9% of PP cats were from homes with no children under the age of 12 and 90% of PP cats were housed indoors. Although no clear risk factors were positively identified in this study future research could help to clarify correlations between environment, social and health risk factors which contribute to FCD. Funding for this study was provided by the Department of the Vice President for Research. **Funding:** Vice President for Research, PRSE Fellowship

DVM Student / Clinical Sciences

## O-38. Effect of a commercially available synbiotic on mycophenolate associated diarrhea

*Kenjiro Fukushima, Hailey Davis, Jason Gagne, Michael Lappin*

mofetil (MMF) is a widely used and effective immunosuppressant in dogs, but gastrointestinal (GI) toxicity is common. Based on a retrospective study in our laboratory, 24.4% of 135 client owned dogs experienced GI toxicity (median dose 17.5 mg/kg/day, median time to onset 10 days). One of the possible mechanisms for MMF induced GI toxicity is GI dysbiosis, but we were unable to document that syndrome in a previous experiment in healthy beagles. However, in that experiment, 9 of 9 beagles developed a fecal score of > 3 over the course of the study. The purpose of this study was to supplement 9 additional beagles that were on the same facility diet as the dogs in the previous experiment with the synbiotic (FortiFlora® SA, Nestlé Purina PetCare) while being administered MMF at 10 mg/kg, PO, twice daily. Individuals masked to the objectives of the study applied a score to each dog's feces daily, using a standardized fecal scoring system. In this system, fecal scores of 4 – 7 are considered abnormal. In the 18-day study, the dogs administered MMF alone frequently had a fecal score of 4 (69 of 153 samples) or 5 (19 of 153 samples) with abnormal stools occurring as early as Day 1. In contrast, the dogs administered MMF and supplemented with the synbiotic only had a fecal score of 4 detected twice in 2 separate dogs (total samples = 157). The frequency of fecal scores of > 3 ($P < 0.0001$) or > 4 ($P < 0.0001$) were statistically different between groups. These results suggest that the mechanism of diarrhea associated with MMF may involve effects on the fecal microbiome that were not detected with the methods used in our previous experiment and support the use of this synbiotic when MMF is used clinically. **Funding:** Nestle Purina PetCare

Resident / Clinical Sciences

## O-39. Evaluation of outcome associated with feline trauma: a Veterinary Committee on Trauma (VetCOT) registry study

*Carly W Gregory, Akaterina M Davros, Darren Cockrell, Kelly E Hall*

*Purpose*: To evaluate outcome (survival to discharge) among trauma types (blunt, penetrating, both) in cats. A secondary objective is to evaluate how other parameters [abdominal fluid score (AFS), animal trauma triage (ATT) score, surgery, glide sign on thoracic point of care ultrasound (T-POCUS), pleural effusion, modified Glasgow coma score (mGCS)] differ between trauma types.

*Materials/methods*: Feline trauma patient data entered into the Veterinary Committee on Trauma (VetCOT) trauma registry by Veterinary Trauma Centers (VTCs) between April 1, 2017 and December 31, 2019 were evaluated. Data entry personnel at VTCs use a standardized data collection method that is entered into a web-based data repository [(Research Electronic Data Capture (REDCap)]. Data collected included patient demographics, trauma type (blunt, penetrating, both), AFS, ATT score, surgical intervention, presence of glide sign on T-POCUS, pleural effusion on T-POCUS, mGCS, and outcome (survival to discharge). A Pearson's Chi-square tests was used to compare variable proportions to trauma type groups and significance was set to $p<0.05$.

*Results*: Data from 3,895 cats were collected over the 30-month period. The most common trauma type was blunt (57.5%), followed by penetrating (35%) and then combined blunt and penetrating trauma (7.4%). If a surgical procedure was performed (33%), the procedure was more commonly performed in the Emergency Room (ER) for penetrating injury (82%), and the Operating Room (OR) for blunt injury (78%). Cats with penetrating trauma had a 90.3% survival rate, blunt trauma had a 79.6% survival rate, and combined (blunt and penetrating) trauma had an overall 68.3% survival rate.

*Conclusions*: All categories of feline trauma have a good prognosis for survival to discharge, with the highest survival rate associated with penetrating trauma. Of the cats requiring a surgical procedure for their injuries, the hospital location of surgery differed between trauma types.

Resident / Clinical Sciences

## O-40. The effect of chiropractic treatment on lameness and concurrent axial skeleton pain and stiffness in horses

*Mikaela D. Maldonado, Samantha D. Parkinson, and Kevin K. Haussler*

Chiropractic care is a common treatment modality used in equine practice to treat back pain and stiffness. Clinically important interactions occur between axial and appendicular regions with regards to poor performance and lameness. The objective of this study was to evaluate the effect of chiropractic treatment on limb lameness and concurrent axial skeleton pain and dysfunction. Our hypothesis was chiropractic care would reduce lameness and spinal pain and stiffness. Twenty horses with grade 1-3/5 lameness (AAEP scale) within at least one limb were randomly assigned to treatment and control groups. Subjective and objective lameness examinations and scores for the quantity and severity of spinal stiffness, muscle hypertonicity, and mechanical nociceptive thresholds were performed on days 0, 14, and 28 by a blinded examiner. Chiropractic treatment was applied on days 0, 7, 14, and 21. Data was analyzed by a mixed model fit separately for each response variable ($p<0.05$). No significant change was observed in measures of fore or hind limb lameness. Spinal pain scores significantly decreased in the number of affected cervical vertebral levels ($p =0.006$) and severity within the cervical ($p = 0.017$) and lumbar ($p = 0.047$) regions. Spinal pain severity ($p = 0.041$) was the only variable to decrease significantly when examined independently. Chiropractic care had no significant effect on lameness in this population of horses; however, a reduction in axial skeleton pain did occur. Further studies are needed to evaluate the effect of chiropractic care in horses with known causes of limb lameness. **Funding:** College Research Council: Racing Commission

DVM Student / Clinical Sciences

## O-41. Dried blood spot-based metabolomics reveals rice bran supplementation modulates weaning infant nutrition and growth in Mali

*Brigitte A. Pfluger, Annika M. Weber, Hillary V. Smith, Hend Ibrahim, Lassina Doumbia, Abdoulaye Bore, Alima Cissoko, Seydou Douyon, Karim Kone, Lansana Sangare, Ababacar Maiga, Ousmane Koita, Kelli Goodman, Annie Evans, Kirk Pappan, Jason Kinchen, and Elizabeth P. Ryan*

Rice bran supplementation provides key macronutrients and micronutrients important for gastrointestinal health, immunity, and infant growth and development in the first 1,000 days of life, a period when poor nutrition can lead to irreversible outcomes. Yet these nutrients are frequently lost during milling, and rice bran is often used in animal feed or discarded. This study investigated the effects of rice bran supplementation on length-for-age (LAZ) and weight-for-age (WAZ) z-scores and metabolomic profiles in weaning infants from 6 to 12 months old in Mali. Healthy infants (n=50) were randomized to a control or rice bran group, which received daily supplementation at monthly dose increases (1-5 grams/day). Dried blood spots (DBS) and anthropometric measurements were collected monthly. Two-way repeated measures ANOVA group comparisons were made at each time point. Diet and time interactions were analyzed at time points 7 to 12 months. Compared to the control, rice bran-fed-infants showed significant improvements in LAZ over the study period and WAZ from 6-8 months and again from 8-12 months. DBS analysis showed changes in metabolites especially at months 9 and 10 compared to baseline. Rice bran-fed infants had significant increases in hypoxanthine, xanthine, and allantoin (month 9) and higher scaled relative abundances of reduced glutathione (months 7-11), nicotinamide (months 7-11), NAD+ (months 10-12), and trigonelline (months 9-12). Both groups had significant increases in DBS threonate (months 8-12), yet rice bran-fed infants had higher fold differences at 7 and 10 months. The fatty acids valerate and caproate significantly increased in rice bran fed-infants (all timepoints) as did DBS for lipid metabolites choline (month 9) and 12, 13-DiHOME (months 10-12). Data analysis of DBS non-targeted metabolomes in infants points to the potential for rice bran to positively modulate metabolomic profiles when acting as a supplement during infant weaning, thus improving infant nutrition, growth, and development. **Funding:** Funding for this study was provided by the Grand Challenges Explorations in Global Heath award from the Bill and Melinda Gates Foundation OPP1043255. Metabolon, Inc. in Durham, NC analyzed DBS to determine metabolite profiles.

Graduate Student / Environmental and Radiological Health Sciences

## O-42. Evaluation of 18F-fluorodeoxyglucose positron emission tomography-computed tomography for staging of canine insulinoma

*Raelyn Walczak, Lukas Kawalilak, and Lynn Griffin*

Canine insulinomas are uncommon functional pancreatic neuroendocrine tumors with a well-recognized propensity to metastasize despite lack of consistent malignant histopathologic features. Diagnostic imaging can aid in staging and surgical planning; however, identification of these small tumors can be unpredictable across modalities. Neuroendocrine tumors often do not have increased avidity on $^{18}$F-fluoro-deoxyglucose positron emission tomography-computed tomography ($^{18}$F-FDG PET-CT) imaging in humans, though this modality has not been investigated for staging dogs with insulinomas. The intent of this prospective, descriptive pilot study was to investigate the utility of $^{18}$F-FDG PET-CT for identification of canine insulinoma lesions alone and in combination with computed tomographic angiography (CTA). Three patients met the inclusion criteria. Patients underwent both CTA and $^{18}$F-FDG PET-CT imaging and had exploratory laparotomy within 1-2 days following imaging. All patients had histologically confirmed primary or metastatic insulinoma lesions. In dog 1, hepatic and regional lymph node insulinoma metastases displayed mild increased avidity (SUVmax 2.33 and 2.79, respectively). In dog 2, a primary pancreatic insulinoma showed minimally increased avidity (SUVmax 2.16). In dog 3, a primary pancreatic lesion had similar avidity (SUVmax 1.54) to adjacent pancreatic parenchyma and was poorly evident on $^{18}$F-FDG PET-CT. These lesions were also apparent on, and in some instances better documented, with CTA, displaying variable attenuation and contrast enhancement patterns. Metastatic hepatic and regional lymph node insulinoma lesions not found with either imaging modality were discovered in patient 2 at surgery. Primary and metastatic canine insulinoma lesions inconsistently exhibited increased avidity on $^{18}$F-FDG PET-CT. These findings are likely attributable to small lesion size and to the variable glucose metabolism of these unconventional tumors. No advantage of $^{18}$F-FDG PET-CT for use in staging of canine insulinomas was determined in this small case series.

Resident / Environmental and Radiological Health Sciences

## 1. Molecular adaptation of SARS-CoV-2 in nonhuman mammalian hosts

*Laura A Bashor, Erick B Gagne, Angela M Bosco-Lauth, Mark D Stenglein and Sue VandeWoude*

SARS-CoV-2 is a virus that originated and evolved in animal hosts. After jumping from probable bat reservoir hosts in late 2019, it has adapted to be exceptionally transmissible in humans. The virus is capable of infecting a broad range of animal hosts, and adapts rapidly to new host environments. Golden hamsters (*Mesocricetus auratus*) and ferrets (*Mustela putorius*) are susceptible to SARS-CoV-2, and are widely used as animal models to aid the accelerated development and testing of vaccines and other therapeutics. Dogs (*Canis lupus familiaris*) are also susceptible. Serial passage of SARS-CoV-2 in mice has been shown to result in increasingly lethal mouse-adapted viral isolates. Our laboratory and others have also found that mutations arise reproducibly when the virus is passaged in tissue culture for use in experimental research. The rapid mutation and host-specific adaptation of SARS-CoV-2 raises important questions about both the potential for companion animals to become disease reservoirs, and the ability of an animal model to authentically reflect human disease for the purposes of vaccine development and validation. We quantified molecular changes in SARS-CoV-2 viral populations following infection in hamsters, ferrets and dogs. We generated full SARS-CoV-2 genomes with high depth-of-coverage and identified viral variants and genomic signatures of selection. Here I will report our methodology and preliminary assessment of SARS-CoV-2 molecular adaptation. We identified multiple amino acid changes unique to each species, in addition to a number of changes that represent reversions from mutations acquired by SARS-CoV-2 in tissue culture. The results of this research will provide insight into the cross-species transmission dynamics of SARS-CoV-2 in translational animal models and companion animal hosts.

Graduate Student / Microbiology, Immunology and Pathology

## 2. Elucidating the Endogenous Opioid Circuit in the Retina

*Casey-Tyler Berezin, Zephyr Lenninger, Nikolas Bergum, Jozsef Vigh*

Long-term opioid treatment remains the standard of care for chronic pain patients, despite the known disruptive effects on circadian and sleep cycles. Intrinsically photosensitive retinal ganglion cells (ipRGCs), which send direct photic signals to brain regions associated with sleep/wake to entrain circadian rhythms to light-dark cycles in the environment (i.e. photoentrainment), are implicated in these effects. Previous work in our lab has shown that ipRGCs express μ-opioid receptors (MORs), the preferred receptor for the endogenous opioid β-endorphin and exogenous opioids like morphine, and that ipRGC firing activity is modulated by opioid binding. In addition, we have shown that β-endorphin is expressed in the retina, and behavioral studies using a mouse line where MORs are specifically knocked-out in ipRGCs (McKO) suggest that modulation of these receptors by endogenous opioids are critical for normal sleep-wake cycles. In this study, we will expand upon these findings to elucidate the molecular mechanisms that underlie the modulation of ipRGC activity by endogenous opioids. We will assess the expression of β-endorphin and MOR mRNA by RNAscope fluorescence *in situ* hybridization, as well as the expression of those proteins by immunohistochemistry. We expect that β-endorphin expression will be circadian (set by time of day rather than light/dark cues), just as it is in other brain regions.

Graduate Student / Biomedical Sciences

## 3. Novel route of morphine-induced sleep disorders

*Nikolas Bergum, Casey-Tyler Berezin, Sierra Curdts, and Jozsef Vigh*

Despite their strong addictive potential opioids remain the most widely used drugs for treating moderate to severe pain. While opioids effectively decrease pain acutely, long term opioid use causes sleep disturbance, that in turn triggers hyperalgesia; this positive feedback loop exacerbates pain-related sleep problems. Sleep disruptions that result from chronic opioid use increase the risk of depression and even suicide. Thus, a better mechanistic understanding opioid-induced circadian disruption could minimize the negative side effects associated with long-term opioid use.

Intrinsically photosensitive retinal ganglion cells (ipRGCs) are the sole synchronizers of circadian rhythms to light. Importantly, past research determined that these cells express μ-opioid receptors (the primary target for opioids) that are accessible for systemically administered opioids. To examine the role that ipRGC μ-opioid receptors (MORs) in modulating circadian behavior, we generated a transgenic mouse line (McKO) in which only ipRGCs lack MORs. Then, we implanted mini telemetry transmitters into McKO and control mice to accurately monitor activity over several weeks. Here, we show that McKO mice have altered circadian activity compared to controls, especially in trials with long-term morphine exposure. Following this chronic morphine paradigm, we will assess the performance of these mice in an assay of depression-like behavior. We predict that McKO mice will exhibit less depression-like behavior compared to controls. These findings would expand on past results, causally linking opioid-induced circadian disruptions to the affective mood associated with chronic opioid use. Moreover, these results implicate ipRGC MORs as a potential therapeutic target to combat the negative side effects associated with long-term opioid use.

Graduate Student / Biomedical Sciences

## 4. Anti-Nucleus Pulposus vaccine for the development of Degenerative Disc Disease

*Andres F. Bonilla, Mitchell Page, Ben Gadomski, Katie Sikes, Christian Puttlitz, Lyndah Chow, Steve Dow, Brian Johnstone, Jeremiah T. Easley*

Low back pain affects 80% of the human population with over 40% of cases attributed to degenerative disc disease (DDD). The pathogenesis of DDD is theorized to have an autoimmune component because nucleus pulposus (NP) tissue is considered to be hidden from the immune system. The purpose of this study is to develop a vaccine containing rabbit NP's proteins that would result in DDD. Twelve New Zealand rabbits will be used for this pilot study. Group 1 (n=4) receives disc puncture at L2-L6, and 3 doses of anti-NP vaccine, group 2 (n=4) receives same doses of vaccine anti-NP without disc puncture, and group 3 (n=4) will serve as an untreated control group. Blood collection for ELISA analysis for detection of IgG against NP will be performed pre-operatively, and at 2, 4, 8, and 12 weeks post-operatively. Magnetic resonance imaging and standard radiographs will be performed pre-operatively and at 8- and 12-weeks. Animals will be humanely euthanized at 12 weeks followed by non-destructive kinematics, biochemical and histological analyses. Preliminary results have shown that the application of the vaccine anti-NP is safe and does not cause side effects to the rabbits. We hypothesize that rabbits with NP exposure (Group 2) will develop a robust immune response to the vaccine resulting in DDD. The successful development of the proposed model will positively impact our understanding of the etiopathogenesis of DDD, advance the implementation of basic science immunological research, and provide novel therapies for patients with spinal disease.

Graduate Student / Clinical Sciences

## 5. Carbon-ion Cancer Radiotherapy: Double-Strand DNA Break Distribution and Repair

*Dylan J. Buglewicz[1], Hirokazu Hirakawa[2], Akira Fujimori[2], Takamitsu A Kato[1]**

The sharp high dose Bragg peak of the carbon-ion beam helps it to deliver the highest dosage to the malignant cells while relatively sparing the surrounding normal healthy cells. However, the precise range in which it distributes dosages that significantly induce double-stranded DNA breaks (DSBs) causing biological effects surrounding its Bragg peak remains unknown. We have developed a technique utilizing γ-$H_2AX$ assay allowing us to examine DSB distribution throughout the full beam length in a single system to address DSB complexity, carbon-ion nuclear fragmentation-induced DSBs, as well as, at different time points to address cellular capability for DNA repair at different beam depths. Furthermore, we addressed DSB repair by comparing the differences in survival fractions of cells deficient in either non-homologous end joining (NHEJ) or homologous repair (HR) DSB repair mechanisms at various depths within the beam range to help identify the ranges in which these repairs may be more important.
**Funding:** Colorado State University College Research Council Grant, Japan Ministry of Education, Culture, Sports, Science and Technology (MEXT) Grants-in-Aid for Scientific Research on Innovative Areas, Grant Number (JP15K21745), Dr. Akiko Ueno Radiobiology Research

Graduate Student / Environmental and Radiological Health Sciences

## 6. CAR T cells targeting the checkpoint molecule B7-H3 for treatment of osteosarcoma in dogs

*Jennifer Cao, Lyndah Chow, Jade Kurihara, Jessica Lake, Micheal Verneris, Steven Dow*

Osteosarcoma (OS) is an aggressive bone cancer that mainly affects children and young adults and is also prevalent in large breed dogs. OS has a high rate of metastasis to the lung and for those patients there is poor prognosis and no good treatment option. Although use of CD19 Chimeric Antigen Receptor (CAR) T cell therapy has revolutionized the treatment of advanced stage B cell leukemia and lymphoma, application of CAR T cell therapy has not shown the same clinical success in solid tumors such as sarcomas and carcinomas. A major barrier to the success of solid tumor CAR T cell therapy is the immune suppressive myeloid cells in the tumor microenvironment (TME) which are inherently defective in the standard Nod SCID gamma mouse model used in preclinical trials. **This study investigates the feasibility of spontaneously occurring canine osteosarcoma (OS) for B7-H3 targeted CAR T cell therapy as a translational model for metastatic pediatric OS**. We found that primary canine OS overexpressed B7-H3 while normal liver and spleen had low to no expression of B7-H3. Canine OS cell lines also expressed B7-H3 to various levels with level of expression correlating to Canine B7-H3 CAR T cells activation as measured by secreted interferon gamma.

DVM/PhD Student / Microbiology, Immunology and Pathology

## 7. Comparison of two TLR activating immune stimulants for induction of interferon responses in cats

*Petra Cerna, Steven Dow, William Wheat, Lyndah Chow and Michael R Lappin*

Immune stimulants in cats are greatly needed to aid in the management of intractable viral diseases of cats, including the coronavirus that causes feline infectious peritonitis (FIP). The purpose of this study was to compare a commercially marketed compound TLR 2/6 activating compound (Polyprenyl immunostimulant; PI; Vetimmune) and a liposomal TLR 3/9 activating immune stimulant (LTC) for their relative ability to stimulate induction of type I (IFN-α, IFN-β) and type II (IFN-γ) interferon immune responses, as these responses are key to controlling viral infections, including coronaviruses. To address this question, *in vitro* assays were conducted using peripheral blood mononuclear cells (PBMC) prepared from blood of healthy, young adult research cats. Cells were incubated with several dilutions of PI or LTC, and production of IFN-α and IFN-β was assessed by RT-PCR, while production of IFN-γ was assessed by both RT-PCR and ELISA. LPS was included as a positive assay control. We found that LTC induced significant production of IFN-γ at all 3 concentrations evaluated, whereas PI failed to induce IFN-γ at any dose. When compared to untreated cells in this study, LTC but not PI induced significant expression of IFN-α ($p=0.0441$), IFN-β ($p=0.0060$), and IFN-γ ($p=0.0152$). These findings suggest that activating the TLR 3 and 9 pathways is a more effective approach to generating broad interferon production in cats, whereas TLR 2 and 6 pathway activation is much less effective for inducing these cytokines. Additional studies are warranted to more broadly assess and compare the immune stimulatory properties of LTC and PI, and to rigorously evaluate their antiviral properties, both *in vitro* and *in vivo*, and their potential effectiveness as treatments for FIP.

DVM Student / Clinical Sciences

## 8. Detection of anti-erythrocyte and anti-platelet antibodies using flow cytometry in experimental *Babesia gibsoni* and *Candidatus* Mycoplasma haematoparvum co-infected dogs

*Nida Chornarm, Sarah Shropshire, Jennifer Hawley, Melissa Brewer, Kristine Kofron, Michael R Lappin*

Canine babesiosis, which is characterized largely by anemia, develops in some dogs after infection of erythrocytes with one of several protozoal agents in the *Babesia* genus, including *Babesia gibsoni* (Bg). Canine hemoplasmosis is a blood-borne erythrocytic bacterial disease associated with anemia in some dogs that is caused by *Mycoplasma haemocanis* or *Candidatus* M. haemaotoparvum (CMhp). Our group recently titrated flow cytometry-based assays to detect anti-platelet and anti-erythrocyte antibodies to help characterize immune and infectious causes of thrombocytopenia or anemia in dogs. The primary purpose of this study was to determine whether dogs experimentally inoculated with Bg and CMhp produce anti-erythrocyte or anti-platelet antibodies. Blood from a Pitbull terrier that was naturally infected with Bg and CMhp was used to initiate infection in 5 research beagles. Over an 8 week-period, a complete blood count, PCR assays for DNA of Bg and CMhp, both flow cytometry assays, and direct Coomb's test were performed on designated time points. An antimicrobial rescue protocol was initiated in all dogs between Day 21 and Day 28 post-infection due to clinical signs of babesiosis. All dogs developed anemia and thrombocytopenia which corresponded to the presence of anti-erythrocyte and anti-platelet antibodies. Direct Coomb's test was positive prior to detection of anti-erythrocyte antibodies in 2 of 5 dogs and was positive in all dogs post-infection. After treatment, platelet count and hematocrit rebounded to normal levels at Day 35 and Day 42, respectively and both flow cytometry assays became negative at Day 42 post-infection. The results support that Bg and CMhp co-infection induces anti-erythrocyte and anti-platelet antibodies which likely relate to pathogenesis of disease and then rapidly resolve with specific treatment for the agents. **Funding:** Center for Companion Animal Studies, Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University

Graduate Student / Clinical Sciences

## 9. Immunohistochemistry characterization of the immune response to LPS in the equine gastrointestinal tract

*Katt Crowdis, Lauren Harrison, Brad Nelson, Rachel Hector, Jeremiah Easley, Daniel Regan*

As one of the leading causes of death in horses, endotoxemia remains a complicated disease process with many unknown complexities. While the clinical presentation and clinicopathologic findings of equine endotoxemia have been well described, [1,2,3,4] limited information exists about the endotoxin-mediated immune response in the equine gastrointestinal (GI) tract. Therefore, we sought to characterize the immunological response to lipopolysaccharide (LPS) in all anatomical segments of the equine GI tract via quantitative immunohistochemistry. Immune cell phenotype and density were characterized in four sex-matched healthy horses and horses following administration of 0.1mcg/kg dose of LPS over 30 minutes each via immunohistochemical labeling (IHC) for the following antigens and cell types: CD3 (pan-T cell), CD79α (B cell), CD204 (macrophages), and FoxP3 (regulatory T cell). Whole-slide images were captured and Visiopharm image analysis software was used to quantitively assess the density of each immune cell type, while standard histological assessment was used to characterize the microanatomical distribution pattern of the cellular infiltrates. LPS injection caused a significant decrease in intestinal CD3+ T cell and FoxP3+ T cell populations, and an increase in B cells and macrophages in both the small and large intestine, as compared to healthy control horses. The lymphocytes were located predominantly within the lamina propria and submucosa layers. These results suggest the local intestinal immune response to LPS may parallel the known systemic pro-inflammatory effects of endotoxemia, and provide an initial foundation for future studies, such as cytokine profiling, that would provide a more complete characterization of the functional consequences of this response. Ultimately, a comprehensive understanding of the intestinal immune response to LPS would allow for the investigation of novel immunomodulatory therapeutics to ameliorate the significant morbidity associated with this clinical condition.
**Funding:** Young Investigators Grant

DVM Student / Microbiology, Immunology and Pathology

## 10. Effect of *Bifidobacterium longum* 999 supplementation on stress associated findings in cats with feline herpesvirus 1 infection

*Hailey Davis, Patricia Franco, Jason Gagné, Ragen TS McGowan, Jennifer Hawley, Michael R. Lappin*

Bifidobacterium longum strain 999 (BL999) is a probiotic (Purina® Pro Plan® Veterinary Supplements; Calming Care) that has been shown to lessen anxiety in dogs and is known to be safe in cats. Feline herpesvirus 1 (FHV-1) is the most common infection of cats and clinical disease can be exacerbated by stress. The primary hypothesis was that cats supplemented with the BL999 containing product would have higher relaxation scores, lower stress markers, and lower FHV-1 clinical scores than cats supplemented with the same product, but without BL999 as a placebo when mild stress was induced by changing the type of housing. This 12-week study enrolled 24 cats with chronic subclinical FHV-1 infection that were randomly divided into two groups. The cats were supplemented with BL999 (group 1) or placebo (group 2) daily. After BL999 was supplemented for 42 days to achieve probable maximal effects, the cats were moved from the individual gang rooms into cages, back into gang rooms, and then back into cages to induce stress over the next 42 days while behavioral, clinical, and biochemical markers were measured. All cats ate a minimum of 75% of both supplements and there was no obvious vomiting or diarrhea. During the stress periods, the cats supplemented with BL999 were significantly less likely to have abnormal serum cortisol concentrations ($P = 0.0059$) or sneezing ($P < 0.00001$). During the times cats were housed in cages, those supplemented with BL999 were significantly more likely ($P < 0.0001$) to reach out to the scorers through the cage bars and were significantly less likely ($P < 0.0003$) to pace in the cages. The results of the study suggest that BL999 is well tolerated by cats, reduces stress, reduces stress associated problems like activated FHV-1, and increases social interactions between cats and people. **Funding:** Sponsored by an unrestricted donation by Nestle Purina PetCare

DVM Student / Clinical Sciences

## 11. Relationship between climate change and waterborne diseases in companion animals: a reconsideration of veterinary surveillance

*Brian M. DeFilippo, Josh K. Moore, Mo Salman, Sheryl Magzamen, Colleen Duncan*

Waterborne diseases present substantial health risks to both humans and animals. A wide range of both infectious and non-infections disease causing agents are known to be transmitted via water sources that are influenced by environmental conditions. Climate change is known to increasingly correlated with the severity, frequency, and distribution of waterborne health hazards. Although water associated risks are well studied for people, the link to animal diseases has been limitedly explored. The objective of this project is to explore the the link between climate change selected indices and water associated diseases in companion animals, and the awareness of animal health community in this relationship with a potential solution. to identify such changes. A scoping literature review was conducted to determine if the most common climate-sensitive waterborne diseases in humans have the potential to impact companion animals. Case examples of climate-sensitive waterborne diseases known to occur in dogs, leptospirosis and harmful algal bloom (HAB) toxicosis, were used to identify challenges related to the study of companion animal diseases. This work highlights a critical gap in the animal health community and offers insight into a platform on which a companion animal surveillance system should be built. Given the current projections for global climate change, veterinarians and other animal health professionals need to be ready to respond effectively to evolving waterborne health hazards. **Funding:** Colorado State University College of Veterinary Medicine and Biomedical Sciences

DVM Student / Microbiology, Immunology and Pathology

## 12. The Benefits and Challenges of Experiencing Homelessness with a Pet in Fort Collins

*Sarah Deluty, Fred Palmer, Janelle Scott, Danielle Frey*

This project investigated the current resources available to people experiencing homelessness with a pet in Fort Collins and the accessibility of those resources. The objective was to identify resource gaps in the community, specifically around access to health care for both the human and the animal. Thirteen clients of the Street Dog Coalition, a free veterinary outreach clinic in Fort Collins, were interviewed about their experiences and their ability to access veterinary care and human medical care. These interviews identified several resource gaps, including a safe place for pets to stay during the day, owner access to medical and other resource facilities, and overall uncertainty about what resources are "pet friendly". In response, a collaboration with the Fort Collins Homeless Resource Guide was established, and a new guide was created clearly demarcating pet friendly resources. Additionally, a literature review regarding the benefits and challenges of experiencing homelessness with a pet was conducted and the results were summarized in an infographic to use as a communication tool. Currently, a day care program and foster care program are being established to provide temporary shelter to pets of people experiencing homelessness, escaping domestic violence, seeking inpatient care, or otherwise hoping to keep their pets safe in the short term. These services are intended to support people while housing is uncertain, unstable or does not permit the animal to stay with the owner. This project focused on needs expressed directly by the members of the Fort Collins community we hope to support and works to establish sustained programs that can fill these needs long-term. **Funding:** Petsmart Access to Care Grant

DVM Student / Biomedical Sciences

## 13. NOD2 expression by mucosal CD11c+ cells is required for a humoral immune response against the *Lactobacillus acidophilus* vaccine platform

*Bridget Eklund, Allison Vilander, Kimberly Shelton, Zaid Abdo*, Gregg Dean**

**Co-mentored by Dr. Abdo and Dr. Dean*

A robust mucosal vaccine platform is an attractive avenue for directly stimulating the mucosal immune response targeted towards pathogens transmitted at mucosal surfaces. We have previously demonstrated the potential of *Lactobacillus acidophilus* as a promising candidate for delivering antigens to relevant mucosal sites via intragastric vaccination. *L. acidophilus* is generally regarded as safe, inherently persists in the gastrointestinal tract, and can be genetically manipulated to express adjuvants and antigens on the cell surface. *L. acidophilus* expresses molecular motifs that stimulate pattern recognition receptors including TLR2, DC-SIGN and NOD2 (Nucleotide-binding oligomerization domain-containing protein 2). Co-culture of *L. acidophilus* with bone marrow-derived macrophages from knock-out mice suggested a key role for NOD2 in the immune activation by *L. acidophilus*. Likewise, the adaptive immune response against prototype exogenous antigens expressed by *L. acidophilus* was abrogated in NOD2 knock-out mice. NOD2 is expressed by both mucosal epithelial cells and antigen presenting cells, therefore we sought to determine the relative importance of NOD2 expression in these two cell types for antigen-specific adaptive responses after mucosal immunization. We employed a Cre/Lox system to generate mice with NOD2 knockout only in CD11c-expressing cells and then immunized with recombinant *L. acidophilus* expressing the MHC class II antigen OVA323-229. Our results show a significant decrease in OVA-specific fecal IgA and serum IgG demonstrating the essential role of NOD2 expression in antigen presenting cells for the mucosal immunogenicity of the *L. acidophilus* vaccine platform.

Graduate Student / Microbiology, Immunology and Pathology

## 14. Characterization of the microbiome associated with *Culicoides sonorensis*

*Abigail Fennell, Christie Mayo, Mark Stenglein, Grace Borlee, Brad Borlee*

*Culicoides sonorensis* is a small biting fly (midge) that is a vector for viral pathogens such as vesicular stomatitis and bluetongue virus. These viruses can cause high mortality in livestock populations and incur significant economic losses. In 2015 alone, bluetongue virus was estimated to cause over $3 billion in global monetary losses. Thus there is a critical need to develop approaches to alter vector competency of the midge. The aim of this investigation was to culture bacteria that have evolved symbiotic relationships with midges. The bacterial isolates were also characterized for antibacterial or antifungal activities and the ability to induce or inhibit cyclic di-GMP and quorum sensing. Bacterial isolates were identified using MALDI-TOF and 16S genetic sequencing. *Acinetobacter* spp., *Aeromonas* spp., *Bacillus* spp., *Elizabethkingia* spp., *Enterobacter* spp., *Microbacterium* spp., *Morganella* spp., *Pseudomonas* spp., *Serratia* spp., and *Shigella* spp. were identified as culturable members of the midge microbiome. Many of these bacteria were also identified in a culture-independent metagenomic analysis of the bacterial community of *C. sonorensis* reared at different temperatures. This investigation marks a significant contribution to understanding the microbial diversity of the *C. sonorensis* microbiome. A more thorough characterization of the midge microbiome is the first step in designing a paratransgenesis strategy to use a symbiont of the vector to reduce or eliminate transmission of viruses.

Undergraduate Student / Microbiology, Immunology and Pathology

## 15. Treatment of ocular feline herpesvirus using a novel topical immunotherapy

*Grace Flynn, Sophie Hopkins, Lyndah Chow, Steven Dow, Kathryn Wotman*

Ocummune, a liposomal toll-like receptor ligand complex, is a novel topical immunotherapy designed to stimulate non-specific immune responses against ocular bacterial and viral pathogens and superficial cancers of the eye with potential for use in veterinary and human medicine. In a pilot study, Ocummune showed promise in treating ocular feline herpes virus-1 (FHV-1) infection in shelter cats. The current study expands upon prior research investigating the *in vitro* efficacy of Ocummune to treat FHV-1 infection in cats. This study hypothesizes that pretreatment of cells with Ocummune at ideal concentrations will increase cell viability after inoculation with FHV-1 and decrease size and quantity of viral plaques in plaque assays. Ocummune was applied to Crandall Reese Feline Kidney (CRFK) cells in varying concentrations 24 hours in advance of FHV-1 inoculation, and both cell viability and plaque formation were quantified 48 hours later. Concurrent *in vivo* studies are assessing Ocummune as a treatment for both cats with experimentally induced FHV-1 infection and shelter cats with naturally occurring conjunctivitis, conjunctival hyperemia, chemosis or mild keratitis. If the results of these studies indicate efficacy of this treatment for FHV-1, Ocummune has the potential to be applied in trials for the treatment of human ocular herpesvirus infections, specifically herpes zoster ophthalmicus and herpesvirus keratitis. **Funding:** Colorado's Advanced Industry Accelerator Proof-of-Concept (AI-POC) Grant Program, Colorado State University's Translational Medicine Institute (TMI) Translation Acceleration Program Grant, NIH Fellowship Grant Trainee

DVM Student / Clinical Sciences

## 16. Viability of equine embryos produced *in vitro* as assessed with zona pellucida measurements

*Caroline Foley, Emma Huggett, Giovana Di Donato Catandi, Kyle Fresa, Elaine Carnevale*

With the development of procedures for intracytoplasmic sperm injection (ICSI) and embryo culture, *in vitro* embryo production has become prevalent in the equine industry. Therefore, there is a growing need to assess *in vitro*-produced embryos, to determine early indications of viability. One way to do this is to use objective parameters, measuring the inner zona pellucida area (IZPA), as an embryo develops in culture. We hypothesized that measurements of the IZPA are indicative of further developmental potential and associated with embryo development stage. For this retrospective study, images of embryos were used that were captured at 200x magnification on an inverted microscope. The measurements were done using an image-assessment program (NIH ImageJ) for IZPA at Day 1, 4/5, 6/7 until degeneration or transfer into a recipient mare (Day 0=day of ICSI). ICSI-produced embryos that do or do not develop into a transferrable embryo (morula or blastocyst stage) were then compared by Day and embryo stage to determine when significant ($P < 0.05$) changes in measurements occur. From this research, we will learn more about equine embryo growth in culture and determine if objective measurements can predict embryo developmental potential, which will allow the equine veterinarian and embryologist to determine which embryos are viable and most suitable for transfer into a recipient mare.

DVM Student / Biomedical Sciences

## 17. Bactcountr: a tool for calculating colony forming units

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

Calculating colony forming units (CFUs) for large studies can take a considerable amount of effort and time. Further, data is often collected and calculated in large excel tables with many calculations, allowing for the opportunity to replicate mistakes. To address these issues, we have developed bactcountr, a novel analysis tool for automatically calculating CFUs based on dilutions used. Bactcountr sifts through the observed data for various experimental groups and finds the CFU observation for each group and replicate that is most appropriate. The final CFU counts are calculated based on dilution factor and resuspended volume. Finally, statistical significance is calculated and the results are plotted. This analysis tool greatly reduces the amount of time to perform analysis. **Funding:** NIH grant 1R01 AI127475-01A1

Graduate Student / Microbiology, Immunology and Pathology

## 18. Vulnerable Populations, Veterinary Telemedicine, and COVID-19: Access to care and telemedicine for vulnerable pet owners during the pandemic era

*Zachary George, Sage Smith, Rachel Wertheimer, Katherine McCaw, Colleen Duncan, Danielle Frey*

When the COVID-19 pandemic began, economic instability, medical supply rationing, and a severe public health risk swept the globe; leaving veterinarians unsupported as they quickly adapted to a new reality. To investigate the impacts of these changes, a survey was developed to better understand how veterinarians shifted their approaches to ensure access to care, particularly to traditionally and newly vulnerable clientele, and how telemedicine was utilized in the process. An anonymous, online survey consisting of 21 multiple choice and 4 free response questions was distributed to veterinarians, technicians, and practice staff through professional and social channels. Results showed that 41% of respondents witnessed increased requests for reduced-cost care, with 43% of those requests left unaccommodated even in spite of a desire to do so. Additionally, 53% of the respondents requested resources on how to better help vulnerable clients. Nearly 50% of respondents reported increased interest in telemedicine due to the pandemic, and 95% of veterinarians said they would continue to use telemedicine, though only 10% learned about it within their curriculum. Seventy-five percent of respondents thought veterinary curricula should adapt in response to the pandemic. Of those respondents, 95% supported adding telemedicine instruction to these curricula changes. These results show that the pandemic has made it harder for clients to access the resources necessary for veterinary care. Further, while telemedicine use increased during the pandemic, most respondents have no formal education on how to legally and efficiently utilize these platforms, highlighting an opportunity to close the knowledge gaps with continuing education options. Finally, a follow-up study on the ramifications of the pandemic on client care-based decisions and development of financial and business literacy education could allow practices to work with vulnerable clients and mitigate the impacts of the pandemic on veterinary practices serving vulnerable populations. **Funding:** Pet Smart Charities

DVM Student / Other

## 19. Neuronal treatment of models of Alzheimer's disease using CBD and Trazodone

*Vincenzo Gilberto, Logan Butler, Charli Geer, Stephanie McGrath and Julie A. Moreno*

Alzheimer's disease (AD) is one of the most common forms of neurodegeneration and is typically characterized by either the presence of Amyloid-Beta plaques (Aβ) or Tau Tangles (NFT). Despite the fact that there is no current cure for AD, it is well known that targeting of signaling pathways involved in reactive oxygen species (ROS) or unfolded protein response (UPR) improves behavioral deficits, glial inflammation and neuronal toxicity. This research aims to utilize specific strains from the model organism *C. elegans* which have been genetically modified to contain two common misfolded proteins found to aggregate and accumulate in AD patients' brains, amyloid-β and the hyperphosphorylation of tau (P-tau). We hypothesize that combinational drug stacking of compounds that target both ROS production and UPR will improve the behavioral qualities associated with AD, and will also extend the lifespan of these neurodegenerative nematode models to that of their control counterparts. To address this hypothesis, we have used CBD and Trazodone to inhibit ROS and the UPR, respectively. Using both one-way and two-way ANOVA our previous experiments have revealed that neurodegenerative *C. elegans* motility is significantly worse compared to their control counterparts, and that early stage exposure to Trazodone significantly improved the motility of the neurodegenerative nematodes. The next steps for this project include; but are not limited to, isolated and combinational drug treatments of both Trazodone and CBD to measure their motility and life span. Once optimal doses are identified, we aim to implement a late stage rescue of neurodegenerative *C. elegans* with combinational drug stacking.

Graduate Student / Environmental and Radiological Health Sciences

## 20. Susceptibility of peridomestic rodents and other small mammal wildlife to infection with SARS-CoV-2 and their potential role in interspecies transmission

*Lauren K. Guilbert, Angela M. Bosco-Lauth, Audrey E. Walker, Stephanie M. Porter, Airn E. Hartwig, Aimee Pepper, Daphne Hawvermale, Richard A. Bowen*

On December 31, 2019, the World Health Organization was alerted to a novel outbreak of unknown origin, which has now been classified as SARS-CoV-2. While the virus is transmissible between humans, an exhaustive host range is not known. Understanding which species are susceptible to the virus as well as how it is transmitted within and between species is important to developing a strategy by which the virus can be treated and its spread controlled. Several common laboratory animals have been evaluated for susceptibility to SARS-CoV-2, including rats, mice, and Syrian hamsters, and hamsters have been demonstrated to be the preferred small animal research model. While laboratory rats and mice are not viable research models, peridomestic wild rodents may shed the virus, as observed in Syrian hamsters. To address this hypothesis, deer mice, wood rats, house mice, and fox squirrels will be inoculated intranasally with SARS-CoV-2. If any of these species are found to be susceptible, similar wildlife species such as prairie dogs, ground squirrels, skunks, and raccoons will also be assessed. Susceptibility will be assessed by monitoring clinical signs and testing for virus shedding in oropharyngeal swabs and organs. Blood samples will be collected at 28 days post infection in certain species to determine an antibody response. If one or more species tested is found to be susceptible to infection and shedding the virus, additional groups of those rodents will be housed together and two infected rodents will be introduced to the enclosure. Contact individuals will be monitored in the same manner as the first groups to determine how readily transmission occurs. This study will be helpful in classifying the host range of SARS-CoV-2. Doing so can aid in the establishment of successful small animal research models and in the identification of potential sources of zoonotic disease related to SARS-CoV-2. **Funding:** Animal Models Core at Colorado State University and Veterinary Summer Scholars (USDA)

DVM Student / Biomedical Sciences

## 21. Osteosarcoma-derived exosomes selectively home to the lung and induce changes in the cytokine profile of the pulmonary microenvironment

*Laurel A Haines, Sophi J Schofield, Eric P Palmer, Alissa B Mathias, Lauren N Alfino, Daniel P Regan*

Osteosarcoma (OS) is the most common bone cancer in children and adolescents. Following resection of the primary tumor, one-third of all OS patients develop metastasis, almost exclusively to the lung. In other tumor types, metastasis is preceded by the formation of a pre-metastatic niche, a process in which distant organs are remotely "primed" for tumor cell seeding by soluble factors released from the primary tumor. Of these secreted factors, nanosized extracellular vesicles, known as exosomes, have been shown to be crucial mediators of this intercellular communication and subsequent induction of pro-metastatic changes to these distant organ microenvironments. However, little is known regarding exosome-induced changes in the pulmonary microenvironment during OS metastasis. We hypothesize that OS exosomes prime resident alveolar macrophages of the lung, which in turn mediate immune and extracellular matrix changes to the lung microenvironment to facilitate OS metastasis. To investigate this, we evaluated human OS exosome bio-distribution *in vivo* in a mouse model, and characterized the effects of *in vitro* OS exosome 'education' of macrophages derived from a human monocyte cell line (THP-1). We show that OS exosomes selectively track to the lung in mice and elicit distinct cytokine changes in the pulmonary microenvironment, including upregulation of IL-6, IL-8, and CCL2. *In vitro* OS exosome-education of macrophages over 72 hours also induces an altered cell phenotype, characterized by changes in macrophage survival/proliferation and cytokine secretion. Our findings demonstrate that OS exosomes alter the phenotypic state of macrophages and the immune microenvironment of the lung. Future studies aimed at determining if and how these immunological changes facilitate OS lung metastasis are currently underway.

DVM/PhD Student / Microbiology, Immunology and Pathology

## 22. Mesenchymal stromal cells from load-bearing connective tissues: a review of cellular properties and laboratory expansion methods for manufacturing cell therapies

*Jacqueline M. Harrison, John D. Kisiday*

Mesenchymal Stem Cells (MSC's) have been vastly studied for their potential as an adjunct to therapy for numerous human and veterinary diseases. Of specific interest is the potential of these cells to aid in the treatment of diseases involving musculoskeletal tissues. Connective tissues represent an anatomical niche for mesenchymal stem cells: MSC's have previously been harvested from sources such as bone marrow and adipose tissue, and more recently have been isolated from load-bearing connective tissues such as bone, tendon and ligament. In this study, we compared MSC phenotypes from different studies that had isolated cells from different tissues: these comparisons were made using the concepts of trilineage differentiation, immunophenotyping, and cell kinetics. We also attempted to explore the emerging concept of "reserved stemness", lending support to a hypothesis that has historically been underappreciated— the prospect of MSC properties coinciding with dedifferentiation of a committed connective tissue phenotype. Our analysis of these different papers suggests that MSC's are similar both phenotypically and in their ability to grow and expand, regardless of which tissue they have been isolated from. We also found evidence that cells from mature load-bearing connective tissues are capable of dedifferentiation to an MSC-like phenotype, which differs from the concept that MSC cultures are derived from resident MSC's that are present in very low density in mature connective tissues. **Funding:** CSU Veterinary Summer Scholars Program

DVM Student / Clinical Sciences

## 23. A large-scale VR deployment: A novel approach to distance education

*Natascha Heise, Katelyn Brown, Jordan Nelson, Chad M Eitel, John P Walrond, Tod R Clapp*

The arrival of COVID-19 and accompanying restrictions posed large challenges to education communities worldwide. In response, a large-scale virtual reality (VR) course was constructed and deployed at Colorado State University (CSU) for a large distance human anatomy course. Enrolled students received a VR capable laptop and head-mounted display (HMD) and participated in synchronous online laboratory and recitation sessions with instructors. The program enabled students to work collaboratively in a common virtual space and learn human anatomy from digital cadavers and volumized medical data (CT and MRI). Qualitative data was collected on student engagement, confidence, and reactions to the new technology. Quantitative data assessed student knowledge acquisition and retention of anatomical spatial relationships when using VR and 2D methods. Results suggested that using VR matched 2D methods in terms of student knowledge acquisition and retention of anatomical relationships. Qualitative data indicated that VR enhanced student engagement and increased opportunities for students to interact with TAs, peers, and the content. Students further reported the unique aspects of the VR program allowed them to explore the content in novel ways. There was a statistically significant increase in student examination scores when compared to previous in-person laboratories. Overall, the virtual classroom maintained the rigor of traditional gross anatomy laboratories without negatively impacting student examination scores and provided a high level of accessibility, without compromising learner engagement. This course may provide an interactive approach to distance education and may further promote research on how to use immersive VR in higher education.

Graduate Student / Biomedical Sciences

## 24. Synaptic Ultrastructure at the Neuromuscular Junction

*Jasmin A Hicks, Uel J McMahan, Noreen E Reist*

Fast and efficient intercellular communication in the brain is orchestrated through the release of neurotransmitter from presynaptic nerve terminals. The “active zone”, a specific presynaptic specialization, is the primary mediator for transmitter release during cell-to-cell communication. Transmitter-filled vesicles dock, prime, and ultimately fuse with the presynaptic membrane at active zones to release transmitter. While the functional roles of several active zone proteins have been determined, the resolution limitations of standard electron microscopy (~50 nm) have limited the identification of spatial relationships. Electron Tomography permits the 3D analysis of 0.5 nm virtual slices through a single 50-70 nm sample. Recently, the 3D active zone ultrastructure at the mouse and frog neuromuscular junction have been determined at a sufficient level of resolution to begin assessing functional spatial relationships. One spatial relationship that has newly been studied, using this high-resolution 3D tomography, is that of the transmitter-filled synaptic vesicles and their associated pre-synaptic membrane at the neuromuscular junctions of frog. This study, conducted by Dr. McMahan’s lab at Texas A&M, revealed that the physical contact area between the vesicle membrane and the plasma membrane (VM-PM) shows a normal distribution. Moreover, the VM-PM contact area distribution shifts to the left when the neuromuscular junctions are fixed during stimulation. These results demonstrate that not only is the VM-PM contact area measurable using electron tomography but that we can also use it as a correlate of synaptic vesicle priming. This proposal aims to exploit the fast, cost-effective, genetic system of Drosophila to test a mutation in synaptotagmin, the $Ca^{2+}$ sensor required for vesicle fusion, hypothesized to play a role in synaptic vesicle priming. We will also use the 3D data to begin identifying the presynaptic ultrastructure at the active zone and start determining the molecular mechanisms mediating neurotransmitter release
**Funding:** Graduate Research Fellowship Program through Society for Neuroscience

Graduate Student / Biomedical Sciences

## 25. Could neurobasal-CTS + B27 be a universal culture media?

*Alexandra E Lake, Brielle H Patlin, Luke A Schwerdtfeger, and Stuart A Tobet*

Maintaining cells, organoids, or tissue explants *in vitro* requires important choices of culture media traditionally utilizing serum supplements for maintenance of healthy samples. Serum, derived from animal blood, renders quality control difficult for repeatability, and alternatives are challenging to optimize. For maintaining organotypic tissue ex vivo, conditions must mimic tissue pH and osmolarity, among other factors. Different cell types and tissues may require different conditions that are not all met in static environments ex vivo. Neurobasal media + B27 supplement (ThermoFisher) contains proteins, vitamins, and other components. Murine brain, pituitary, ovary, adrenal, and intestinal slices, as well as human intestinal slices have been used in separate experiments with this media to maintain slices for multiple days. Successful usage of this media model across these tissues shows utility across multiple single systems and suggests potential for experimentation across multiple organs in combinatorial systems. The current study explores expanding neurobasal media use to lung slices. Ideal media for culturing lung slices *ex vivo* would promote cell growth and normal proliferation minimizing apoptosis. In preliminary experiments, animals were perfused with warm PBS, lungs were infiltrated with 2% agarose, embedded in blocks of 8% agarose, and cut 300 µm thick to create organotypic lung slices. Culturing lung tissue using adult neurobasal-CTS + B27 media resulted in maintenance of tissue morphology. Minimal cell death across the tissue as marked by membrane impermeant DNA binding dye, ethidium homodimer, was observed. Future improvements of the technique will facilitate research on changes within infected lung slices.

Graduate Student / Biomedical Sciences

## 26. Neuropathogenesis in Guinea Pigs Exposed to Mycobacterium tuberculosis

*Amanda Latham, McKenzie Richards, Arielle Hay, Forrest Ackart, Brendan Podell, Randall Basaraba, and Julie Moreno*

Tuberculosis (TB), a disease caused by the bacterium *Mycobacterium tuberculosis*, is one of the top ten leading causes of death worldwide. Tuberculosis infection of the meninges (TBM) is the most severe form of this disease, especially in children and immune compromised patients, such as those diagnosed with HIV/AIDS. Our long-term goal is to therapeutically treat burdened individuals. Currently, we aim to develop a clinically relevant animal model of TBM using the guinea pig, investigate an alternative route of central nervous system infection by *Mtb*, as well as demonstrate neuropathogenesis caused by peripheral infection. Guinea pigs exposed to *Mtb* are a well-characterized laboratory model of infection and most closely resemble naturally occurring TB disease in humans compared to other common animal models. Further, we hypothesize that *Mtb* bacteria are able to penetrate the brain, bypassing the blood brain barrier, by infecting olfactory neuronal precursor cells in the nasal cavity, which can travel into the brain and differentiate into neurons. We are able to isolate these cells in the guinea pig and, via flow cytometry, verify their infection with a BSL-2 fluorescently labelled strain of *Mtb*. Preliminary data also shows that infection by aerosolized *Mtb* causes damage and inflammation in the brain. Through immunohistochemistry and various staining techniques we have shown that peripherally infected guinea pigs demonstrate toxic misfolding of proteins and amyloid aggregation as well as gliosis. From this data we hope to uncover a better understanding of how TBM occurs and the mechanisms behind its pathogenesis.

Graduate Student / Environmental and Radiological Health Sciences

## 27. The use of altrenogest releasing intravaginal rings in the mare for the purpose of estrus suppression

*Jessica D Lederman, Jeremiah T Easley, John A Moss, Marc M Baum, Jennifer N Hatzel*

This study proposes an alternative method for delivering altrenogest, a synthetic progestin, to decrease adverse behavior in mares through hormonal manipulation. We hypothesize that an intravaginal ring designed for the mare will provide a novel and efficacious method for sustained release of altrenogest to suppress adverse behavior commonly associated with estrus. Importantly, it will allow the mare to resume normal cyclicity upon removal for pursuit of reproductive procedures. Mares often demonstrate undesirable behaviors during their estrus phase (5-7 days) of their 21-22 day estrus cycle, due to elevated estrogen levels produced by the dominant ovarian follicle. Following ovulation, progesterone levels increase, overcoming effects of estrogen for 11-14 days; often alleviating the undesirable behavior. Altrenogest, currently delivered orally or by injection, provides estrus suppression through similar biologic activity as native progesterone. Mares often resist daily oral administration or injections, leading to noncompliance. The safety and ease for the application of intravaginal rings between the diameters of 13cm and 18cm was determined through pilot trials of control rings in the summer of 2020. Ring size (14.2 cm) and shape was effective for both mare retention and safety. The next phase will begin in the spring breeding season of 2021 to evaluate intravaginal altrenogest pharmacokinetic and pharmacodynamic values at the onset, steady-state and tail-end of vaginal altrenogest delivery. Treatment groups will receive varying dosages of altrenogest to determine the optimal level. Outward estrus behavior and physiological changes will be assessed through exposure to stallions, routine transrectal ultrasound examination and hormonal assessment. The overall goal is retention, steady-state levels of altrenogest at or above 0.5 ng/mL and behavioral estrus suppression for a 30 day duration. Comparative data will be analyzed using the Shapiro-Wilcoxon signed-rank test. Statistical significance will be set at $P \leq 0.05$.
**Funding:** Internal funding

Graduate Student / Clinical Sciences

## 28. Adult equine chondrocytes are capable of extensive *in vitro* expansion and express CD146 with time in culture

*Bethany E Liebig, Laurie R Goodrich, John D Kisiday*

Culture expansion of chondrocytes induces dedifferentiation to a fibroblastic phenotype. Such dedifferentiation has been reported to coincide with the adoption of mesenchymal stromal cell (MSC) properties. Therefore, chondrocytes may be a source for generating MSC-like cells. However, the propensity of equine chondrocytes to adopt MSC properties has not been investigated. Therefore, the objective of this study was to evaluate whether equine chondrocytes are MSC-like in their ability to undergo extensive expansion, and expression of CD146, a pericyte marker that has been associated with MSCs. We hypothesized that rapid growth coincides with CD146 expression. Equine articular chondrocytes were expanded in monolayer culture. Cells were seeded at 500 cells/cm$^2$ alphaMEM supplemented with 10% fetal bovine serum and 2ng/ml fibroblast growth factor. Cells were subcultured every 3-5 days. At each passage, cells were immunostained with a CD146 antibody and analyzed via flow cytometry. Cultures were maintained for 47 days. Primary cells were polygonal and proliferated in confluent patches. Therefore, cultures were passaged on days 3 and 7. By day 12, cell morphology shifted from polygonal to fibroblastic, and cells dispersed across the growth surface during proliferation. Hereafter, cultures were passaged every 5 days. Chondrocytes proliferated through 65 population doublings (PD) in 47 days, which is consistent with equine bone marrow derived MSCs. The percentage of CD146+ cells increased from ~4% at 0 PD to ~42% at 19 PD. CD146 expression remained >34% for the remainder of the timecourse. The increase in chondrocyte CD146 expression over time suggests a temporal phenotype transition. Our methods resulted in unusually rapid and sustained growth, indicating potential for clinical manufacturing. Future experiments will investigate properties that are important in regenerative medicine, such as immunomodulation.

DVM/PhD Student / Clinical Sciences

## 29. Evaluation of the cattle fever tick eradication program in the Texas/Mexico border region

*Brooke MacNeill, John Picanso, Gay Miller, Jason Lombard, David Miller, Andy Schwartz, David Hewitt, Mike Short, Pete Teel, Ross Wilson, Denise Bonilla, Mo Salman*

The Cattle Fever Tick Eradication Program (CFTEP) was developed in 1906 with the goal of eradicating bovine babesiosis, a tick-borne illness, that was eradicated from the United States in 1943; however, a permanent quarantine zone remains along the Texas/Mexico border. Economic losses from an extended outbreak in the United States is estimated at above $1 billion. The United States Department of Agriculture: Animal Plant Health Inspection Service: Veterinary Service (USDA:APHIS:VS) determined that an external review of their long-term funded CFTEP was necessary to evaluate the program's efficacy. The objective of this evaluation was the construction of an analytical tool, specifically a multidimensional matric, to determine the overall efficiency of various mitigation strategies used by the CFTEP, with the aim being to constrain the spread of Cattle Fever Ticks. A comprehensive table of global tick mitigation strategies was developed using available literature and technical reports, with each strategy being scored to determine the suitability of the mitigation strategy for use by the CFTEP. Selected new promising mitigation strategies will be evaluated using a simulation stochastic model to assess their potential performance. A list of performance indicators was developed for use within the matric for the assessment of the mitigation activities. Historical data from 2009-2020 program records were compiled and analyzed using descriptive statistics to look for program trends. Once the tools and technical reports are completed, the CFTEP will have a better understanding of the efficiency of their current mitigation activities, potential new mitigations, and combinations of strategies with increased efficacy. It is anticipated that the applied analytical tools will be used in the future to assess new or modified mitigation strategies before they are put into operation. This has the potential to reduce the burden of these ticks and their associated animal pathogens in the United States. **Funding:** Texas Animal Health Commission, United States Department of Agriculture (USDA:APHIS:VS)

DVM Student / Clinical Sciences

## 30. Predisposing Factors of Nasal Fistulas and Osteonecrosis Development Following Stereotactic Radiation Therapy: 91 cases (2010 - 2020)

*Patricia Marquez-DiPaulo, Thomas Lee, Sangeeta Rao, Lynn R Griffin*

Many canine patients diagnosed with naturally occurring nasal tumors are treated with stereotactic radiation therapy (SRT). There is potential to develop quality of life-limiting late radiation side effects such as nasal fistulas and osteoradionecrosis following treatment. The goal of this project was to determine if there are underlying factors that predispose individual patients to these late side effects. Medical records of canines treated with SRT for nasal tumors over the past ten years at the Colorado State University Veterinary Teaching Hospital were reviewed. Multiple factors were compared in dogs that developed osteoradionecrosis and nasal fistulas to those that did not. This study aims to determine if there is a way to prevent the development of complications in future patients treated with SRT for nasal tumors.

DVM Student / Environmental and Radiological Health Sciences

## 31. Investigating the impact of osteosarcoma exosomes on the lung microenvironment

*Alissa B. Mathias, Laurel A. Haines, Eric P. Palmer, Daniel P. Regan*

Osteosarcoma (OS), the most common primary tumor of bone, has seen no improvement in patient outcomes since the 1980's, primarily due to our inability to treat recurrent disease. Once OS patients develop recurrence, which occurs almost exclusively in the form of lung metastasis, their 5-year overall survival rate drops to 20%. Exosomes (EVs) have emerged as critical mediators of intercellular communication and have been shown to target non-malignant cells of distant microenvironments to aide in the development of metastatic lesions. Based on previous research, EVs released by OS cells are targeting cells in the lung microenvironment to promote metastatic niche formations by increasing pro-inflammatory cytokine production. These increases in pro-inflammatory cytokines target resident alveolar macrophages (AMs) in paracrine fashion to induce transcriptional changes that can be detected via RNAseq, allowing AMs to serve as a biological diagnostic tool to detect pre-metastatic niche formation in patients with OS due to their priming by OS-derived EVs. Nude mice were injected with OS labeled EVs or tumor cells and lungs were collected for flow cytometry analysis and IHC staining to characterize immune cell infiltrates, as well as EV biodistribution in the lung. BAL fluid was collected and analyzed by ELISA for EV-induced changes in cytokine levels. Preliminary work shows injections with OS EVs and tumor cells change the BAL composition with changes in pro-inflammatory cytokine production and immune cell infiltrates within the lung microenvironment. This work shows that OS EVs may impact the lung microenvironment to help establish a premetastatic niche formation that is able to be detected in the BAL fluid by changes in cytokine levels. Future work will include educating human AMs and other lung cell types with OS EVs and analyzing cytokine production and transcriptional differences using RNAseq to understand the impact of OS EVs in the lung.

Graduate Student / Microbiology, Immunology and Pathology

## 32. The impacts of climate change on animal health: a gap analysis

*Jon C Maxwell, Sadie Skeels, Colleen Duncan, and Tuula Hollmen*

The effects of climate change on human health have drawn extensive scholarly inquiry and governmental consideration since the formation of the Intergovernmental Panel on Climate Change in 1990. While many of the emerging climate issues facing humans, such as increased disease ranges, increased rates of extreme weather events, increased thermal stress, and changes in air and water quality, are essentially identical to those of animals, the impacts on animals have historically received less attention. The goals of this literature review are to elucidate how climate change effects are being discussed within animal health sectors and to supply context to determine areas that require action or investigation. Articles gathered from the search will be analyzed based on the animal type, the climate change impact, and the year of publication. As the effects of climate change become more pronounced, the problems facing domestic and wild animals will require more focus, particularly in areas at an increased risk of modification. The northern latitudes are already experiencing vulnerabilities due to climatic shifts and associated ecological impacts on animals. The second part of this project focuses on Alaska, which, according to the Fourth National Climate Assessment, has warmed at twice the rate of the rest of the United States since 1950. A survey was dispersed to Alaskan veterinarians focusing on climate change impacts on animals they serve and where they perceive gaps of knowledge and resources. The analysis and survey provide a baseline understanding of how the scientific community has focused on climate-related challenges to date and where we must direct focus in order to mitigate future instabilities. **Funding:** Veterinary Summer Scholars Program and the CSU One Health Institute

DVM Student / Other

## 33. Pet ownership as a barrier for finding housing: a review of pet policies across homeless shelters in the United States

*Isabella Mazariegos, Rebecca Ruch-Gallie, Danielle Frey, Jon Geller*

People experiencing homelessness have limited access to resources in their communities, including access to temporary housing, transportation, and health care. Limitations in access are exacerbated when they own a pet. Currently, there are no comprehensive or easily accessible resources with data on pet-friendly shelters in the United States. This information is vital for homeless pet owners seeking shelters that would allow them to enter with their companion animals. This project is meant to fill that gap through the creation of a database of animal-friendly homeless shelters in the United States' one hundred most populous cities. This study found that only six percent of shelters in these cities allow individuals to enter with their pets. The information gathered was combined with previously acquired information on pet-friendly transportation options, low-cost or free veterinary clinics, and pet food pantries within the same cities to create a comprehensive list of resources for this population. The team is now using the results of this research to create a hotline, housed under Colorado's 2-1-1 hotline and run by veterinary student volunteers, to direct individuals to the resources they need. This project was funded by PetSmart Charities.
**Funding:** Pet Smart Charities

DVM Student / Other

## 34. Impacts of the COVID-19 Pandemic on Veterinary Education

*Katherine McCaw, Zachary George, Sage Smith, Rachel Wertheimer, Danielle Frey, Colleen Duncan*

The COVID-19 pandemic has profoundly impacted all aspects of society, including the provision of veterinary medical education. Students and institutions have been pressed to navigate a novel response to an outbreak of this magnitude that will mitigate the health and academic ramifications for students, as well as capitalize on the opportunities the situation presents for evolution and advancement in the veterinary field. The objective of this study was to capture the self-reported effects of the COVID-19 pandemic on students prior to the fall 2020 semester. It aims to elucidate the impacts on the delivery of veterinary education to allow for a comprehensive assessment of the educational effects, student sentiments and intervention opportunities in the wake of the outbreak. An anonymous online survey was disseminated to veterinary students at AVMA accredited institutions using listservs and online veterinary platforms. Data was collected from July 2nd through August 10th of 2020 and a total of 1511 participants were included in the final analysis. Survey respondent demographics corresponded with national averages for veterinary students. Most students felt their mental health (86%), clinical preparedness (83.1%), physical health (71.8%) and finances (63.8%) were impacted by the pandemic. Additionally, the majority of participants reported that the pandemic had changed their perceptions of telemedicine and remote delivery of care (77.7%), access to human medical care (68.2%), health disparities (67.6%), human-human disease transmission (60.2%), and their role in public health (56.2%). These findings suggest that pre-existing academic and non-academic stressors have been compounded and health perceptions have been influenced by the pandemic. The results from this study can be utilized to understand the impacts on student career interests, mental health, physical health, clinical preparedness, career aspirations, and finances, to tailor current and future responses to be efficient, dynamic, and conscientious of the emerging needs of students. **Funding:** PetSmart Charities

DVM/MPH Student / Microbiology, Immunology and Pathology

## 35. Urine and renal cultures from middle aged to older cats with and without chronic kidney disease

*Lia McCoy, Kenny Siu, Patricia Franco, Hayley Clark, Josh Daniels, Michael Lappin*

Chronic kidney disease (CKD) is common in geriatric cats and bacterial infection is a contributing cause; however, cultures of renal tissues are rarely reported. In this study, we performed bacterial culture on urine from the bladder and the renal pelvis of cats as part of an ongoing study of renal metabolomics. With owner consent and humane euthanasia, cadavers were transported on ice for sample collection using aseptic technique. Urine was collected from the bladder via cystocentesis and new sterile scalpel blades were used to penetrate the pelvis of both kidneys to aid placing a swab from a commercially available transport system to collect samples. Aerobic, anaerobic, and *Mycoplasma spp*. cultures were performed by the Bacteriology Section of the CSU Veterinary Diagnostic Laboratory. Serum creatinine concentration and urine specific gravity were used to classify cats as normal or different International Renal Interest Society (IRIS) stages of CKD. Among the 10 cats classified as normal or IRIS Stage 1, one was positive for Mycoplasma growth in urine but neither kidney. Among the 12 cats classified as IRIS stages 2, 3 and 4, bacteria were isolated upon aerobic culture (4 cats; *Escherichia coli or Enterococcus spp*.) or anaerobic culture (1 cat; [> 4 isolates] were isolated from the urine of 5 cats (41.7%). The cat with anaerobic bacteria in urine also yielded *E. coli* from the left kidney. The same bacterium in urine was cultured from one or both kidneys in 3 other cats. One additional cat had a heavy growth of *E. coli* from the left kidney but not urine. These results suggest that bacterial culture of urine may be considered in the workup of cats with IRIS stages 2-4 and that a negative urine culture may not rule out pyelonephritis. **Funding:** Center for Companion Animal Studies

DVM Student / Clinical Sciences

## 36. Regionally biased CNVs accompany large-scale chromosomal rearrangements in *S. cerevisiae*

*Sean Merriman, Ane F. B. Zeidler, Matthew Dilsaver, Ruthie Watson, 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.

Graduate Student / Environmental and Radiological Health Sciences

## 37. 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, 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

## 38. Characterizing nanoparticle localization in *M. tuberculosis* infected lungs

*Camron Pearce, Anne Lenaerts, Mercedes Gonzalez-Juarrero*

*Mycobacterium tuberculosis* (*Mtb*) is the causative agent of tuberculosis (TB), which is one of the top 10 causes of death and the leading cause of death from a single infectious agent. During the course of infection, a variety of *Mtb* filled lesions and granulomas form that create heterogenous microenvironments within the lungs, and result in bacterial populations with different phenotypes. Varying lesion characteristics can affect how an administered drug will distribute throughout the lungs and likely prevents a bactericidal dose from reaching most of the bacilli. This project utilized fluorescent nanoparticles to deliver a drug payload directly to the *Mtb* filled lesion, with an aim to characterize the specific nanoparticle localization and cellular uptake/delivery of these particles to the site of infection. The results of this include a novel computational approach that utilized an automated image analysis software, combined with an R based data pipeline, to provide a quantitative and qualitative analysis of the fluorescent particle distribution in *Mtb* infected lungs. This study is laying the groundwork for a deeper understanding of particle localization within the lesion microenvironment. On-going studies are in progress that will determine how the lesion-directed particles may improve the bactericidal effect of the drug. **Funding:** Akagera Medicines, Inc.

Graduate Student / Microbiology, Immunology and Pathology

## 39. High rate of feline leukemia virus spillover in North American pumas

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

Feline leukemia virus (FeLV) is a gammaretrovirus that is horizontally transmissible between many species of felids. A subset of animals infected with FeLV experience progressive infections, which can result in mortality in wildlife and domestic cats. As outbreaks of FeLV are of significant concern for conservation, we conducted an extensive survey of FeLV infection in free-ranging pumas and bobcats, as well as domestic cats presented to shelters to assess risk of infection in each species and transmission dynamics between species. Samples from 651 pumas, 307 domestic cats, and 212 bobcats were tested for the presence of FeLV using a standardized quantitative PCR assay. Proviral load was quantified in positive samples by normalizing with the CCR5 gene, and an FeLV env genome segment was amplified by PCR and sequenced to assess individual infections. We identified 22 positive samples in pumas (3.34% prevalence), 23 positive samples in domestic cats (7.17%), and 1 positive bobcat sample (0.47%). Regional differences were detected, with the highest prevalence in domestic cats, bobcats, and pumas in Florida. Data obtained from analysis of the env region of the viral genome demonstrates variation between viral sequences in and indicates two distinct clades of FeLV-A that are present in each region studied. A six-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 each 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

## 40. Use of activated mesenchymal stromal cells (MSC) to treat drug-resistant septic arthritis in horses

*Lynn Pezzanite[1], Lyndah Chow[1], Jason Stoneback[2], Lauren Schnabel[3], Jess Gilbertie[3], Tom Schaer[4], Julie B. Engiles[4], Natasha Werpy[5], Valerie Johnson[6], Josh Daniels[1], A Russell Moore[1], C. Wayne McIlwraith[1], Gregg Griffenhagen[1], Sherry Johnson[1], Jennifer Phillips[1], Laurie Goodrich[1] and Steven Dow[1]*

Septic arthritis causes significant morbidity and mortality in human and veterinary patients and is increasingly complicated by the rising incidence of multidrug resistance. Mesenchymal stromal cells (MSC) are antimicrobial and immunomodulatory through antimicrobial peptide secretion and paracrine recruitment of immune effector cells. Using *in vitro* assays and an equine model of joint infection, our objectives were to 1) evaluate effects of Toll-like and NOD-like receptor ligand stimulation of equine MSC to enhance antibacterial and immunomodulatory properties *in vitro*, and 2) determine if intra-articular (IA) administration of TLR3-activated bone-marrow-derived MSCs improved clinical outcomes and reduced bacterial burden and inflammatory biomarkers in an equine model of septic arthritis compared to antimicrobials alone. *In vitro*, MSC were stimulated with TLR (pIC, LPS) or NLR agonists (IE-DAP) 2h and plated ($1x10^5$ cells/well, 24-well plate, 24h). MSC-conditioned media (MSC-CM) were collected and assessed for antimicrobial peptide cathelicidin/LL-37 and cytokine production, bactericidal action against multidrug-resistant planktonic and biofilm *S. aureus* and neutrophil phagocytosis. TLR-3 pIC MSC activation was most effective to enhance antibacterial and cytokine responses of stimuli evaluated and was assessed further in an equine model of joint infection. Two pilot horses were inoculated in one tarsocrural joint with *S. aureus* ($1x10^6$ CFU) and treated on day 1 and 4 with either TLR-activated equine MSC or saline. Outcome parameters included inflammation/pain scoring, serial complete blood count and synovial fluid analyses, quantitative bacterial counts in synovial fluid and synovium, inflammatory biomarkers, imaging (radiographs, ultrasound, MRI), macroscopic scoring at end-term and histology of osteochondral and synovial tissues. Overall pain/inflammation scores, joint circumference and quantitative bacterial counts from synovium at end-term were improved in the TLR3-MSC-treated horse. Continued investigation in an additional fourteen horses is ongoing. These findings demonstrate that TLR MSC activation is a simple approach to enhance antibacterial activity towards improving treatment of antibiotic-resistant infections. **Funding:** Grayson Jockey Club Research Foundation, ACVS Zoetis Dual Training Grant, 5TL1TR002533-02, 5T32OD010437-19, Verdad Foundation, Shipley Family Foundation, and Carolyn Quan and Porter Bennett.

Graduate Student, Post-doctoral Fellow / Clinical Sciences

## 41. Testing the Validity of Using a Biochemical Analytical Assay for Diagnosing Canine Cognitive Dysfunction, as a Translational Model for Alzheimer's Disease

*McKenzie Richards, Amelia Velarde, Breonna Thomas, Brittney MacQuiddy, Amy Nalls, Candance Mathiason, Damar Taylor, Nicole Kruh-Garcia, Stephanie McGrath and Julie A. Moreno*

Canine Cognitive Dysfunction (CCD) is a well-recognized neurodegenerative disease that affects 80% of dogs >8 years of age. Aged canines develop many features of human aging and Alzheimer's disease (AD) including cognitive decline and neuropathology. Similar to the neurodegenerative disease shown in humans and mouse models, dogs have an accumulation of misfolded proteins and neuronal loss in postmortem analysis of tissue, like immunohistochemistry. The only antemortem diagnostic available for veterinarians and diagnosing CCD is magnetic resonance imaging (MRI). Diagnosing early cognitive dysfunction in dogs is challenging due to low concentrations of these misfolded proteins like phosphorylated tau (P-tau) and amyloid beta (Aβ) in blood and cerebrospinal fluid (CSF). To date, not much is known regarding inflammation in the brains of canines and if these inflammatory markers, like GFAP, and misfolded proteins can be detected in antemortem samples. The objective of this project is to define an early non-invasive, antemortem novel diagnostic method as well as have a greater understanding of the progression of neurodegeneration in dogs. We will use an amplification assay RT-QuIC utilized in prion diseases to amplify the misfolded proteins within canine blood and CSF samples. In addition to the amplification assay, extracellular vesicles will be extracted using exosome prep to detect an accumulation of P-tau, Aβ, and various inflammatory markers from blood and CSF samples to diagnose CCD. These sensitive and specific diagnostic assays would improve the ability of veterinarians to accurately diagnose CCD early. Due to canine cognitive dysfunction being a large animal model for AD in humans, the translational power of this project will contribute to the progression of human medicine as well.

Staff / Environmental and Radiological Health Sciences

## 42. The expanding problem of pet owner vulnerability in the era of COVID-19

*Sage Smith, Zachary George, Katherine McCaw, Rachel Wertheimer, Colleen Duncan, and Danielle Frey*

As with all health and economic disparities, certain vulnerable populations experience the burden of barriers to accessing veterinary care more than others. The global pandemic and subsequent health and economic crises brought forth by SARS-CoV-2 redefined vulnerability by disproportionately impacting certain groups. While there has been investigation into the impact of the pandemic on the veterinary profession, how it has reshaped what vulnerability means for pet owners remains largely unexplored. We hypothesized that the pandemic increased these barriers to care for both traditionally vulnerable groups as well as for newly vulnerable groups emerging as a result of the pandemic. To explore this, we conducted an online survey of pet owners using Amazon Mechanical Turk and interviewed professionals working within organizations that interact with vulnerable populations through animal health services or animal assisted therapy. Survey responses were collected from 1,011 pet owners, with the majority of respondents indicating that their personal health risk made it more challenging to receive veterinary care for their pet during the pandemic. Vulnerable pet owners were more likely to surrender or consider surrendering their pet, and were more likely to utilize veterinary telemedicine services during the pandemic. Seventeen professionals were interviewed, and 80% of organizations represented in these interviews stopped all services at the onset of the pandemic. Common themes identified include the challenge of balancing services with the health and safety of communities and staff, new opportunities to strengthen community support and organization efficiency, and the increased comradery within organizations, communities, and the profession as a whole. These findings indicate an expanding vulnerable population of pet owners in the wake of the COVID-19 pandemic, and highlight the role of veterinary professionals to help inform and contribute to the support of these vulnerable pet owners in order to ensure their animals get adequate veterinary care. **Funding:** PetSmart Charities

DVM Student / Other

## 43. A randomized dose escalation, safety, tolerability, and drug interaction study of cannabidiol administration in dogs with intractable epilepsy

*Justine Stalnaker, Breonna Thomas, Lisa Bartner, and Dan Gustafson, Stephanie McGrath*

Idiopathic epilepsy affects nearly 5% of the canine population, with nearly a third of those afflicted demonstrating uncontrolled seizures on the standard drugs available. Cannabidiol (CBD) has demonstrated antiepileptic properties in humans and canine patients recently and could therefore present itself to be an effective adjunctive medication to standard anti-epileptic drugs (AEDs). The purpose of this clinical trial is to discover the effective oral dose at which CBD, in adjunct to the patient's standard AED protocol, will reduce seizures by 50%. We will also be looking at the pharmacokinetic and pharmacodynamic properties of CBD. Through this study we expect to find the effective oral dose of CBD to reduce monthly seizure activity by 50%. We also expect that CBD will result in measurable and predictable plasma concentrations. We will be enrolling patients that are having two or more seizures a month for at least 3 months while receiving standard AEDs. Patients will be randomly assigned to one of 3 dosing groups for 12 weeks with CBD doses given BID (twice a day) as follows; 5mg/kg, 10mg/kg, and 20mg/kg. Clients will be recording seizure activity daily and we will monitor the animal's health every 4 weeks through physical exams, CBC, and chemistry panels. CBD plasma levels will be collected every 4 weeks throughout the study period. With this study we expect to be able to determine the effective oral dose of CBD that will reduce average monthly seizure activity by 50% or more in dogs with uncontrolled idiopathic epilepsy when used in adjunct to standard anticonvulsive therapy.

DVM Student / Clinical Sciences

## 44. Elucidating the regulation and function of *Mycobacterium tuberculosis* RelBE toxin-antitoxin systems

*Julie M Starkey, Clinton C Dawson, and Richard A Slayden*

With over a million deaths reported each year, tuberculosis continues to put a significant strain on public health around the world despite decades of disease control efforts. To improve disease management and treatment efficacy, it is critical to further understand the pathogenesis of *Mycobacterium tuberculosis* (*Mtb*). Phylogenomic studies have shown that the bacterium harbors an abundant number of regulatory elements known as Toxin-Antitoxin (TA) systems. TA systems have been implicated in *Mtb*'s adaptive responses to the complex host environment initiated by immune responses. This adaptation leads to non-replicating persistence and drug tolerance, resulting in difficult to treat infections. In this study we investigated the functionality and regulation of RelBE TA loci using a combination of transcriptional, biochemical, and *in vivo* experimental techniques. Transcriptional profiling of *relBE* in response to environmental stresses was conducted using global RNA-seq and targeted RT-qPCR. The dynamics of *relBE* expression and *Mtb* growth during infection were assessed *in vitro* under defined conditions, *ex vivo* using a THP-1 macrophage model, and *in vivo* in murine models of infection. Western and northern blotting along with 5′/3′ RACE were incorporated to further characterize RelBE protein interactions and regulatory mechanisms of RelBE. Here we show that *relBE* loci are transcriptionally active under various host-associated stresses such as alternative carbon sources, low pH, and drug exposure. Additionally, coordination and regulation of the RelBE TA system are mediated through prototypical protein interactions mechanism and a newly discovered antisense RNA, asRelE2. These findings support the hypothesis that RelBE TA systems play a role in *Mtb* persistence and are transcriptionally coupled to metabolic adaptation during infection. This study also highlights the discovery of cooperative regulatory mechanisms in response to host environmental conditions. This work was supported by NIH-NIAID (Contract No: 75N93019D00005).

Graduate Student / Microbiology, Immunology and Pathology

## 45. Measuring Systemic Instability in Saccharomyces cerevisiae

*Joseph A Stewart and J Lucas Argueso*

In evolution, gradualism is described as the slow accumulation of mutations that over time leads to genetic diversification. Although this Darwinian model is well supported and widely accepted, additional modes of mutation accumulation have also been proposed (Darwin 1859, Eldredge and Gould 1972). Our lab has recently published two studies that offer support for a mode of rapid and non-independent accumulation of chromosomal rearrangements. As described in both Heasley et al. 2020 and Sampaio et al. 2020, evidence of punctuated bursts caused by systemic genomic instability (SGI) may also be the cause of the genomic landscapes that are observed in evolution. In these papers, our lab used a yeast model to track structural genomic rearrangements such as loss of heterozygosity (LOH) and whole chromosome aneuploidy. The chances of a single mutation happening are very low, however, in these papers, my colleagues in the Argueso lab found multiple mutations that co-occurred over a very short period of time. The chances of multiple mutations happening are even lower, so it raised the question on why this occurred. Systemic instability is the idea of some event causing instability to the entire cell and offers and explanation as to how these multiple experiments occurred. However, the timing and frequency of these events has not been explored. Using a unique model in yeast, we are creating a system that is able to monitor specific LOH events across the genome. Using this information, we will be able to measure the frequency and timing of both gradualism and punctuated bursts, thus gaining insight on how complex genomes, such as in tumor cells, arise over time. **Funding:** NIH

Graduate Student / Environmental and Radiological Health Sciences

## 46. Ecology of Vesicular Stomatitis Virus in North America

*Nicole E. Towner and Christie E. Mayo*

Vesicular Stomatitis Virus (VSV) is a rhabdovirus that affects horses, cows, pigs and various wildlife populations with blisters, sores and sloughing of the skin. When VSV is found on a property, triggers a required quarantine and creates economic problems for producers and owners. The epidemiology and transmission of VSV is not well understood but many insect vectors, including sand flies (*Lutzomyia spp.*), black flies (*Simulidae spp.*), mosquitos (*Culex nigripalpus*), midges (*Culicoides spp.*) have demonstrated the ability to transmit VSV after feeding on infected lesion and be infected themselves. With little understanding of epidemiology and transmission. Understanding the ecology of potential vectors is next step. To understand the ecology of the different potential vectors, Mosquito Magnet traps were set up at 4 bovine locations and 4 equine locations in Northern Colorado. Samples were collected after approximately 48 hours and seperated into mosquitoes and others. The seperated samples were sent to the USDA lab in Manhattan, KS for species identification by PCR. We predict to see a difference in the numbers of potential vectors in on equine vs. bovine properties. This could be due to management of the property, due to spraying for flies or manure management. Having and understanding of the ecology of these different vectors is important for understanding of the epidemiology and transmission of VSV **Funding:** USDA

DVM Student / Microbiology, Immunology and Pathology

## 47. Neurotoxic signaling due to air pollutant exposure

*Stephanie A Townsend, Tanner Murphy, Athbah Al Owaifeer, Katriana Popichak, Ronald B. Tjalkens, Joshua Schaffer and Julie A Moreno*

There are more than 50 million individuals with dementia worldwide and the number is expected to continue growing fast. Alzheimer's disease is the most prevalent type of dementia and may account for 60-70% of cases. Several studies state that air pollution, especially at particulate matter 2.5 micrometers (PM2.5) and smaller, contributes to Alzheimer's related changes in the brain. These changes in the brain include neuroinflammation, tau phosphorylation, microglia activation, and oxidative injury. Pollution related changes in the brain were investigated in study designs including *in vitro*, *in vivo*, and brain autopsies after sudden death. Our ultimate goal is to determine if dust and particulate matter from rural Northern Colorado increases neurotoxicity. To address this, we plan to expose primary neuronal and glial cells with PM collected outside dairies in the Northern Colorado area. We will then assess the levels of inflammatory signaling and neurotoxicity in neurons and mixed glial cultures (astrocytes and microglia cells). We will expose cells to PM and examine mRNA and protein expression of common inflammatory markers, C-C Motif Chemokine 2 (CCL-20), C-C Motif Chemokine 5 (CCL-5), and Nitric Oxide Synthase 2 (NOS2), to name a few. Individual and co-culture studies will also be performed using primary neuronal cultures to determine the role of glial cells on neurotoxicity following pollutant exposure. These studies will increase the understanding of neurotoxic mechanism caused by common pollutants.

Graduate Student / Environmental and Radiological Health Sciences

## 48. Treatment of thymoma with intensity-modulated stereotactic body radiation therapy or non-modulated hypofractionated radiation therapy: retrospective study of fifteen canines

*Erin Trageser, Susan LaRue, Tiffany Martin, Del Leary, and Mary-Keara Boss*

Canine thymomas are routinely treated with radiotherapy (RT). We investigate the response and toxicity of canine thymoma treated with hypofractionated intensity-modulated stereotactic body radiation therapy (SBRT) relative to dogs treated with hypofractionated non-modulated radiation therapy (NMRT). We theorized that SBRT would result in fewer radiation toxicities and tumor control would be improved or comparable to NMRT. A retrospective study was performed of dogs with thymoma treated with RT (n=15; SBRT n=8, NMRT n=7). SBRT protocols ranged from 20-30 Gy delivered in 1 to 3 daily fractions; NMRT protocols ranged from 24-36 Gy delivered in 2 to 5 daily or weekly fractions. Following SBRT, 2 dogs (50%) experienced partial responses (PR) and 2 (50%) had stable disease (SD). Following NMRT, 1 dog had PR (33%) and 2 dogs had SD (66%). Median PFS was 116 days (range 66-727 days) for the SBRT group and 134 days (range 10-405 days) for the NMRT group. The MST for the SBRT group was 218.5 days (range 1-727 days) and 169 days (range 10-405 days) for NMRT. Reported acute and late side effects were limited to the lungs and heart and were more common in the NMRT (71%) compared to the SBRT group (13%). The decreased incidence of side effects in the SBRT group was statistically significant when compared to the NMRT group ($p=0.04$). We suggest similar treatment efficacy may be provided for canine thymoma treated with either approach, but SBRT provides the clinical benefit of decreased incidence of side effects and completion of RT in a shorter time.

Resident / Environmental and Radiological Health Sciences

## 49. Evaluation of SARS-CoV-2 infection and transmission in domestic livestock

*Audrey E. Walker, Lauren Guilbert, Stephanie Porter, Airn Hartwig, Daphne Hawvermale, Aimee Pepper, Richard A. Bowen, Angela Bosco-Lauth*

SARS-CoV-2 (COVID-19) has spread to nearly every country worldwide and concerns regarding the impacts of this virus continue to rise as human case numbers increase. SARS-CoV-2 likely originated through spillover from an animal reservoir, but the exact mechanism remains unknown. Thus, it is important to understand whether other species can serve as reservoir hosts or become clinically infected. Livestock play a crucial role in the human food supply and are frequently in close contact with the human population. Therefore, infection of livestock could cause major ramifications to animal and human health, food security, the economy and trade if SARS-CoV-2 becomes established in livestock populations. Due to these concerns, this study evaluated susceptibility of goats and cattle to SARS-CoV-2 infection. We found that goats and calves do not become clinically ill when intranasally inoculated with SARS-CoV-2 and infectious virus could not be detected from nasal or rectal swabs. However, SARS-CoV-2 was isolated from the trachea of a calf three days post-inoculation, which indicates some level of susceptibility to infection. Further studies involving serology and viral tissue loads are ongoing and will be crucial to evaluate to what extent livestock are susceptible to SARS-CoV-2 and what role they may play, if any, as reservoirs for the virus.
**Funding:** Animal Models Core

DVM Student / Biomedical Sciences

## 50. Microenvironmental immune effects of stereotactic radiotherapy and immunotherapy in canine solid tumors

*Remy Watts, Lauren Harrison, Leone Hopkins, Erin Trageser, Steven Dow, Mary-Keara Boss*

Purpose: Combining radiotherapy with immunotherapy is a novel therapeutic approach to treating cancer. In this study, we characterize the microenvironmental immune effects of canine tumors treated with stereotactic body radiation therapy (SBRT) and local injection of agonistic OX40/TLR9 immunotherapy (SBRTi) compared to SBRT+saline control (SBRTc).

Methods: A randomized case-matched prospective study was performed in dogs with solid tumors. Tumor samples were obtained pre- and 2 weeks post-treatment. Immunohistochemistry (IHC) analysis of immune cell density was performed with antibodies against CD3 (T cells), CD79α (B cells), CD204 (macrophages), and FoxP3 (Tregs) using VIS image analysis software. Nanostring gene expression analysis was performed using a customized 48 gene immune panel.

Results: Tissue samples from the first 5 dogs of the study were analyzed: SBRTc (n=2, melanomas), SBRTi (n=3; melanoma, carcinoma, sarcoma). From IHC analysis, the mean percentage of intratumoral Tregs increased following SBRTc (+111.5%; range= 67.4-155.5%) and decreased following SBRTi (-78.7%; range= -71.4 to -86.1%). The mean percentage of T cells increased following SBRTc (+68.2%; range= 3.4- 133.3%) and decreased following SBRTi (-57.1%; range= -37.4 to -76.7%). The mean percentage of macrophages decreased post-treatment for both SBRTc (-51.3%; range= -32.3 to -70.4%) and SBRTi cases (-39.8%; range= -15.3 to -64.2%). No pattern was found for intratumoral B cells. There is an emerging trend where expression of TReg-related genes (CTLA4, FoxP3, GATA3, Lag3) is increased post-SBRTc and decreased post-SBRTi.

Conclusion: These results suggest that SBRTi suppresses immunosuppressive Tregs in the tumor microenvironment compared to SBRTc.

DVM Student / Biomedical Sciences

## 51. Polyunsaturated fatty acid metabolism contributes to age-related impairment of cardiac mitochondrial calcium tolerance

*maxwellLuke A Whitcomb, Lance C Li Puma, Philip T Zilhaver, Cheyenne S Izon, Adam J Chicco*

Acute myocardial ischemia and subsequent reperfusion experienced during a heart attack causes a pathologic increase in cardiomyocyte mitochondrial calcium ($Ca^{++}$) concentration, which triggers a series of events that contribute to cell death and myocardial necrosis. Previous studies in our lab and others have shown that metabolites of arachidonic acid (AA), an omega-6 polyunsaturated fatty acid, contribute to mitochondrial permeability transition pore (mPTP) opening in response to $Ca^{++}$ overload, leading to mitochondrial swelling, rupture, and release of reactive oxygen species (ROS). We hypothesized that previously reported age-related increases in these parameters result in part from greater mitochondrial production of AA from its PUFA precursor linoleic acid (LA) in response to $Ca^{++}$ overload. To test this hypothesis, we evaluated the effects of 50-400 μM $Ca^{++}$ on $O_2$ consumption, ROS release and mPTP opening in cardiac mitochondria isolated from young (2-3 months) and older (24 months) aged BALB/c mice in the presence or absence of a pharmacological inhibitor of delta-6 desaturase (D6D), the rate-limiting enzyme in the production of AA from LA. Results confirmed that cardiac mitochondria from old mice release more ROS during oxidative phosphorylation and undergo more mPTP opening in response to $Ca^{++}$ overload than mitochondria from young mice. D6D inhibition significantly attenuated these responses in both young and old mitochondria ($P < 0.05$), but had greater effects in old, largely abolishing the effect of aging on both outcomes. These findings extend recent evidence from our lab that heterozygous knockdown of the D6D gene (Fads2) improves cardiac mitochondrial $Ca^{++}$ tolerance and reduces myocardial injury following ischemia, while Fads2 overexpression exacerbates mitochondrial $Ca^{++}$ intolerance and ischemic injury. Taken together, these studies identify D6D as a potential therapeutic target for mitigating the pathological effects of mitochondrial $Ca^{++}$ overload during myocardial ischemia-reperfusion and perhaps other cardiovascular pathologies.

Graduate Student / Biomedical Sciences

## 52. Human Clinic vs Animal Agriculture: Comparison of *Escherichia coli* Antimicrobial Resistance

*Reed Woyda, Ade Oladeinde, Zaid Abdo*

Antimicrobial resistance is on the rise worldwide. The CDC estimates that per year at least two million people will become infected by a drug-resistance bacteria and at least 23,000 will die as a result. The World Health Organization recently reported that during the COVID-19 pandemic 72% of hospitalized patients received antibiotics while only 8% demonstrated overlying bacterial or fungal co-infections. Additionally, the pandemic is causing disruptions to treatments, for example patients with tuberculosis, which leads to a potential increase in drug-resistant pathogens. There is an urgent need to better understand the spread of antimicrobial resistance in human populations, how resistance-conferring genes are shared among bacterial pathogens and how prevalent these are within human and agricultural settings. Agricultural use of antimicrobial agents is known to impact the treatment of human disease. Increased prevalence in the food-borne pathogens *Salmonella*, *Escherichia coli* and *Campylobacter* has led to limitations in therapeutic agents and has resulted in an increase in treatment failures and unfavorable clinical outcomes. To understand the relationship between antimicrobial resistance elements in agricultural and human pathogens we have surveyed over 700 *Escherichia coli* isolates taken from both poultry agriculture and human clinical isolates in the United States. Through our study we have identified antimicrobial resistance elements common to both poultry agricultural and human clinical isolates, determined their distribution within the United States and evaluate if transfer of antimicrobial resistance elements is likely between these two populations. **Funding:** NIH T32 Fellowship

Graduate Student / Cell and Molecular Biology

## 53. Impacts of the COVID-19 Pandemic on Access to Veterinary Care Within Animal Shelters, Rescues and Humane Societies

*Rachel E. Wertheimer, Katherine A. McCaw, Zachary W. George, Sage Smith, Colleen Duncan, Danielle Frey*

While the COVID-19 global pandemic generated significant fear and uncertainty for people and businesses both nation- and worldwide, the animal sheltering industry faced an entirely unique set of challenges. To better understand these challenges, we conducted an anonymous survey of volunteers and staff members of animal shelters, foster-based rescues, and humane societies. The survey – consisting of 26 multiple choice, matrix table, and open-ended questions – was designed to explore various impacts of the pandemic on these organizations, with an emphasis on changes in their ability to access veterinary care. Respondents to this survey were asked a variety of questions relating to their experiences with caring for unowned animals, both before and after the start of the pandemic. In addition to describing previously existing barriers to care, services offered, and operational concerns, this survey also sought to parse out changes which arose as a direct result of the pandemic. The survey was completed by 1,226 volunteers and employees of animal shelters and rescues across all 50 states. Overall, the study revealed widespread challenges associated with declining levels of funding, staff and volunteer support, which were further compounded by fixed to increasing demands for services. Despite these considerable obstacles, a positive shift towards the utilization of technology was also noted, with 93% of organizations planning to keep their newly integrated telemedicine or remote adoption services even after the threat of the pandemic has passed. Although it may still be many years until we are able to fully describe the true impacts of the COVID-19 pandemic, the insights and strategies gleaned from this research serve as a foundation of understanding on which animal care organizations can continue to build, grow, and prepare for the future. **Funding:** PetSmart Charities

DVM Student / Other

| Name | Date | Poster | Oral |
|---|---|---|---|
| Alfino | 1/30 | | 2:00, Session 2 |
| Auerbach | 1/30 | | 12:00, Session 2 |
| Bashor | 1/28 | 11:30, Session 2 | |
| Baxter | 1/30 | | 2:15, Session 2 |
| Berezin | 1/28 | 11:30, Session 3 | |
| Bergum | 1/28 | 12:00, Session 2 | |
| Bonilla | 1/27 | 6:30, Session 1 | |
| Buglewicz | 1/28 | 1:00, Session 3 | |
| Cao | 1/27 | 4:00, Session 3 | |
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| Cooper | 1/30 | | 2:30, Session 2 |
| Crowdis | 1/28 | 3:00, Session 1 | |
| Curtis | 1/30 | | 12:15, Session 2 |
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| Davis | 1/27 | 5:00, Session 3 | |
| DeFilippo | 1/28 | 1:00, Session 2 | |
| Deluty | 1/28 | 11:00, Session 1 | |
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| Eklund | 1/27 | 6:00, Session 3 | |
| Fennel | 1/27 | 4:00, Session 1 | |
| Flores | 1/30 | | 3:30, Session 2 |
| Flynn | 1/27 | 5:00, Session 2 | |
| Foley | 1/27 | 4:30, Session 1 | |
| Fox | 1/27 | 6:30, Session 3 | |
| Frank | 1/30 | | 12:5, Session 1 |
| Fukushima | 1/30 | | 1:00, Session 1 |
| Geldert | 1/30 | | 3:30, Session 1 |
| George | 1/28 | 10:30, Session 1 | |
| Georges | 1/30 | | 12:00, Session 3 |

| Name | Date | Poster | Oral |
|---|---|---|---|
| Gilberto | 1/28 | 3:00, Session 2 | |
| Gregory | 1/30 | | 1:15, Session 1 |
| Guilbert | 1/27 | 5:30, Session 2 | |
| Haines | 1/28 | 11:30, Session 1 | |
| Harris | 1/30 | | 12:15, Session 3 |
| Harrison | 1/28 | 2:30, Session 2 | |
| Hayes | 1/30 | | 12:30, Session 3 |
| Heise | 1/28 | 11:00, Session 2 | |
| Henry | 1/30 | | 12:30, Session 2 |
| Hicks | 1/28 | 2:00, Session 3 | |
| Labb | 1/30 | | 12:45, Session 2 |
| Lake | 1/28 | 1:30, Session1 | |
| Latham | 1/28 | 2:00, Session2 | |
| Liebig | 1/28 | 10:30, Session 3 | |
| Logsdon | 1/30 | | 12:45, Session 3 |
| MacNeill | 1/27 | 5:30, Session 1 | |
| Maldonado | 1/30 | | 1:30, Session 1 |
| Manchester | 1/30 | | 1:00, Session 3 |
| Marquez-DiPaulo | 1/28 | 2:30, Session 1 | |
| Mathias | 1/28 | 12:30, Session 1 | |
| Maxwell | 1/28 | 1:00, Session 1 | |
| Mazariegos | 1/28 | 10:00, Session 2 | |
| McCaw | 1/28 | 10:00, Session 1 | |
| McCoy | 1/27 | 6:00, Session 1 | |
| Merriman | 1/27 | 6:30, Session 2 | |
| Moore | 1/30 | | 1:15, Session 3 |
| Moskaluk | 1/30 | | 1:00, Session 2 |
| Nealon | 1/30 | | 1:30, Session 3 |
| Omar | 1/30 | | 1:15, Session 2 |
| Parkinson | 1/30 | | 12:00, Session 1 |
| Pearce | 1/28 | 12:30, Session 3 | |
| Petch | 1/27 | 6:00, Session 2 | |
| Pezzanite | 1/27 | 5:00, Session 1 | |

| Name | Date | Poster | Oral |
|---|---|---|---|
| Pfluger | 1/30 | | 2:00, Session 1 |
| Richards | 1/28 | 2:30, Session 3 | |
| Rutten | 1/30 | | 2:00, Session 3 |
| Sauerwein/Koycheva | 1/30 | | 2:15, Session 3 |
| Schlein | 1/30 | | 2:30, Session 3 |
| Schlemmer | 1/30 | | 2:45, Session 3 |
| Smith | 1/28 | 10:00, Session 3 | |
| St Clair | 1/30 | | 3:00, Session 3 |
| Stalnaker | 1/28 | 1:30, Session 2 | |
| Starkey | 1/28 | 3:30, Session 1 | |
| Stewart, H. | 1/30 | | 3:15, Session 3 |
| Stewart, J. | 1/28 | 12:30, Session 2 | |
| Timkovich | 1/30 | | 3:30, Session 3 |
| Towner | 1/28 | 12:00, Session 3 | |
| Townsend | 1/28 | 3:00, Session 3 | |
| Trageser | 1/28 | 11:00, Session 3 | |
| Vick | 1/30 | | 3:15, Session 1 |
| Walczak | 1/30 | | 2:15, Session 1 |
| Walker | 1/28 | 12:00, Session 1 | |
| Watts | 1/28 | 3:00, Session 3 | |
| Waugh | 1/30 | | 1:30, Session 2 |
| Wertheimer | 1/28 | 10:30, Session 2 | |
| Whitcomb | 1/27 | 4:00, Session 3 | |
| Williams | 1/30 | | 2:30, Session 1 |
| Wilson | 1/30 | | 2:45, Session 1 |
| Worthington | 1/30 | | 3:00, Session 1 |
| Woyda | 1/27 | 4:00, Session 2 | |
| Lederman | 1/28 | 2:00, Session 1 | |
| | | | |