Scientific Proceedings

17TH ANNUAL RESEARCH DAY
JAN. 30, 2016
HILTON FORT COLLINS

Colorado State University
College of Veterinary Medicine
and Biomedical Sciences

SPONSORS

ROYAL CANIN

MERIAL

zoetis

BioMARC Biopharmaceutical Manufacturing & Academic Resource Center

CSUVENTURES

Colorado State University
College of Veterinary Medicine and Biomedical Sciences

ON THE COVER: Colorado State University graduate student Rachel West is among more than 150 undergraduate students, graduate students, veterinary residents, and post-doctoral fellows participating in the 2016 Research Day. The day gives trainees in the College of Veterinary Medicine and Biomedical Sciences a showcase for their research efforts and findings.

CONTENTS

OUR 17TH ANNUAL RESEARCH DAY showcases the work of more than 150 aspiring scientists in Colorado State University's College of Veterinary Medicine and Biomedical Sciences. The day gives our rising stars vital experience presenting their research findings to a scientific audience through poster displays and talks. The day also provides young researchers with an avenue for feedback to help them develop ideas that, in many cases, will become lifelong scientific pursuits. In a sign of significance, the research projects on display are sponsored by two dozen well-respected companies, foundations, and institutions concerned with improving human, animal, and environmental well-being. Thank you for supporting and engaging with our presenters – undergraduate students, graduate students, veterinary residents, and post-doctoral fellows – as they pursue research that will help animals, people, and the planet!

SCHEDULE of Events

11:30-NOON	POSTER SET UP	SALON III, IV
NOON	OPENING REMARKS – Dr. Sue VandeWoude Associate Dean for Research	SALON II
12:10 p.m.	ZOETIS RESEARCH EXCELLENCE AWARD WINNER – Dr. Sheryl Magzamen	SALON II
12:45 p.m.	BREAK	
1-5 p.m.	ORAL SESSION I: Clinical Science	SALON I
1-5 p.m.	ORAL SESSION II: Basic Science	SALON II
1-5 p.m.	ORAL SESSION III: Basic Science	SALON V
1-2:45 p.m.	POSTER SESSION I JUDGING: Odd-Numbered Posters	SALON III, IV
3-5 p.m.	POSTER SESSION II JUDGING: Even-Numbered Posters	SALON III, IV
5-6 p.m.	SOCIAL HOUR	SALON III, IV
6 p.m.	AWARDS	SALON III, IV

DEPARTMENTAL ABBREVIATIONS

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

Just breathe
Epidemiologist studies factors impacting lung health

BY KRISTEN BROWNING-BLAS

FOR RESPIRATORY RESEARCHER SHERYL MAGZAMEN, ASKING BIG QUESTIONS IN EPIDEMIOLOGY IS AS NATURAL AS BREATHING.

The assistant professor in CSU's College of Veterinary Medicine and Biomedical Sciences enjoys looking at the big picture, as well as detailed data about the effects of air pollution and pesticides on lung health.

"I started long-distance running in high school. Running totally cleared my mind, because I was so focused on getting in my next breath. Usually, we just take breathing for granted, it's something we don't pay attention to until it doesn't function properly," Magzamen said. "It's been something that has carried me since I started my research in 1998. There's no shortage of good questions to ask."

These days, Magzamen is investigating the combined influence of air pollution and pesticides on childhood asthma and the lung ailment known as chronic obstructive pulmonary disease – research that has attracted funding from the National Institutes of Health. On Jan. 30, she will receive the Zoetis Research Excellence Award from her college and will be the guest faculty speaker at its annual Research Day.

UNDERGRAD CLASS SPARKED CAREER

The rising researcher was inspired to start her career path when she took a class about comparative health policy while pursuing a bachelor's degree in biology from Cornell University.

"I started to understand how different countries plan their health-care systems, and it was fascinating. That was the first time I understood health from a macro viewpoint, where supporting health wasn't necessarily about interacting with a patient, but planning a system that could influence an entire population," she recalled.

Drawn to the idea of large-scale decision-making, she went on to earn a master's degree in public health from Emory University. Magzamen worked for a time on tobacco-control policy in the political arena but discovered she prefers the rigors of science.

So back to school she went, this time earning a Ph.D. from the University of California, Berkeley, School of Public Health.

"Epidemiology has been such a good fit for me. It's really about puzzles because most epidemiology tends to be observational in nature. We look at people as they live in their community – what are they exposed to, how they get sick, how they behave," said Magzamen, who is based in the Department of Environmental and Radiological Health Sciences.

NIH GRANT SUPPORTS COMPLEX THINKING

In 2014, she won a $461,000 Career Development Award from the National Institutes of Health for a three-year project to study the effects of vehicle emissions and pesticide use on children with asthma in California's San Joaquin Valley, where busy highways intersect with heavy commercial agricultural use.

The challenge in studying environmental mixtures like air pollution is, "How do we capture the whole environment?" Magzamen said. "In science, we tend to look at one factor at a time and try to capture the impact of that one exposure, but we know that doesn't happen in real life.

"The idea is that if you have high levels of some kind of pesticide and high levels of diesel pollution, what is your risk of having decreased lung function compared to someone with high levels of diesel pollution but low levels of pesticide? Or, high levels of pesticide but low levels of diesel? The more things we study, the more infinitely complex this gets."

2016 ZOETIS RESEARCH EXCELLENCE AWARD

Sheryl Magzamen, an assistant professor of epidemiology in the Department of Environmental and Radiological Health Sciences, has been honored with the 2016 Zoetis Research Excellence Award. Magzamen will kick off 2016 Research Day with a keynote speech about her work in respiratory disease. Magzamen will receive a plaque and $1,000 honorarium. In keeping with proud tradition, global animal health company Zoetis sponsors Research Day and the Research Excellence Award.

Magzamen often uses the phrase "infinitely complex," yet the effort to understand complexities motivates her.

"The part I'm excited about is looking at these combinations and our analytic strategies to capture the total environment. So not just one exposure at a time, but multiple exposures – smoke, pesticides, wildfires, lead, arsenic in the soil," she said.

CLASSROOM, COLLABORATION BALANCE RESEARCH LOAD

Magzamen reconciles the infinite patience required for such longitudinal research with the more immediate rewards of interacting with students in her graduate-level class, Geographic Information Systems and Health.

"That's one of the fun things about being here – the incremental approaches you take in research are balanced out by the immediate gratification of teaching. I couldn't imagine just doing research and being patient enough to wait for the long term outcomes. Here, I also get to work with great students, and that's a really nice balance."

She credits CSU's collaborative environment for support that led to the NIH grant, one of seven currently funded nationally for promising young researchers in the environmental health sciences.

One of her collaborators is Stephen Reynolds, director of the High Plains Intermountain Center for Agricultural Health and Safety. "Sheryl is good at building partnerships and she brings a new perspective from epidemiology," Reynolds said. "She's really creative, so it adds a spark."

SESSION 1: Clinical Science

1–5 p.m. | SALON I

1:00	Ball	Genetic modification of mesenchymal stem cells with scAAV-equine-BMP-2 to induce osteogenesis: an "off the shelf" treatment for fracture repair | **CS**
1:15	Barron	Investigation of the pharmacokinetics of transdermal ondansetron in normal purpose-bred cats | **CS**
1:30	Contreras	Evidence for genetic predisposition to Borrelia burgdorferi infection in purpose-bred beagles | **CS**
1:45	Cooley	Survey of subcutaneous fluid practices in cats with chronic kidney disease | **CS**
2:00	Doster	Investigating the effect of tulathromycin exposure on potential microbial community function in feedlot cattle during the early feeding period using shotgun metagenomics | **CS**
2:15	Herdrich	Accuracy of the single needle technique to the three compartments of the equine stifle | **CS**
2:30	Hunvald	Novel immunotherapy utilizing cancer stem cell targeted vaccine for improved immune system control of cancer | **CS**
2:45		BREAK
3:00	Ledesma-Feliciano	Feline foamy virus infection of domestic cats: immune cell phenotyping and IgG antibody response | **MIP**
3:15	Martin, K	Evaluation of factors influencing accelerometry activity data in dogs | **CS**
3:30	Martin, L	The impact of local weather on European badger (Meles meles) capture success: implications for bovine tuberculosis management | **CS**
3:45	Ouyang	Utility of Electronic Medical Record Data for Healthcare-Associated Infection Detection with Fever Sequella | **CS**
4:00	Sato	A retrospective study on use of leflunomide in dogs with immune mediated diseases | **CS**
4:15	Stroda	The Pharmacokinetics of Cyclophosphamide Administered Orally, Intravenously, or Intraperitoneally in Cats | **CS**
4:30	Summers	Assessment of repeated administration of a feline herpesvirus-1, calicivirus, and panleukopenia virus vaccine as a model for interstitial nephritis | **CS**
4:45	Wennogle	Fluorescence in situ hybridization identifies occult bacterial infection in gallbladder mucoceles | **CS**

SESSION 2: Basic Science

1–5 p.m. | SALON II

1:00	Adney	Vaccination of Camels Against MERS Coronavirus and Camel-to-Camel Transmission of Virus **| MIP**
1:15	Bacon	Identification of clathrin and dynamin II in porcine oocytes support the presence of clathrin-mediated endocytosis **| BMS**
1:30	Bromberek	A golden opportunity: using dogs to understand human diseases | MIP
1:45	Chiu	Feline leukemia virus: a risk to endangered felids? **| MIP**
2:00	Dejyong	Analysis of risk of African swine fever virus introduction into Thailand during 2015 - Development of strategy **| CS**
2:15	Dietz	International Surveillance of Antimicrobial Resistant Bacteria in Diverse Farm, Water, and Wastewater as Sources for Human Exposure **| ERHS**
2:30	Eddy	Enzymatic isolation and viability assessment of canine ovarian primordial follicles **| BMS**
2:45		BREAK
3:00	Fagre	Serosurvey for infectious pathogens in horses in Colorado **| CS**
3:15	Fauver	Description of the virome of Anopheles gambiae mosquitoes from West Africa **| MIP**
3:30	Hernandez	Whole genome sequence analysis of canine transitional cell carcinoma of the bladder **| CS**
3:45	Hughes	Gene expression signature of T zone lymphoma\leukemia in dogs **| MIP**
4:00	Johnson, S	Racing performance in Quarter Horses undergoing prosthetic laryngoplasty **| CS**
4:15	Kane	The good, the bad, and the ugly of the prion protein **| MIP**
4:30	Klippenstein	Chemotherapeutic targeting of molecular pathways associated with osteosarcoma metastatic potentia**l | CS**
4:45	Krajacich	Use of mosquito bloodmeals as epidemiological tools to study malaria transmission **| MIP**

SESSION 3: Basic Science

1–5 p.m. | SALON V

1:00	Lakin	Artificial intelligence in biomedical science: utilizing machine learning for antimicrobial resistance gene discovery in metagenomic data | **CS**
1:15	Li Puma	FADS2 expression modulates myocardial ischemic tolerance in mice | **BMS**
1:30	Lowery	Histological features of California sea lion (Zalophus californianus) pups from a die-off at San Miguel Island, California | **MIP**
1:45	Malmberg	Evidence for frequent lentiviral transmission from bobcats to mountain lions in California and Florida: Implications for emergence of lentiviral epidemics | **MIP**
2:00	Mangalea	Breaking biofilms: nitrate inhibits biofilm formation in Burkholderia pseudomallei | **MIP**
2:15	McWhorter	LIN28 regulates androgen receptor in the placenta | **BMS**
2:30	Miller	Novel vaccination strategies for feline immunodeficiency virus | **MIP**
2:45		BREAK
3:00	Schwerdtfeger	Intestinal – Microbial Interactions in an Ex Vivo Slice Model | **BMS**
3:15	Selwyn	Uncovering prion strain structural differences via examination with epitope-mapped antibodies and chaotropic agents | **MIP**
3:30	Shivley	Associations between average daily gain and calf health, feeding and management practices in preweaned dairy heifer calves in the U.S. | **CS**
3:45	Smith, M	Association of genetic risk alleles and the loss of anergic, high affinity, insulin-specific B cells in type 1 diabetes | **MIP**
4:00	Stenkamp-Strahm	Climate, lactation, and treatment factors influence shedding of virulent dairy Escherichia coli O157 | **CS**
4:15	West	The Lin28B-let-7-Hmga2 axis regulates human trophoblast cell differentiation | **BMS**
4:30	Willett	Tumor microenvironment: A critical determinant of cancer progression and therapeutic efficacy that can be maintained ex vivo | **BMS**
4:45	Zingale	Effects of iatrogenic blood contamination on cerebrospinal fluid total nucleated cell count and protein concentration | **MIP**

POSTER PRESENTATIONS

SESSION 1 | ODD-NUMBERED POSTERS | 1-2:45 p.m.
SESSION 2 | EVEN-NUMBERED POSTERS | 3-5 p.m.

NOTE: Poster numbers precede names and correspond to numbers listed on the floor plan at the back of this document

1	Adams	Indoor hockey officials' hearing threshold shifts and effect of helmet visor length on exposure to whistle noise **| ERHS**
2	Ali	CRISPR/Cas9-based Genome Editing to Investigate the Role of Lin28A and Lin28B in Regulation of Human Trophoblast Cell Differentiation **| BMS**
3	Alturki	TERRA in the Telomeric DNA Damage Response **| ERHS**
4	Alyami	Different factors contribute to increased expression of IGF2BP1 in human and canine osteosarcoma **| CS**
5	Andrade	PI3K-Akt signaling pathway association with oocyte competence **| BMS**
6	Arrieta	Communication among the three compartments of the equine stifle joint **| CS**
7	Barbosa	Effect of cryoprotectants and maturation status of oocytes on post-thaw cleavage and blastocyst rates **| BMS**
8	Bartner	Bartonella spp. PCR assay results using cerebrospinal fluid of naturally exposed dogs with central nervous system disease **| CS**
9	Bender	Crispr/Cas9 as a treatment for prion disease **| MIP**
10	Benham	Timing of superovulation and embryo collection in North American bison **| BMS**
11	Bickett	Innate immunity induced by BCG **| MIP**
12	Boostrom	Canine Cutaneous Plasmacytosis: A Retrospective of 21 cases (2005-2015) **| CS**
13	Borresen	Public health nutrition for chronic disease control and prevention with rice bran and beans **| ERHS**
14	Brody	Dual-energy x-ray absorptiometry scans of sedated and awake cats with the VetMousetrapTM device **| CS**
15	Brown	Metabolomics Investigation of Xenobiotics and Metabolic Pathway Networks in Tumors, Adjacent Mucosa and Stool from Colorectal Cancer Patients **| ERHS**
16	Bunkers	Cystolith dissolution in cats using a commercially available diet **| CS**
17	Caress	Investigation of whether Leptospira vaccinal antibodies react with Borrelia peptides used in a commercial assay **| CS**

18	Charley	Targeting of the cellular exoribonuclease XRN1 appears to be a shared strategy among several families of RNA viruses | **MIP**
19	Cheng	Influence of bone protein vaccine on macrophage recruitment and healing following allograft reconstruction of a massive bone defect | **CS**
20	Cleymaet	Opioids attenuate light-evoked firing of intrinsically photosensitive retinal ganglion cells via increasing a voltage-gated K+ current | **BMS**
21	Cunningham	Mercury and selenium partitioning in Steller sea lion blood compartments | **CS**
22	Del Monte	Clinical data & prevalence of pathologic levels of copper in canine hepatic cytology | **MIP**
23	Dirsmith	Low pathogenicity avian influenza virus maternal antibody transfer among captive mallards (Anas platyrhynchos) | **MIP**
24	Dubin	Correlation of Mycoplasma quantitative PCR to severity of conjunctivitis in cats | **CS**
25	Earnest	In vitro comparison of three suture methods for closure of pelvic flexure enterotomy in normal horses | **CS**
26	Edmondson	Genome mapping for loci that control differential strain susceptibility to lymphoid and non-lymphoid hematopoietic neoplasms in mice | **ERHS**
27	Faulhaber	Patterns of PD-L1 Expression by Canine Tumors and Association with T Cell and Myeloid Cell Infiltrates | **CS**
28	Fletcher	Effector MAIT cells in Johne's Disease Cattle | **MIP**
29	Foos	Time Motion Evaluation of Repeated Lumbosacral Flexion in Dairy Workers | **ERHS**
30	Gallegos	Optimizing Digital Droplet PCR to quantify mRNA expression levels in naïve and Mtb infected mouse models | **MIP**
31	Garcia	Fgfr1 and Fgfr2 protein expression in canine osteosarcoma | **MIP**
32	Glapa	Comparison of blood progesterone values obtained from an in-house one hour enzyme linked fluorescent immunoassay (ELFA) or radioimmuno assay (RIA) | **CS**
33	Gonzalez-Castro	Sorting of equine sperm using a microfluidic device as a method of sperm selection for IVF and ICSI | **BMS**
34	Gullberg	Intracellular lipid content impacts Dengue particle infectivity | **MIP**
35	Heck	A methyl-specific RNA-binding protein is highly expressed in induced pluripotent stem cells | **MIP**
36	Heim	Seasonal cold exposure modulates metabolic phenotype and mitochondrial function in obese golden-mantled ground squirrels | **BMS**
37	Hein	A high fat diet versus regular chow drives an inflammatory cytokine profile in fat depots in obese guinea pigs | **MIP**
38	Hennet	Localization of bacteria during cases of equine bacterial endometritis |**CS**
39	Herndon	Pharmacokinetics of intravenous and subcutaneous dolasetron and pharmacodynamics of subcutaneous dolasetron in purpose-bred cats | **CS**
40	Hill, D	Preliminary investigation of overgrown hooves in Colorado cervids | **MIP**

41	Hill, E	The predictive value of in vivo drug assays against Mycobacterium abscessus | **MIP**
42	Hura	The Use of Equine Bone-Marrow Derived Stem Cells as a Potential Treatment Against Preformed Biofilms | **CS**
43	Jalkanen	Post-transcriptional mechanisms coordinate expression of zinc finger protein mRNAs | **MIP**
44	Jeon	Evaluation of antineoplastic effects of JQ1 against a panel of canine lymphoma-derived cell lines | **CS**
45	Johnson, T	Coagulopathy in Prairie Rattlesnake (Crotalus viridis) envenomation: with carbon monoxide releasing molecule - 2 increases clot strength and attenuates fibrinolysis | **MIP**
46	Knox	DNA damage response signaling inhibition differentially affects canine osteosarcoma cell radiosensitivity | **ERHS**
47	Kopanke	Effects of Low-level Brodifacoum Exposure on the Feline Immune Response | **MIP**
48	Lake	PharmCat: a physiologic-based pharmacokinetic (PBPK) model to study virtual drug dosing in cats | **CS**
49	Lakey	Guinea Pig Bone Marrow Derived Macrophages Alter Glucose Metabolism Under Mycobacterium tuberculosis Infection | **MIP**
50	LeCureux	Increased mucosal immunogenicity of L. acidophilus expressing HIV MPER and utilizing adjuvants IL-1β or FliC | **MIP**
51	Lee, E	Thermal Imaging as an Alternative to PIT Tagging for Monitoring Body Temperature and Clinical Disease Progression in Mice | **MIP**
52	Lee, J	The value of diversity: A genomic analysis of historical and contemporary blue tongue virus isolates | **MIP**
53	Lesser	Evaluation of the equine mental foramen nerve block: foramina anatomic positioning and cadaveric evaluation of needle placement and injectate distribution | **CS**
54	Liebig	Fertility may depend on conceptus-derived signals in lactating Holstein cows | **BMS**
55	MacMillan	Prevalence of select infectious disease agents in client owned cats in Moscow, Russia | **CS**
56	Malmlov	Robust expression of early innate immunity genes in dromedary camel plasmacytoid dendritic cells in response to MERS-CoV | **MIP**
57	Manchester	Coxiella burnetii DNA not identified in fleas from domestic cats in Australia and the United States | **CS**
58	Mann	[18F]-FDG positron emission tomography – an innovative technique for the diagnosis of canine lameness | **ERHS**
59	Martin	Transposon mutagenesis of potential cyclic-di-GMP metabolic genes in Burkholderia pseudomallei to characterize their function in biofilm formation and motility | **MIP**
60	McKenna	Telomere length and telomerase activity as biomarkers in astronauts | **ERHS**
61	McNamara	Differentiation of canine induced pluripotent stem cells into neural progenitor cells | **MIP**
62	McNeilly	Developing 3D tractography maps for identification of parafascicular corridors to improve surgical access to deep brain tumors in dogs | **CS**
63	Miller	Assessment of digital venograms in 24 polo horses | **CS**
64	Minor	Ectoparasites and vector-borne pathogens of dogs in Baja California Sur | **CS**

65	Mirassou-Wolf	Public Health Needs Assessment and Proposed WaSH Solutions: Leadership Training in the Tro Pang Cho Commune, Cambodia | **ERHS**
66	Moezzi	Relationship of Hepatic Copper Concentrations and Histological Changes in the Dog | **CS**
67	Montgomery	Biomarkers for antimicrobial resistance: A pilot study identifying novel antimicrobial drug resistance markers for developing clinically-applicable detection and therapeutic tools | **CS**
68	Moore	A canine model for studying the role of the cellular prion protein in cancer cells | **MIP**
69	Morrissey	Prediction of pregnancy survival after embryo transfer based on initial ultrasound pregnancy examination | **CS**
70	Nealon	Rice bran in the presence and absence of probiotics differentially alters the porcine large intestinal and serum metabolome for enhanced protection against human rotavirus infection | **ERHS**
71	Nelson	Recognition and processing of telomeric double strand breaks in human cellsm | **ERHS**
72	Ortega	Detection of Prions on Plants Collected from Rocky Mountain National Park | **MIP**
73	Pannone	Desmin immunostaining does not differentiate mesothelial hyperplasia from malignant mesothelioma in dogs | **MIP**
74	Porter	Experimental infection of horses and sheep with MERS coronavirus | **BMS**
75	Powers	Feline leukemia virus distribution in a privately held colony of domestic cat-leopard cat hybrids | **MIP**
76	Pyuen	In vitro effects of PI3K/mTOR inhibition in canine hemangiosarcoma | **CS**
77	Racchini	Sensory substitution via electro-tactile stimulation of the tongue | **BMS**
78	Radakovich	Evidence of inflammaging and gastrointestinal bleeding in old dogs | **MIP**
79	Ramos	The mini-pig as a neonatal TB vaccine efficacy animal model| MIP
80	Rout	Unique features of immunoglobulin gene use and mutation status in Boxers with chronic lymphocytic leukemia | **MIP**
81	Schilling	Reference ranges in blood hematology in the Alaskan sled dog | **CS**
82	Schwartz	The Effect of C-DIM12 on Mitigating the Development of Inflammation in Cultured Equine Chondrocytes and Synoviocytes | **CS**
83	Seaman	Repeat Ivermectin Mass Drug Administrations for the control of Malaria (RIMDAMAL): a randomized pilot safety and efficacy study | **MIP**
84	Sedam	Inflammatory response to advanced glycation end products in the murine subcutaneous air pouch model | **MIP**
85	Shields	Emerging Roles of Synaptotagmin: Modeling Neurogenic Disease in Drosophila | **BMS**
86	Shivley	Evaluation of doctor of veterinary medicine program curricula on animal welfare, animal behavior, and animal ethics courses | **CS**
87	Shropshire	Serial evaluation of thromboelastography and platelet aggregometry in healthy dogs | **CS**
88	Smith, S	In vitro evaluation of the effects of pre-conditioning on female canine adipose-derived mesenchymal stem cell cytokine production | **CS**

89	Smith, A	The neural regulation of feeding: anorexigenic circuits | **BMS**
90	Stout	Detection of tuberculosis antibodies and clinical signs in lions in Kruger National Park, South Africa | **CS**
91	Stutzman-Rodriguez	Comparison of qPCR and ELISA for assessment of Felis catus gammaherpesvirus 1 infection of domestic cats | **MIP**
92	Su	Induction of Cytotoxic and Genotoxic Responses by Novel Glycerol Glucoside | ERHS
93	Tangtrongsup	Effect of serum and N-acetyl-L-cysteine on chondrogenesis of equine bone marrow-derived mesenchymal stem cells | **CS**
94	Taylor	Effects of venom from the Prairie Rattlesnake (Crotalus viridis) and the Western Diamondback Rattlesnake (Crotalus atrox) on coagulation and fibrinolysis in equine plasma: in vitro evaluation using thromboelastography | **MIP**
95	Thomas	The effect of micropatterned surfaces on biofilm formation in dogs with indwelling urinary catheters | **CS**
96	Trundell	Establishment of minimum inhibitory concentrations (MIC) for ciprofloxacin for bacterial organisms cultured from the mare reproductive tract | **CS**
97	Vallejos	Age-Dependent Shal Channel Stability in Neurons | **BMS**
98	Varnum	Creating awareness of tuberculosis caused by Mycobacterium bovis | **CS**
99	Verma	Reversal of phenotypic resistance of Anti-mycobacterial Compounds against Non tuberculosis mycobacteria | **MIP**
100	Wesley	Myxoma virus causes cytopathic effects in canine osteosarcoma cells | **MIP**
101	Whitaker	FADS2 Overexpression Promotes Metabolic Syndrome in Mice: Influence of Maternal Dietary Polyunsaturated Fatty Acid Composition | **BMS**
102	Willingham	Elucidating mother to offspring transmission of chronic wasting disease using a transgenic mouse model | **MIP**
103	Wolfel	Central corneal pachymetry measurements in dogs using the Pentacam Scheimpflug topography system | **CS**
104	Worcester	Lactobacillus paracasei fermentation of rice bran extract reduces Salmonella Typhimurium growth in vitro | **ERHS**
105	Zellar	A comparison of tricaine methanesulfonate and alfaxalone as an immersion anesthetic in two tropical fish species | **CS**
106	Zhang	Localization and Stability of a Toxic mRNA in a Cell Culture Model of Type I Myotonic Dystrophy | **MIP**

VETERINARY SUMMER SCHOLARS PROGRAM

DVM STUDENTS DIVE INTO RESEARCH WITH PROJECTS AND FIELD TRIPS

APPLY BY 2 P.M. FEB. 5, 2016!

BY THE NUMBERS

- 25 scholars in the 2015 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.
- 247 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
- About 60 faculty mentors

SPONSORS OF THE 2015 PROGRAM:

Merial Limited
National Institutes of Health
Morris Animal Foundation
American Society of Lab Animal Practitioners
University of Alaska, Fairbanks
Royal Dick School of Veterinary Science, Scotland
CSU College of Veterinary Medicine and Biomedical Sciences

Students in the CSU Veterinary Summer Scholars Program visit Rocky Mountain National Park to learn about opportunities in veterinary wildlife research.

VETERINARY SUMMER SCHOLARS PROGRAM was initially established through support from Merck-Merial to provide an opportunity for veterinary schools to expose students, in their first and second years, to biomedical research. The current Veterinary Student Scholars Program gives veterinary students hands-on exposure to veterinary medical research to introduce them to potential research careers. The application deadline is Feb. 5 for the summer 2016 program!

The College of Veterinary Medicine and Biomedical Sciences, which hosts the program, recently received funding from the National Institutes of Health to expand the very successful program next year!

Last year, 25 veterinary students from CSU and abroad participated in the 2015 CSU Veterinary Summer Scholar Program. Students spent the summer working in research labs, attending weekly research seminars, and going on field trips to other CSU, federal, and state research facilities. Many of the projects conducted by CSU students last summer are being presented today at the CVMBS Research Day.

Merial, a multinational animal health company, supports 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 2015, or to apply for the summer 2016 program, please visit the website at: http://csu-cvmbs.colostate.edu/dvm-program/Pages/Veterinary-Scholars-Program.aspx

YOUNG INVESTIGATOR GRANT PROGRAM: FUNDING RESEARCH AND BOOSTING VET STUDENTS

CENTER FOR COMPANION ANIMAL STUDIES, DEPARTMENT OF CLINICAL SCIENCES

Dr. Dan Smeak is one of the 25 Colorado State University faculty members supporting veterinary students with a research project that was funded through the Young Investigator Grant Program.

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 2015, corporate and non-corporate sponsors donated more than $77,000 to the program. This funding was distributed to 25 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 nine 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 D.V.M. 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 D.V.M. students in important clinical research.

To view the grants funded in 2015 or to make a donation, please visit the Center for Companion Animal Studies website at: http://csu-cvmbs.colostate.edu/vth/veterinarians/research/companion-animals/Pages/student-projects.aspx

2015 YOUNG INVESTIGATOR GRANT PROGRAM SPONSORS

PLATINUM SPONSORS

Merial Limited
Royal Canin

GOLD SPONSORS

Bayer Animal Health
Boehringer Ingelheim Vetmedica
Dechra
HESKA Corporation
IDEXX Laboratories
Merck Animal Health
Nestle Purina PetCare
Zoetis Animal Health
Veterinary Centers of America
Vetoquinol

SILVER SPONSORS

Hill's Pet Nutrition and SCAVMA

BRONZE SPONSORS

Canine Rehabilitation Institute
International Veterinary Seminars

ORAL PRESENTATIONS Clinical Science

1–5 p.m. | SESSION I - SALON I

Genetic modification of mesenchymal stem cells with scAAV-equine-BMP-2 to induce osteogenesis: an "off the shelf" treatment for fracture repair

Alyssa N. Ball, Jennifer N. Phillips, R. Jude Samulski, and Laurie R. Goodrich

Fracture treatment in horses is fraught with difficulties. Bone marrow derived mesenchymal stem cells (BMDMSCs) have shown efficacy in their ability to accelerate healing of connective tissue injuries, including bone. Further, literature supports an osteo-induction of BMDMSCs in response to bone morphogenic protein-2 (BMP-2). The objective of this study is to develop an "off-the-shelf" BMDMSC product for osteogenesis that will decrease time to callous formation, and result in accelerated fracture repair. Equine BMDMSCs were transduced with scAAV-equine-BMP-2 or scAAV-GFP in cell culture monolayer with doses ranging from 4,000-48,000 viral particles per cell. Transduction efficiency was confirmed on days 4 and 7 qualitatively through fluorescent imaging of scAAV-GFP treated cells, or quantitatively through BMP-2 ELISA. Cells treated with scAAV-equine-BMP-2 were also given a morphology score, stained for calcium matrix deposition and alkaline phosphatase activity, and subjected to alkaline phosphatase enzyme activity. scAAV-GFP cells were cryopreserved, thawed after liquid nitrogen storage, and then subjected to flow cytometry. Cells were compared to scAAV-GFP transduced cells that were not subjected to cryopreservation. Statistics were performed using one-way ANOVA with Tukey's post hoc. Data suggests BMDMSCs should be transduced with 48,000 viral particles per cell (vpc) as their morphology appeared most osteogenic at this dose. Further, by day 7, cells transduced with 48,000 vpc stained more osteogenic than cells transduced with a lower dose. Cells transduced with 48,000 vpc also produced significantly more BMP-2 protein on days 4 and 7 post transduction than cells transduced with other doses ($p<0.0001$). Through flow cytometry quantification and by fluorescent microscopy we confirmed scAAV-GFP genetically modified cells retain their induced phenotype and do not have differences in GFP expression following cryopreservation. Genetically modifying equine BMDMSCs prior to cryopreservation induces sustained secretion of equine BMP-2. After cryopreservation, cells may enhance fracture repair when combined with current clinical standards.

Graduate Student/ Clinical Sciences

Investigation of the pharmacokinetics of transdermal ondansetron in normal purpose-bred cats

Lara Zajic Barron, Andrea Herndon, Liberty Sieberg, Amber Caress, Leigh Davis, Ryan J Hansen, Luke A Wittenburg, Daniel L. Gustafson, and Jessica M. Quimby

Ondansetron is a 5-HT3 receptor antagonist used as an anti-emetic in ill cats. Ondansetron can be dosed orally, IV, or subcutaneously but has previously been demonstrated to have poor oral bioavailability and a short elimination half-life requiring frequent dosing (every 6-8 hours). Because of the impracticality of dosing (>3 doses/day) at home, it is often resigned to in-hospital administration. Ondansetron is a candidate for a transdermal medication because it is small in size (294 Daltons) and is moderately lipophilic (log p ~ 2.1). The purpose of this study was to assess the pharmacokinetics of transdermal ondansetron administration in healthy, purpose-bred cats. Five purpose-bred cats with unremarkable CBC, biochemistry and urinalysis were utilized. 2 mg transdermal ondansetron Lipoderm gel was applied once to the internal ear pinna (total volume of 0.1ml). Blood samples were collected via jugular catheter over a 48 hour period following administration (0, 15min, 30min, 1hr, 2hr, 4hr, 8hr, 12hr, 24hr and 48hr). Serum was separated and frozen prior to analysis. Ondansetron was measured via liquid chromatography coupled to tandem mass spectrometry. Analysis revealed no appreciable drug was present in serum after transdermal administration of 2 mg ondansetron, indicating that this is not an acceptable method of drug delivery despite the characteristics of the drug that imply that it would adequately pass the skin barrier. This study highlights the importance of assessing the potential of each medication for transdermal administration.

DVM Student/ Clinical Sciences

Evidence for genetic predisposition to Borrelia burgdorferi infection in purpose-bred beagles

Elena T. Contreras, Scott Moroff, and Michael R. Lappin

A genetic predisposition to *Borrelia burgdorferi* (BB) infection or clinical illness has been suggested in people and dogs with Lyme nephritis (Bernese mountain dogs, Retriever breeds). In vaccine studies, infestation with wild-caught *I. scapularis* ticks usually results in at least a 75% BB infection rate. A current *I. scapularis* infestation study resulted in only a 29% infection rate. The purpose of this study was to investigate genetic relatedness and seropositive status in those dogs. Ten female and 14 male purpose-bred beagles, 9-12 months of age, and negative for antibodies against *A. phagocytophilum* (AP), BB, and *E. canis* (Accuplex-4 BioCD system; Antech Diagnostics), were infested for 7 days with *I. scapularis*. Blood was collected weekly and screened for antibodies. Canine pedigrees were tabulated. Statistical analyses included Wilcoxon rank-sum and Fisher's exact tests. BB antibodies were detected in 29.2% of dogs (n=7/24); all positives were females (p=0.0003); there were no significant differences in the dogs' ages or number of ticks recovered or fed. AP antibodies were detected in 41.7% of dogs (n=10/24). Previous publications using the same source of dogs, ticks, and model demonstrated 77.8% (BB) and 55.5% (AP) seropositivity, respectively. Infection rates in the current versus historical study were similar for AP but significantly lower for BB in the current study (p = 0.004). A sibling or parent was the most recent common ancestor (MRCA) for 6 of the 7 (86%) BB-positive dogs versus 8 of the 17 (47.1%) BB-negative dogs (p=0.09). None of the BB-positive dogs had a MRCA sibling or parent in common with any of the BB-negative dogs. These findings provide further evidence that a genetic predisposition to *B. burgdorferi* infection exists. As studies have investigated common genotypes and alleles in human Lyme disease, this should also be considered in future studies of canine Lyme borreliosis.

Postdoctoral Fellow/ Clinical Sciences

Survey of subcutaneous fluid practices in cats with chronic kidney disease

Crystal M. Cooley, Liberty G. Sieberg, Sarah Caney, and Jessica M. Quimby

Chronic kidney disease (CKD) is common in elderly cats. The purpose of this study was to describe subcutaneous fluid (SQ) administration practices of owners of CKD cats to help more owners successfully give SQ fluids to their cat. An anonymous web-based survey was advertised via list serves, websites and social media. Owners of 468 cats with CKD participated. 87% of cats were 10 years or older. Cats were IRIS stage I (1%), II (20%), III (37%), IV (17%), and unknown (25%). 95% of owners said they discussed giving fluids with their veterinarian. 399 respondents stated they gave SQ fluids, 57 did not, and 12 tried but could not. Only 42% of owners were given additional educational resources. 79% said the process was ok/easy to learn. Once experienced, 15% said it was still somewhat/highly stressful on them, and 11% said it was somewhat/highly stressful for the cat. To improve tolerance 57% used food for positive reinforcement with 59% stating this improved tolerance, 60% warmed the fluids and 83% felt warming fluids increased tolerance. 74% felt that length of time it took to administer fluids affected tolerance. 82% said needle size affected tolerance. 40% of owners checked hydration status daily or twice daily and 18% of owners did not know how. 43% said they skipped/added fluids based on hydration assessment. The majority of owners were successful in administering fluids but additional education materials could be provided. Variables such as needle size, warming fluids, and length of time of administration may improve tolerance.
Resident/ Clinical Sciences

Investigating the effect of tulathromycin exposure on potential microbial community function in feedlot cattle during the early feeding period using shotgun metagenomics

Enrique Doster, Pablo Rovira, Noelle R. Noyes, Brandy A. Burgess, Xiang Yang, Maggie Weinroth, Lyndsey Linke, Roberta Magnuson, Kenneth Jones, Christina Boucher, Jaime Ruiz, Keith E. Belk, and Paul S. Morley

Shotgun metagenomics, facilitated by next-generation sequencing, represents a novel approach to investigate bacterial communities. The goal of this study was to use bioinformatic analysis to understand the impact of metaphylactic tulathromycin (Draxxin) exposure on the microflora of cattle in the early feeding period. Tulathromycin is a macrolide antibiotic, the most commonly used class of antibiotics in livestock production, and is commonly used to treat bovine respiratory disease. Two pens of cattle in a Texas feedlot were selected for this study. One pen was chosen to receive 800 mg of tulathromycin while the other was chosen for the control. Individual fecal samples from the rectal-anal junction were collected at arrival processing and 11 days into the feeding period. Selected fecal samples from treated (n=30) and control (n=30) animals from both sampling times were subjected to total DNA extraction for metagenomic sequencing. Sequenced reads were trimmed for poor quality nucleotides and filtered to remove bovine DNA. To evaluate the microbial community, the bioinformatic tools BWA, Humann2, and Kraken as well as various genetic databases were utilized to identify differences in the number of sequences aligning to known antimicrobial genes (resistome), metabolic functional genes, and genomes for taxonomic profiling (microbiome) respectively. Statistical comparison of community data matrices from metagenomic samples necessitates using multiple techniques such as cumulative sum scaling, Hellinger transformation, and the employment of both zero-inflated multivariate models and non-metric multidimensional scaling of Euclidean distances. Preliminary results suggest that exposure to tulathromycin during arrival processing exerts a relatively small effect on the microflora composition in treated cattle whereas the transition into the feedlot exerts a greater effect on the composition of microbial communities in all cattle. Shotgun metagenomics allows an extraordinary glimpse into complex bacterial communities, but the challenge lies in accurately interpreting the biological relevance of next-generation sequencing results.
DVM/PhD Student/ Clinical Sciences

Accuracy of the single needle technique to the three compartments of the equine stifle

Meredith R.A. Herdrich, Shelby E. Arrieta, Brad B. Nelson, David D. Frisbie, and Valerie J. Moorman

Intra-synovial stifle joint injections are commonly utilized in equine veterinary medicine, and there are many reported techniques for injection into the medial femorotibial (MFTJ), lateral femorotibial (LFTJ), and femoropatellar (FPJ) joints. A single needle approach to all three compartments has been described, but exact needle placement and accuracy of the technique have not been previously reported. The authors hypothesize that this single needle technique allows accurate injection into each of the three compartments of the equine stifle. Four individuals performed this technique on 24 cadaver stifles. Thirty mL of contrast, tap water, and food coloring was injected into each compartment. Radiographs were obtained following each injection to determine needle and contrast location. Each stifle was then dissected to determine color distribution. Descriptive statistics were calculated. Needle insertion depth and angle of insertion were determined, as well as accuracy of injection. Joint injection accuracy was 91.67% (22/24), 91.67% (22/24), and 100% (24/24) for the MFTJ, LFTJ, and FPJ, respectively. The most common anatomic needle location was axial femoral condyle (MFTJ and LFTJ) and middle third of the femoral trochlea (FPJ). The average proximal-distal needle angle was 82° (MFTJ), 80° (LFTJ), and 16.6° (FPJ), the average medial-lateral needle angle was 27.5° (MFTJ), 7.2° (LFTJ), and 6.8° (FPJ), and the average needle depth was 5.71cm (MFTJ), 5.83cm (LFTJ), and 5.58cm (FPJ). Results of this investigation demonstrate the high accuracy of the single injection technique and provide guidelines for accurate needle placement both externally and using radiographic guidance.

DVM Student/ Clinical Sciences

Novel immunotherapy utilizing cancer stem cell targeted vaccine for improved immune system control of cancer

Stacey J. Hunvald, Genevieve Hartley, Lyndah Chow, Daniel Regan, Amanda M. Guth, and Steven W. Dow

Background/Rationale. Cancer stem cells (CSCs) are a small but highly persistent subset of tumor cells that are resistant to chemotherapy and radiation therapy and are an attractive target for immunotherapy. Finding ways to guide the immune system to fight resistant CSCs is critical in combating the recurrence and metastasis that is usually the cause of cancer mortality in both human and veterinary patients. *We hypothesize that targeting CSCs specifically with CSC vaccines will more effectively control tumor growth than conventional cancer vaccines*. Approach: BALB/c mice (n = 5 per group) bearing CT26 colon carcinomas and C7BL/6 mice bearing PyMT mammary sarcomas received weekly vaccine treatments derived from tumor cell lines cultured in either conventional medium or CSC enrichment medium. Vaccines were created by combining tumor cell lysates with optimized cancer vaccine adjuvant. Antitumor immune responses were analyzed via tumor measurement, T-cell response assays and immunohistochemical analysis of CSC populations in tumor tissues Results. Animals vaccinated with lysates from tumor cells grown in stem cell enrichment conditions had suppressed tumor growth, greater T cell responses, and reduced populations of CSC than unvaccinated mice or mice immunized with conventional tumor vaccines. Discussion. These results indicate that tumor vaccines targeting CSC antigens can more effectively induce tumor immunity than conventional cancer vaccines. A CSC vaccine study is forthcoming in dogs with cancer to determine the translational relevance of these findings.

DVM Student/ Clinical Sciences

Feline foamy virus infection of domestic cats: immune cell phenotyping and IgG antibody response

Carmen Ledesma-Feliciano, Ryan Troyer, Esther Musselman, Martin Löchelt, and Sue VandeWoude

Feline foamy virus (FFV) is a retrovirus that has been regarded as apathogenic despite persistent infection in domestic cats. Because of this, FFV carries potential applications in vaccine vector and gene therapy development. To verify apathogenicity and further understand immune response we inoculated cats (n=4/group) with either wild-type FFV ($TCID_{50}$ of 2.78E5 IU/ml) or sham control and collected blood over 176 days. For immune cell phenotyping, whole blood was processed through the Q-Prep method to antibody-label and fix white blood cells that were afterwards analyzed by flow cytometry. Two panels were devised: Panel A determined CD4+ and CD8+ T lymphocyte numbers expressing CD25+ or CD134+ (activation) or Fas (apoptosis) markers. Panel B assayed CD56+ (natural killer cells), CD21+ (B cells), and CD14+ (monocytes) expressing MHC class II (activation) or Fas markers. Specific antibody-fluorophore combinations were: A) CD4-FITC, CD8-PE, CD25-PE/Cy7, CD134-647, Fas-APC/Cy7; B) MHCII-FITC, CD14-PE, CD21-PE/Cy7, CD56-APC, Fas-APC/Cy7. To determine IgG antibody response against FFV Gag structural and Bet accessory proteins, cat sera was subjected to a novel enzyme-linked immunosorbent assay (ELISA) using glutathione crosslinked to casein as the capture protein to bind recombinant Gag and Bet proteins fused to bacterial N-terminal glutathione S-transferase (GST) and compared to reference sera. Cutoff values were determined by comparing negative control and antigen absorbance values. Regarding immunophenotyping, there were no consistently statistically significant differences between wild-type and control cats. IgG response against FFV Gag and Bet proteins was detected using the GST-capture ELISA method and correlated to presence or absence of peripheral blood mononuclear cell proviral DNA viremia (PCR.) Based on this, we can conclude that GST-capture ELISA is a reliable detection assay. Immunophenotyping comparisons between negative and wild-type infected cats did not produce discernible differences. Antibody response and white blood cell population trends over time are currently being analyzed.

Postdoctoral Fellow/ Microbiology, Immunology and Pathology

Evaluation of factors influencing accelerometry activity data in dogs

Kyle W. Martin, Anastasia M. Olsen, Colleen G. Duncan, and Felix M. Duerr

Accelerometry-based activity monitoring is a promising new tool in veterinary medicine used to objectively assess activity in companion animals. It is unknown whether device orientation and attachment of a leash to the collar holding an accelerometer will affect activity monitoring. It was our goal to evaluate whether attachment methods of accelerometers affect activity monitoring. Eight healthy, client-owned dogs were individually fitted with two identical neck collars to which two identical activity monitors were attached using six different methods of attachment. For trials where the effect of leash attachment to the collar was not being studied, the leash was attached to a harness. Activity data obtained from separate monitors within a given experiment were compared using Pearson correlation coefficients. The correlation between sensors was compared across all experiments using a Kruskal-Wallis Test with post-hoc pairwise comparisons. Significance levels were adjusted using the Bonferroni correction. There were poor correlations between sensors in three experiments: when the leash was fastened to the collar that held an activity monitor, when one activity monitor was housed in the manufacturer-provided protective casing, and when one activity monitor was loosely zip-tied to the collar rather than threaded on using the provided metal loop. While accelerometer-based activity monitors are useful tools to objectively assess physical activity in dogs, care must be taken when choosing a method to attach the device. The attachment of the activity monitor to the collar should be standardized and remain consistent throughout a study period.

DVM Student/ Clinical Sciences

The impact of local weather on European badger (Meles meles) capture success: implications for bovine tuberculosis management

Laura E. R. Martin, James O'Keeffe, Andrew W. Byrne, and Francisco J. Olea-Popelka

Bovine tuberculosis (bTB) affects livestock and wildlife around the world. Badgers (*Meles meles*) are an important wildlife reservoir in Ireland and Britain, which complicates disease eradication efforts. In the Republic of Ireland, badgers have been managed through culling to control bTB, but vaccination using the intramuscular Bacillus Calmette-Guérin (BCG) vaccine is becoming the new goal. However, for vaccination to be effective, badger trapping success must be high to deliver vaccines to a large proportion of badgers in a given population (coverage). In this study, we examined the effect of local weather on badger capture success. We compiled data from badger captures during 2010-2013 as part of a bovine TB badger vaccine trial in County Kilkenny, Ireland. We compared Poisson, zero-inflated Poisson, negative binomial, and zero-inflated negative binomial models to evaluate factors affecting badgers capture numbers. In our preliminary analysis, we found that badger captures were significantly higher in drizzle (25%, $p = 0.01$), rain (22%, $p = 0.02$), and heavy rain (32%, $p = 0.006$), and significantly lower in snow (72%, $p = 0.03$) compared to dry weather. There was a quadratic relationship between temperature and badger captures. We are currently continuing to analyze additional data to account for variation trapping effort (traps laid per sett). Our results provide valuable information for prioritizing trapping based on local weather conditions. Effectively vaccinating badgers will be a crucial aspect and a key step toward the goal of eradication of bovine TB in Ireland.

Graduate Student/ Clinical Sciences

Utility of Electronic Medical Record Data for Healthcare-Associated Infection Detection with Fever Sequella

Ben Ouyang, Brandy Burgess, and Paul Morley

Healthcare-associated infections (HCAI) are a major concern for infection control programs. HCAI surveillance is often complicated by variations in subjective interpretations of the patient's clinical status. Fever is a common indicator of infectious processes, and is consistently recorded in the electronic medical record (EMR). Patients that develop a fever during their stay in the hospital are more likely to have acquired HCAI. This study reports the usability of patient rectal temperatures in HCAI surveillance. Data for rectal temperatures (> 102.5F) were extracted from the EMR for a 30 month period. On average, there was 1 temperature recorded per day per patient. 50,926 (53.79%) visits had temperatures entered into the EMR. 92.87% of visits without recorded temperatures lasted one day or less and were excluded from the analysis. 1,122 (2.65%) canine visits and 216 (2.51%) feline visits exhibited temperature patterns suspicious for HCAI. Examination of the dataset shows that not all data is captured by the EMR. More critical patients often receive multiple temperature checks throughout the day. However, only one temperature is entered into the EMR per day for each patient. Further, a large number of visits do not have temperatures recorded. While a large proportion of these visits last one day or less, best practices would encourage a physical exam and entry of findings into the EMR, regardless of length of stay. In spite of missing data, patient temperatures may still be useful in detecting HCAI. Rectal temperatures are one diagnostic component used to assess patient health. While limited to one recording per day, temperatures are consistently recorded for visits of length greater than 1 day. This population of visits is at a higher risk of developing HCAI. Records of all temperatures taken during patient visits would likely improve the detection of HCAI with fever sequella.

Resident/ Clinical Sciences

A retrospective study on use of leflunomide in dogs with immune mediated diseases

Masahiko Sato, Julia K. Veir, Marie Legare, and Michael R. Lappin

The purpose of this retrospective study was to report safety and efficacy of leflunomide for the treatment of naturally occurring immune mediated diseases in dogs. Medical records from 1995-2014 at a USA Veterinary Teaching Hospital were retrospectively searched for dogs with immune mediated diseases administered leflunomide. Data that were extracted from the medical records included signalment, bodyweight, underlying indication for leflunomide, dose of leflunomide, treatment duration, concurrent medications, treatment response, and adverse events. A total of 92 cases were included. The mean starting dose of leflunomide was 1.79 ± 0.8 mg/kg/day. The median duration of the use of leflunomide was 23.5 weeks. Adverse events which could be related to leflunomide administration included diarrhea (3 of 92, 3.3%), lethargy (2 of 92, 2.2%), suspect blood dyscrasia (3 of 92, 3.3%), thrombocytopenia (2 of 31, 6.5%) and increased liver enzyme activities (1 of 16, 6.3%). Leflunomide was discontinued due to the possible adverse events in 6 dogs (6.5%). Significant dose differences between dogs with adverse events (n=11, 2.6 ± 0.8 mg/kg/day) and dogs without adverse events (n=81, 1.7 ± 0.8 mg/kg/day) were found ($P < 0.001$). The treatment response could be evaluated in 9 dogs with suspected immune mediated polyarthritis (IMPA), 7 dogs with immune mediated thrombocytopenia (IMTP), and 1 dog with cutaneous histiocytosis (CH). Of these 17 dogs, 12 dogs (70.5%; 7 dogs with IMPA, 4 dogs with IMTP, 1 dog with CH) had an apparent positive response to the use of leflunomide. There was no significant difference ($P=0.18$) in doses between dogs that responded to leflunomide (2.0 ± 0.8 mg/kg/day) and those that did not respond (1.5 ± 0.4 mg/kg/day). A lower starting dose of leflunomide of 2 mg/kg/day than the current suggested dose of leflunomide of 3-4 mg/kg/day is recommended based on the findings in this study.

Resident/ Clinical Sciences

The Pharmacokinetics of Cyclophosphamide Administered Orally, Intravenously, or Intraperitoneally in Cats

Katherine Stroda, Susan Lana, Jackie Murphy, Elizabeth Atencio, Lisa Brownlee, Ryan Hansen, and Daniel Gustafson

Introduction - Cyclophosphamide is a chemotherapeutic drug used commonly in many species. It can be administered intravenously, orally or intraperitoneally. It is assumed that systemic exposure to the active metabolite, 4-hydoxycyclophosphamide (4-OHCP), is the same with any route of administration. No pharmacokinetic data exists in the cat to confirm this. The objective of this study was to characterize the pharmacokinetics of cyclophosphamide and 4-OHCP in the plasma of normal cats when administered orally, intravenously, or intraperitonealy. We hypothesized that no difference would be detected in the quantity of the active metabolite, 4-OHCP. Methods – Six normal cats were used. They were randomly assigned to receive 200 mg/m2 of cyclophosphamide via one of the 3 dosing routes and then after a 28 day wash out period, were crossed over to the other dosing group. Plasma samples were collected at various time points for 8 hours following dosing. Samples were immediately treated with semicarbazide hydrochloride in order to trap the 4-OHCP in stable form. Cyclophosphamide and 4-OHCP were measured using mass spectrometry. Exposure to parent drug and metabolite was calculated and maximum concentration (Cmax), clearance (CL), area under the curve (AUC0-t), half-life (t1/2), and time to maximum concentration (T_{max}) determined. Toxicity was monitored and graded according to VCOG-CTCAE 1.1. Results- Cyclophosphamide was well tolerated regardless of route used. Only grade 1 GI toxicity was seen. Bioavailability was 100% for intraperitoneal and 81% for oral administration. Conclusion- Bioavailability for 4-OHCP was equivalent for intraperitoneal and intravenous routes. Oral administration was less bioavailable, likely due to variability in absorption.

Resident/ Clinical Sciences

Assessment of repeated administration of a feline herpesvirus-1, calicivirus, and panleukopenia virus vaccine as a model for interstitial nephritis

Stacie C. Summers, Shannon McLeland, Jennifer R. Hawley, Jessica Quimby, Randall Basaraba, Catriona MacPhail, and Michael R. Lappin

Interstitial nephritis (IN) is the primary cause of feline chronic kidney disease. The Crandell Rees feline kidney (CRFK) cell line is commonly used to grow feline herpesvirus-1 (FHV-1), calicivirus, and panleukopenia virus used in (FVRCP) vaccine production. Previous studies have shown that cats administered parenteral FVRCP vaccines develop antibodies against CRFK lysates and alpha-enolase and these antibodies can bind to feline renal cell lysates. In addition, three of six cats that were hyperinoculated with CRFK lysates over two years had IN on renal biopsy collected two weeks after the last booster. The primary objective of this study was to determine whether IN could be induced over 16 weeks by repeatedly administering a market leading parenteral FVRCP vaccine to potentially use as a short term model to study biomarkers associated with IN in cats. A total of six one-year-old purpose bred cats were included in the study. On Week 0, blood, serum, and urine were collected for biomarker assays and a wedge kidney biopsy for histopathological evaluation and alpha-enolase immunohistochemistry was obtained. All cats were administered a commercially available FVRCP vaccine on Weeks 2, 4, 6, 8, 10, 12, and 14 and samples were collected for biomarker assays. On Week 16, renal biopsies were repeated. Haematoxylin and eosin stained sections were provided to two board-certified pathologists that were masked to the timing of the biopsies. Anti-CRFK and anti-enolase antibodies levels in serum were determined by ELISAs. All 6 cats developed progressively increasing anti-enolase and anti-CRFK serum antibodies. Histological evidence of IN was not detected by light microscopy in any of the tissue biopsies. Significant biochemical or urinalysis changes during the study were not detected and the cats were adopted to private homes. Results of selected biomarkers and enolase immunohistochemical staining will be used to further evaluate this potential IN model.

Resident/ Clinical Sciences

Fluorescence in situ hybridization identifies occult bacterial infection in gallbladder mucoceles

Sara A. Wennogle, David C. Twedt, and Kenneth W. Simpson

Gallbladder mucocele is a commonly recognized form of gallbladder disease in the dog that is characterized by cystic mucinous hyperplasia and insidious accumulation of viscous bile and mucus within the gallbladder. Although several predisposing factors have been proposed, the underlying etiology of gallbladder mucocele formation is unknown. Previous studies have found limited associations with cholecystitis or bacterial infection. The aim of this study was to utilize culture-independent, fluorescence in situ hybridization (FISH) to determine the presence of bacteria in gallbladder mucoceles with and without concurrent cholecystitis. Electronic medical records at Colorado State University were reviewed for cases of histopathologically confirmed gallbladder mucocele between December 2010 and January 2015. 29 cases were available and suitable for evaluation. Formalin-fixed, paraffin-embedded gallbladder sections were mounted on charged glass slides and evaluated by FISH using a eubacterial probe (5Cy3-EUB-338) concurrently with a control probe (56 FAM-Non-EUB-338). Sections were examined on a BX51 epifluorescence microscope, and images were captured with an Olympus DP-7 camera. Histopathology revealed cystic mucinous hyperplasia in all cases, with associated cholecystitis noted in 13/29(45%) cases. Bacteria were detected by eubacterial FISH in 8/29 (28%) cases. Bacteria were visualized as multifocal clusters of short rods within the mucus (n=1), adherent to the wall (n=1), or within the parenchyma (n=8) in 6/29 (21%) gallbladders. In 2 cases, a single bacterium was observed within the gallbladder parenchyma. Of the 6 cases with multifocal bacteria, 4 had concurrent cholecystitis. Bacterial culture of mucocele contents was positive in only 1/24 cases, yielding moderate growth of *Escherichia coli*. FISH detected the presence of bacteria in 28% of mucoceles evaluated. Our results reveal that bacterial infection is more common than previously thought, and support the need for further studies to examine the relationship between gallbladder mucoceles, cholecystitis, and bacterial infection.

Resident/ Clinical Sciences

ORAL PRESENTATIONS Basic Science

1–5 p.m. | SESSION II - SALON II

Vaccination of Camels Against MERS Coronavirus and Camel-to-Camel Transmission of Virus

Danielle R. Adney, Vincent J Munster, Laurie A. Baeten, Ralph Baric, Neeltje van Doremalen, Trenton Bushmaker, Megan Miller, Emmie de Wit, and Richard A. Bowen.

The Middle Eastern respiratory syndrome coronavirus (MERS-CoV) was detected in 2012 and is associated with severe respiratory disease in humans. Efficient human-to-human transmission is possible, as documented by the recent outbreak in South Korea. However, continuous zoonotic introductions from camels appear to play an important role in transmission. Up to 100% of camels are seropositive in some areas, indicating widespread and efficient circulation of MERS-CoV. Experimentally inoculated animals develop a transient upper respiratory tract infection and shed large quantities of virus in nasal secretions. This study examined the hypothesis that vaccination with a Venezuelan equine encephalitis replicon expressing the MERS-CoV spike protein will protect camels from infection and virus shedding. Four subadult dromedaries were vaccinated and boosted by subcutaneous injection on days 0 and 21, and intranasally boosted on day 56. Two control subadult dromedaries were vaccinated in tandem with a replicon expressing H1N1 hemagglutinin. None of the vaccinated animals developed neutralizing antibodies against MERS-CoV. Thus, we decided to examine the additional hypothesis that inoculated animals would efficiently transmit MERS-CoV to uninfected animals. The four vaccinated dromedaries were infected with a human isolate of MERS-CoV on day 79, and the two control animals were cohoused with the inoculated animals beginning on day 81. All of the inoculated dromedaries shed virus beginning 1 day after infection, while both control animals shed virus beginning 2 days after exposure to infected animals. This study indicates that this vaccine platform is a poor candidate for protection of camels and that infected dromedaries efficiently transmit virus to contacts.

Graduate Student/ Microbiology, Immunology and Pathology

Identification of clathrin and dynamin II in porcine oocytes support the presence of clathrin-mediated endocytosis

Margaret L. Bacon and Douglas C. Eckery

Primordial follicles represent the entire reproductive potential of a female during her lifetime. The feral swine population in the United States causes an estimated $1.5 billion annually in damages and control. Destroying primordial follicles in the porcine ovary could be a permanent fertility control method used to address this overly abundant wildlife population. Relatively little is known about what types of cellular communication mechanisms are utilized in primordial follicles. The purpose of this study was to investigate whether primordial follicles utilize a form of receptor mediated endocytosis known as clathrin-mediated endocytosis (CME). This process, if present, could be manipulated as a method to deliver ovotoxins to the primordial follicle pool. This study focused on locating two components of this process, clathrin and dynamin II. Ovaries from 6 piglets and 6 gilts were bisected longitudinally, fixed in formalin, embedded in paraffin, and cut and mounted on slides. Fluorescent immunohistochemistry (IHC) was performed on the mounted tissues. The antigen of interest, clathrin or dynamin II, was bound by antibodies to allow visualization via fluorescein isothiocynate (FITC). Expression of clathrin and dynamin II was revealed in the cytoplasm of oocytes of all follicular stages, suggesting that CME could be a mechanism of cell signaling in porcine oocytes. Using this information, future research will focus on investigating the ligands that are taken up by primordial follicles and on the formulation of reproductive modulators that could cause permanent sterility.

Graduate Student/ Biomedical Sciences

A golden opportunity: using dogs to understand human diseases

Julia Bromberek, Ingegerd Elvers, Janna Yoshimoto, Jeremy Dossey, and Anne Avery

Non-Hodgkin lymphoma (NHL) is the most common hematopoietic neoplasm in both humans and dogs. NHL is a heterogeneous disease with an uncertain etiology; very few risk factors in humans or dogs have been identified. Some rare human NHL subtypes that are challenging to study are relatively common in dogs, presenting an ideal setting to use dogs as a model of human disease. T zone lymphoma (TZL) appears to have a striking predilection for Golden Retriever dogs, suggesting a genetic risk factor for this disease. Purpose: To identify genetic risk factors for canine TZL. Methods: We performed a genome-wide association study of Golden Retrievers aged 9 years and older, including 100 cases of TZL and 155 controls. The Illumina CanineHD BeadChip was used for genotyping. Odds ratios and p-values were calculated using Plink software, adjusting for population stratification. A significance level of 10^{-4} was used. Results: Eleven SNPs on chromosome 14 spanning 9.7M to 11.4M base pairs were significantly associated with TZL, with ORs ranging from 0.79–0.82. This region includes the gene POT1, which is responsible for regulating telomere length and protecting chromosome ends from unstable recombination. In addition, four SNPs spanning 11.7–11.8M were significantly associated with TZL, with ORs of 1.21–1.22. Multiple genes encoding hyaluronidases, including SPAM1 and HYAL4, are found in this region. Conclusions: The pathogenesis of canine TZL may be related to dysregulation of telomere length and hyaluronidase. We will explore this finding by assessing whether the function of telomerase and hyaluronidose is altered in dogs with TZL.

DVM/PhD Student/ Microbiology, Immunology and Pathology

Feline leukemia virus: a risk to endangered felids?

Elliott Chiu, Mark Cunningham, Miles McKenna, and Sue VandeWoude

Feline leukemia virus (FeLV) is an important pathogen affecting feline health worldwide. Once considered the most common fatal infectious disease in domestic cats, FeLV infection often results in macrocytic anemia, lymphoma, red blood cell aplasia, and other cytological dyscrasias. In the 2000's, the virus spilled over from domestic cats to their wild counterparts, and disease outbreaks were recorded in the Florida panther (*Puma concolor coryi*) and in the Iberian lynx (*Lynx pardinus*). FeLV isolated from the Florida panther outbreak was genetically characterized, which indicated that infection resulted from exposure to a highly pathogenic domestic cat-origin strain. Four additional Florida panthers have recently tested positive for FeLV antigenemia, representing a new outbreak of FeLV in the fragile Florida panther population. We used quantitative PCR (qPCR) to determine FeLV tissue load in bone marrow, lymph node, spleen, and/or thymus of the four individuals described above. To determine the impact of FeLV pathogenesis in wild felids compared to domestic cats we infected panther cells in vitro and monitored FeLV infection based on three assays: enzyme linked immunosorbent assay (ELISA) to detect FeLV capsid antigen, qPCR to determine viral copy number, and fluorescent in situ hybridization (FISH) to visualize integration site with host nuclei. Preliminary findings demonstrate widespread viral infection in puma lymphoid tissues following natural infection and differences between domestic cat and puma cell FeLV susceptibility. This work lays the foundation for evaluating genetic factors that relate to FeLV species-specific disease patterns.

DVM/PhD Student/ Microbiology, Immunology and Pathology

Analysis of risk of African swine fever virus introduction into Thailand during 2015 - Development of strategy

Tosapol Dejyong, Mo Salman, Kachen Wongsathapornchai, Joleen Hadrich, and Sangeeta Rao

African swine fever (ASF) is a highly contagious disease which infects swine species and highly impacts the pig industry. ASF outbreaks have been reported in some European and African countries during 2015. Thailand has an extensive pig industry and imports pigs and pig products from European countries. Risk analysis is the key measure to mitigate the introduction of ASF into Thailand. The objective of our study is to estimate the potential risk of introduction of ASF virus into Thailand in 2015, utilizing qualitative risk assessment approach with an aim to provide specific control and preventive measures. This study will demonstrate a conceptual framework of risk analysis, generated by utilizing information from the Department of Livestock Development (DLD) and published literature. Later, specific risk pathway with risk question will be identified taking into account the socio-economic impact of the disease. Questionnaires will be administered to experts at each node of the pathway for ASF. This data will be combined with the data collected from literature, DLD, Food and Agriculture Organization of the United Nations (FAO-UN) and World Organization for Animal Health (OIE). This study will develop a risk analysis process which will comprise hazard identification, qualitative risk assessment, risk mitigation and risk communication. Suitable categories of likelihood, uncertainty of risks, combined risk estimation and risk matrix tables will be developed and used in the risk assessment. The descriptive results on importation of pigs and pig products reveal that Thailand has imported 69,983,691.35 kilograms of pig products such as internal organs, pig skin, and pork from 17 countries, one of them being an ASF outbreak country (4,210,425.10 kilograms); and imported 237 pigs from 2 countries a during 2015. The strategy developed during the study will be beneficial in assessing the risk of ASF introduction into Thailand.

Graduate Student/ Clinical Sciences

International Surveillance of Antimicrobial Resistant Bacteria in Diverse Farm, Water, and Wastewater as Sources for Human Exposure

Megan A. Dietz, Matthew Sabel, Richard Bowen, and Elizabeth P. Ryan

Antimicrobial resistant bacteria (ARB) are a global threat, but coordinated international action is lacking. An interdisciplinary team is identifying ARB in environmental sites from Fort Collins, León and Chapel Hill for the presence of *Methicillin resistance Staphylococcus aureus* (MRSA), *Vancomycin-resistant Enteroccocus* (VRE) gram positive bacteria, and *Extended Spectrum beta Lactamase Enterobacteriaceae* (ESBL) and *Carbapenem-resistant Enterobacteriaceae* (KPC) resistant gram-negative bacteria. Samples from livestock, crop soils, sewage water inlets and outlets of treatment plants, hospital and community wastewater, and/or surface waters are used. Enumeration is done for all *E.coli* and other total coliforms, *Staphylococcus aureus* and enterococci and of resistant MRSA, VRE, ESBL and KPC. Relative abundance and antimicrobial resistance properties are measured phenotypically by Kirby-Bauer disk diffusion susceptibility methods and by molecular characterization using multiplex PCR assays and Random amplified polymorphism PCR. Mean recoveries are compared for equivalence using paired t-tests or non-parametric Mann-Whitney. Results from Nicaragua and North Carolina showed that 95% of the suspected ESBL *E. coli* were real ESBL producers of the TEM, SHV and CTXM betalactamases families and 5% as Amp-C broad spectrum betalactamases producers. 90% of the suspected KPC *E. coli* were confirmed by the Hodge modified assay to be KPC producers. For clonality, between ESBL *E. coli* from Leon and Chapel Hill, there were 32 common clones that cluster more than 2 isolates and 18 unique isolates. Out of these 32, 4 clones clustering Chapel Hill and Leon isolates, with 3 recovered from Hospital, raw and secondary sewages. Other clone cluster isolates were from Hospital, raw and secondary sewages of Chapel Hill and Leon and from the stools of healthy children of Leon. We have begun sample collections from Fort Collins and will provide initial results accordingly. Conclusions indicate that related ARB exists across international communities and human infections may derive from hospital and sewage sources.

Graduate Student/ Environmental and Radiological Health Sciences

Enzymatic isolation and viability assessment of canine ovarian primordial follicles

Kathleen M. Eddy, Darcy S. Mora, James K. Graham, Terry R. Spraker, Jason E. Bruemmer, Terry M. Nett, and Douglas C. Eckery

Seventy-five percent of the world dog population is free-roaming; within the United States, damages caused by these dogs amount to greater than $620 million annually. High volume spay/neuter clinics are cost prohibitive in areas lacking resources, such as Indian reservations, and lethal culls take place yearly. Current non-surgical sterilization methods have limited efficacy, requiring supplemental applications. The finite population of ovarian primordial follicles represents the total reproductive potential of an individual; depletion would result in permanent sterility. The objective of this study was to develop a method to isolate viable canine primordial follicles. Canine ovaries were collected from local veterinary clinics and processed the same day. Cortical tissue was removed, finely chopped, and placed into an enzymatic digest solution. To optimize the isolation method, different types and concentrations of collagenases and incubation times were tested. To date, best results achieved followed incubation in a solution containing collagenases I (16.21 mg/ml), II (10.79 mg/ml), IV (10 mg/ml) and DNase (5 mg/ml), at 37 degrees Celsius for 45 minutes with mechanical tissue agitation every 10 minutes. Enzymatic digestion was terminated by addition of 10% fetal bovine serum in 10 mls of phosphate-buffered saline with calcium and magnesium. The tissue slurry was then filtered through cell strainers of decreasing mesh size: 70, 40, and 20um. Primordial follicles are 20-30um in diameter; using this method, 492 +/- 134 (standard deviation) follicles were isolated per ovary. Isolated follicular viability was found to be high, but there were many somatic cells still present. Efforts are continuing to increase primordial follicle yield while decreasing the number of unwanted cells. Future studies will utilize isolated primordial follicles to investigate mechanisms involved in cell survival and death. The overall aim of this research is identification of compounds that can be used to cause primordial follicle depletion and permanent sterility.

Graduate Student/ Biomedical Sciences

Serosurvey for infectious pathogens in horses in Colorado

Anna C. Fagre, Christie E. Mayo, Kristy L. Pabilonia, and Gabriele A. Landolt

Leptospirosis and vesicular stomatitis virus (VSV) are both economically important and zoonotic pathogens of horses. They also potentially have similar risk factors, including access to bodies of water. Despite the potential for heavy disease burden and financial losses, there is a lack of published information documenting the seroprevalence of *Leptospira spp.* in horses in Colorado. VSV is a reportable disease resulting in low mortality, high morbidity, and significant financial losses due to testing and quarantine measures. During a statewide VSV outbreak in 2014, 495 horses tested positive for VSV via qRT-PCR. In 2015 to date, 231 equine samples have been tested for VSV at Colorado State University's Veterinary Diagnostic Laboratory (CSU VDL). The results demonstrated that 153/231 (66.2%) were qRT- PCR-positive and 101/257 (39.3%) were positive as determined by complement fixation assay, indicating that many horses in Colorado have been exposed to VSV. The objectives of this study were to: 1. characterize the seroprevalence to Leptospira spp. and VSV in the state of Colorado; and 2. spatially assess the distribution of seropositive as compared to seronegative animals. Banked serum samples (May -September 2015) were acquired from the CSU VDL, including branch labs. All samples were tested for exposure to six *Leptospira spp.* serovars (Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, Pomona, and Bratislava) using a microagglutination assay. Of the 30 preliminary sera samples, 25 had titers greater than 1:100 for at least one of the six *Leptospira spp*. serovars (83.3%). All sera were also tested for exposure to VSV utilizing a serum neutralization assay. Of the 30 samples tested, 7 (23.3%) were positive for the New Jersey strain and 0 (0%) were positive for the Indiana strain. Results to date suggest evidence that Colorado horses are exposed to Leptospira spp. and VSV, two zoonotic and financially important infectious diseases.

Resident/ Clinical Sciences

Description of the virome of Anopheles gambiae mosquitoes from West Africa

Joseph Fauver, Nathan Grubaugh, Benjamin Krajacich, Steve Lakin, James Weger, Joe Diclaro, Lawrence Fakoli III, Fatorma Bolay, Brian Foy, Doug Brackney, Greg Ebel, and Mark Stenglein

Insect specific viruses (ISVs) are known to infect many species of medically important mosquitoes, including mosquitoes in the genus *Anopheles*. Currently, no ISVs have been described from natural populations of the malaria mosquito, *Anopheles gambiae*. Adult bloodfed *Anopheles gambiae* mosquitoes were collected during multiple field studies conducted across West Africa in order to monitor pathogens infecting humans in the area. Mosquitoes and/or midgut contents were pooled and RNA-Seq was performed and the data was analyzed for the presence of ISVs. Within the data set, multiple putative insect specific viruses new to *Anopheles gambiae* were discovered. These viruses span a wide range of genera. In this study, we report the genetic characterization of these viruses. In particular we (1) assembled the novel viral genomes, (2) reconstructed phylogenies, (3) determined the field prevalence's of select viruses in our study areas, and (4) determine the infectivity of select viruses in both invertebrate and mammalian cell lines. Our data demonstrate that the novel viruses presented in this study are likely insect specific based on phylogenetic analysis and replicative ability. The results of this study further demonstrates the sizable diversity of viruses that occur in medically important insects.

Graduate Student/ Microbiology, Immunology and Pathology

Whole genome sequence analysis of canine transitional cell carcinoma of the bladder

Belen Hernandez, Katherine Cronise, James Costello, Joe Brown, Susan E. Lana, Rodney Page, Ken L. Jones, and Dawn L. Duval

Transitional cell carcinoma (TCC) is the most common bladder cancer in both humans and canines. Previous studies of human TCCs identified genetic defects that may provide diagnostic and therapeutic purposes in human bladder cancer. Therefore, spontaneous canine TCC was evaluated for these values by assessing cancer gene mutations and their role in pathogenesis and progression of disease. Genomic DNA was isolated from 11 archived canine TCCs, 3 matched normal tissue samples, and 2 canine TCC cell lines. Whole exome capture was conducted using the Agilent Sure-select in-solution capture system designed for the canine genome. The final sequences were mapped to the CanFam3.1 reference genome. Somatic mutations were identified using Freebayes and characterized and compared to the Cancer Gene Census (COSMIC). Nonsense, missense, and insertion/deletion mutations were identified in 126 genes shown to be involved in human cancer. The genes exhibiting these types of mutations were further screened using SIFT. Those with SIFT scores < 0.45 in at least 2 sites or 2 samples were: BRAF, RPL5, RANBP2, EWSR1, NONO, PTPRB, LYL1, JAK1, MSH2, PER1, PIM1, and WRN. More specifically, an activating BRAF V to E mutation was identified in 5 of the tumors and both cell lines. Drug sensitivity assays using the BRAF V600E targeting drug, Vemurafenib, were conducted in BRAF mutant canine and human cell lines. BRAF mutant canine lines had an IC50 value ≥ 10 µM, 100 times higher than the sensitive human A375 melanoma cell line. RT-qPCR and Western Blot analysis confirmed mutant BRAF protein expression (99% homologous to human) in canine cell lines. Therefore, Vemurafenib insensitivity is not due to differences in the expressed canine BRAF. These data indicate that although constitutively active BRAF is expressed in canine TCC, other factors may contribute to pathogenesis.

DVM Student/ Clinical Sciences

Gene expression signature of T zone lymphoma/leukemia in dogs

Kelly L. Hughes, Janna Yoshimoto, Jeremy Dossey, Julia L. Bromberek, and Anne C. Avery

T zone lymphoma is an indolent lymphoma identified in dogs by a homogeneous expansion of T cells with loss of CD45 antigen expression on flow cytometry. There are several subtypes of T cells including T-helper cells that can differentiate into Th1, Th2, Th17, regulatory T cells, and follicular Th cells,as well as naïve, effector and memory CD8 T cells. Different types of human T cell lymphoma have been shown to arise from different types of differentiated T cell subsets. For example, gene expression profiling revealed that angioimmunoblastic T –cell lymphoma in humans arises from follicular T-helper cells. Our purpose was to investigate the gene expression pattern and origin of T zone disease in dogs with the hypothesis that T zone lymphoma arises from a differentiated T cell subset that can be determined by gene expression profiling. Neoplastic cells were sorted and RNA was isolated from 35 diagnosed lymph node and peripheral blood samples of T zone lymphoma\leukemia in dogs received at the Colorado State University Clinical Immunology (CSU-CI) laboratory from December 2013 to March 2015. For comparison, RNA was isolated and CD4 and CD8 T cell subsets were sorted from 8 young, healthy, hound mix dogs. Nanostring technology was used to measure the difference in expression of 200 genes, chosen because they are differentially expressed in different T cell subsets. Unsupervised hierarchical clustering divided the samples into cases and controls with several genes showing significant fold difference. The transcription factor GATA-3, which drives Th2 development, was significantly higher in all cases ($p < 0.005$). All other transcription factors, as well as genes that are upregulated in differentiated CD8 T cells, were higher in the controls than in the cases. Gene expression profiling for T zone lymphoma\leukemia in dogs shows a unique molecular profile that suggests a T-helper 2 subset origin.

Postdoctoral Fellow/ Microbiology, Immunology and Pathology

Racing performance in Quarter Horses undergoing prosthetic laryngoplasty

Sam W. Johnson, Clarisa R. Krueger, and Eileen S. Hackett

Laryngeal hemiplegia is a common upper airway disorder in horses caused by recurrent laryngeal neuropathy that leads to exercise intolerance and a decline in performance. This laryngeal neuropathy leads to paralysis of the muscles associated with the arytenoid and can effectively decrease upper airway diameter during inspiration. Surgical correction of the disorder typically involves a prosthetic laryngoplasty, or “tie-back”. Thoroughbred racehorses affected with laryngeal hemiplegia undergoing prosthetic laryngoplasty have been shown to race at the same level as their unaffected counterparts. However, no study to date exists evaluating racing Quarter Horses undergoing laryngoplasty or the affects on their racing careers. The aim of this study was to determine if racing Quarter Horses undergoing prosthetic laryngoplasty are able to race and perform at the same level as their unaffected peers. Medical records of 168 racing Quarter Horses undergoing prosthetic laryngoplasty for laryngeal hemiplegia between the years 2000-2015 were obtained from 5 veterinary referral centers and compared to 336 matched controls. Statistical analysis including Chi Square test, Kaplan-Meier, and Tukey Ladder test were used to evaluate career longevity, concurrent co-morbidities, earnings before and after surgery, number of starts and the effect of degree of paralysis. Preliminary results show that horses undergoing laryngoplasty versus controls had higher average earnings prior to surgery ($35,729 v $35,106), post-surgery ($20,374 v $19,069), and an average career length of 3 years versus 2.6 years. Control horses had more starts compared to the surgical cohort prior to surgery (9.73 v 8.77) and similar starts after surgery. These results indicate that racing Quarter Horses undergoing prosthetic laryngoplasty can perform at the level of their unaffected peers, and have similar racing careers and career longevity.

DVM Student/ Clinical Sciences

The good, the bad, and the ugly of the prion protein

Sarah J. Kane, Taylor K. Farley, and Mark D. Zabel

The immune system functions to eliminate diseases and threats to the body. However, what happens when normal processes "go bad"? In the present work, we aimed to identify certain parts of the immune system which help combat bacterial infections, yet we also identified these agents as playing a Jekyll and Hyde role. Specifically, the present work provides data to suggest that prions, a type of infectious protein, use these components of the immune system to their advantage. Prior to reaching their end destination, the brain, prions hide out and spread in the lymphoid tissues such as the spleen and lymph nodes. We believe we identified Complement Receptors 1/2, also known as (CR1/2 or CD21/35), as proteins which trap and help prions establish infection. Using mouse models which express only CD21 or its splice variant CD35 showed increased survival, yet it appears CD21 is more important for prions to establish disease. These findings imply follicular dendritic cells and B cells residing in the lymphoid tissues as important cell types for establishing disease. Furthermore, circulating cells which express CD35 also play a role, albeit to a lesser extent than CD21-expressing cells. In summary, these results highlight an example of normally good and necessary function for combatting certain types of infection could be used "against" the host in another type of infection.

Graduate Student/ Microbiology, Immunology and Pathology

Chemotherapeutic targeting of molecular pathways associated with osteosarcoma metastatic potential

Laird Klippenstein, Jared S. Fowles, Sierra K. Lear, and Daniel L. Gustafson

Osteosarcoma (OSA) is the most prevalent primary bone tumor and accounts for approximately 2% of childhood cancers. Forty percent of all OSA patients succumb to metastatic disease within 5 years. Canine OSA occurs about 10 times more frequently than human OSA and has been shown to be very similar to pediatric OSA biologically, histologically, molecularly, and in treatment. This suggests that canine OSA is an excellent pre-clinical model to investigate better treatment options for metastatic OSA. We compared in-vitro markers of metastatic potential with gene expression data in eight canine OSA cell lines to identify molecular pathways that will be potential chemotherapeutic targets to treat and prevent metastatic OSA. The metastatic potential of 8 canine OSA cell lines was characterized utilizing Incucyte Zoom based migration and invasion scratch wound assays. Gene expression analysis was performed using Affymetrix GeneChip Canine Genome 2.0 Arrays. Genes that were statistically correlated with migratory and invasive phenotypes were used to enrich molecular pathways described in several databases utilizing Enrichr software. Altered pathways and associated targeted agents that would inhibit the pathways enriched by the correlated gene sets were identified. Chemotherapeutics targeting significantly enriched pathways ($p < 0.05$) {Map Kinase/ Selumetinib, Focal Adhesion/Dasatinib, Erbb2&3/Lapatinib, mTOR/Rapamycin, STAT3/Stattic, BMPR2/Tacrolimus, ALK/Crizotinib, VEGFR/Vandetanib, ITK/Ibrutinib, PDPK1/ Celecoxib} were screened for their ability to inhibit metastatic potential. Dasatinib (IC50 18nM - 214nM) and Stattic (IC50 2.4 uM - 4.6 uM) were the only inhibitors screened that inhibit migration of the osteosarcoma cell lines at clinically relevant doses. Rational combinations of drugs will be explored for enhanced effectiveness. An orthotopic murine model is currently being tested to further explore the utility of efficacious chemotherapeutics *in vivo*.

Graduate Student/ Clinical Sciences

Use of mosquito bloodmeals as epidemiological tools to study malaria transmission

Benjamin J. Krajacich, Alvaro Molina-Cruz, Carolina Barillas-Mury, and Brian D. Foy

Vaccines and drugs that block malaria transmission from humans to biting mosquitoes will be essential to eliminate malaria in endemic regions. Thus, understanding how mosquitoes become infected and diagnostic monitoring of these events in the field is crucial. Currently, most diagnostics and epidemiological studies in this area focus on identifying transmissible parasite stages in human finger blood samples, and fail to consider the habits of blood feeding vectors within the complexities of the human environment and human behaviors. As an alternative, we have been sampling blood fed mosquitoes caught in African villages with novel, intronic gene targets using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), and quantitative Nucleic Acid Sequence Based Amplification (QT-NASBA). Using these approaches on RNA extracted from fresh bloodmeals, we were able to detect *P. falciparum* transcripts in mosquito bloodmeals up to 48 hours post-ingestion and down to concentrations of 10-100 gametocytes/mL at 12 hours post-ingestion with both approaches. We see a variety of uses for these assays, including correlating human gametocytemia (presence of sexual stage parasites) from human blood spots to the presence of early sexual forms in mosquitoes that bite upon these same individuals, performing sensitive spatial and temporal microepidemiology, and investigating biting tendencies of wild mosquitoes as they relate to human gametocytemia in a natural setting. The strength of these assays is that it will most closely represent the wild transmission cycle of the parasite, with the least amount of bias of any current methodology. This could lead to a more complete understanding of *Plasmodium* transmission, function as an important tool to validate transmission-blocking interventions, and could be a way to identify areas of transmission untargeted or poorly targeted by current interventions.

Graduate Student/ Microbiology, Immunology and Pathology

ORAL PRESENTATIONS Basic Science

1–5 p.m. | SESSION III - SALON V

Artificial intelligence in biomedical science: utilizing machine learning for antimicrobial resistance gene discovery in metagenomic data

Steven Lakin, Rob Raymond, Noelle Noyes, Enrique Doster, Zaid Abdo, Keith Belk, Paul Morley, and Christina Boucher

Finding genes in Next Generation Sequencing (NGS) data is an area of activate research. Because genes evolve over time, the genes of interest in clinical data are often not identical to gene sequences in reference databases. Therefore, it is vital to use techniques that capture genes of interest while allowing for degenerate sequence regions. In particular, we are interested in finding genes conferring antimicrobial resistance (AMR). However, this technique can be applied to any set of sequences. A manually curated database of AMR genes was used to train a Hidden Markov Model (HMM) to recognize AMR sequences. A simulated dataset was produced from the database for validation. The HMM was tested on three verified datasets (Forsburg 2012, 2014, and Moore, 2013). Results were compared to performance on the same data using sequence alignment and ResFams, a widely-used protein-level HMM. Our HMM showed approximately 20% higher sensitivity and specificity than ResFams on the test datasets. Additionally, our method found a broader set of genes at higher precision with more detailed classifications than ResFams. Both ResFams and our HMM outperformed alignment-based approaches. HMMs can detect highly degenerate sequences from raw NGS data, which is an improvement on alignment-based approaches that rely on sequence identity. Therefore, it is not surprising that our HMM and ResFams outperformed alignment. Because HMMs rely on quality and abundance of data during training, using DNA sequences in our HMM outperformed the protein-level approach used by ResFams. Additionally, our training database represents the most curated antimicrobial database to date and includes classes of AMR genes not present in the ResFams database, which is a valuable contribution to the study of resistance. HMMs need not be limited to our application, and suggestions for how others might use HMMs will be covered in the presentation.

Graduate Student/ Clinical Sciences

FADS2 expression modulates myocardial ischemic tolerance in mice

Lance C. Li Puma, Connor M. Whitaker, Adrian J. Olson, Amanda J. Evans, Christopher M. Mulligan, and Adam J. Chicco

FADS2 haplotypes and serum evidence for systemic hyperactivity of its gene product delta-6 desaturase (D6D) predict cardiovascular morbidity in humans. D6D regulates long-chain polyunsaturated fatty acid (PUFA) biosynthesis and is upregulated in animal models of heart disease and diabetes, but its role in the pathogenesis of these disorders has remained speculative. Our lab recently established colonies of mice with global transgenic overexpression (FADS2-tg) or heterozygote ablation (FADS2+/-) of the D6D gene to evaluate its effects on cardiometabolic function. FADS2-tg mice develop mild cardiac hypertrophy paralleled by moderate glucose intolerance and hyperlipidemia compared to wild-type (WT) mice fed a chow diet. FADS2+/- mice have no overt cardiometabolic phenotype at baseline. The present study employed these gain- and loss-of-function models to test the hypothesis that systemic FADS2 expression modulates cardiac responses to ischemia/reperfusion stress, with a focus on myocardial infarct size, a primary determinant of cardiovascular mortality in humans with and without metabolic disease. To evaluate intrinsic tolerance of the heart to ischemic insult, mouse hearts were isolated and Langendorff perfused with physiologic buffer at 37C for a 45/120 min protocol of global ischemia/reperfusion ex vivo. Myocardial infarct size was assessed by triphenyltetrazolium chloride staining and expressed as percent of left ventricular tissue mass. Hearts from FADS2-tg mice were found to exhibit ~15% larger infarcts following ischemia/reperfusion compared to WT ($P < 0.05$; N = 4-5/group), while FADS2+/- hearts exhibit ~ 30% smaller infarcts compared to their respective WT ($P< 0.05$; N=10/group). These studies demonstrate an important influence of systemic FADS2 expression on intrinsic cardiac responses to metabolic stress, consistent with epidemiological evidence linking altered D6D activity to cardiometabolic risk in humans. The mechanisms and potential interaction of dietary lipid composition on these effects are currently an intense focus of investigation in our laboratory.

Graduate Student/ Biomedical Sciences

Histological features of California sea lion (Zalophus californianus) pups from a die-off at San Miguel Island, California

Isabella M. Lowery, Eugene T. Lyons, Tetiana A. Kuzmina, Robert L. DeLong, and Terry R. Spraker

Over the last 3 years, California sea lion pups (*Zalophus californianus*) have experienced unusually high mortality. One hypothesis is that nutritional stress, caused by a combination of fluctuating ocean temperatures and productivity, and competition with humans for fishery resources, has contributed to the high mortality observed in recent years. Pups have been most severely affected by this increased mortality and have been found dead at rookeries, or admitted to rehabilitation centers along the California coast. The majority of these pups are severely emaciated, and are dealing with burdens of parasites, such as hookworms, that present additional nutritional and physiological stress. We wish to use gross necropsy and histology on a sample of deceased pups to determine their health status at the time of death, as well as cause of death. We hypothesize that these California sea lion pups will exhibit characteristics of starvation, as well as infections from parasitic and bacterial agents. In January and February 2015, we collected and performed gross necropsies of dead pups at San Miguel Island, California, and collected tissue samples for fixation. In the summer of 2015 we examined the fixed tissues histologically, and recorded all characteristics and lesions. Characterizing the state of health of these pups and describing any histological lesions will give us a better understanding of why these animals are dying. Understanding the cause of mortality in pups may also shed light on challenges faced by adult sea lions. Ultimately, this data may help us to change human behaviors and management practices to reduce mortality in this population in the future.

DVM Student/ Microbiology, Immunology and Pathology

Evidence for frequent lentiviral transmission from bobcats to mountain lions in California and Florida: Implications for emergence of lentiviral epidemics.

Jennifer L. Malmberg, Justin Lee, Sahaja Templin-Hladky, Ryan M. Troyer, and Sue VandeWoude

Owing to a long history of complex host-parasite coevolution, naturally occurring lentiviruses typically exhibit a high degree of species specificity. Cross-species lentiviral transmission is rare, yet the processes and circumstances that promote such events are highly relevant in the context of the emergence and evolutionary origin of human immunodeficiency viruses (HIVs) from simian immunodeficiency viruses (SIVs) in nonhuman primates. We have documented frequent natural cross-species transmission of a strain of feline immunodeficiency virus, puma lentivirus A (PLVA), between bobcats (*Lynx rufus*) and mountain lions (*Puma concolor*) in California and Florida. A second clade of puma lentivirus, PLVB, infects mountain lions at a higher prevalence across their entire geographic range and has never been detected in a bobcat. In this study we investigate host selection pressures, estimate within-host viral fitness based on proviral load, and examine phylogenetic relationships of both viral clades. Here we identify ongoing selection pressures and low viral fitness of PLVA in the mountain lion, providing evidence that the presence of PLVA in mountain lions is largely dependent on contact with infected sympatric bobcats. We further suggest a historical departure from this transmission pattern and hypothesize that a unique lineage of PLVA circulated amongst relic Florida panthers (*Puma concolor coryi*) based on phylogenetic relationships of regional viral isolates. Collectively, our results provide empirical evidence that PLVA and PLVB represent unique viral species associated with two primary feline hosts. PLVA in mountain lions thus represents a non-adapted lentivirus in a novel host, providing a unique opportunity for further investigation of the ecological and evolutionary pressures involved in lentivirus host-range expansion.

Graduate Student/ Microbiology, Immunology and Pathology

Breaking biofilms: nitrate inhibits biofilm formation in Burkholderia pseudomallei

Mihnea R. Mangalea, Brooke Plumley, and Brad Borlee

The opportunistic pathogen *Burkholderia pseudomallei* is a saprophytic bacterium inhabiting wet soils in tropical regions and is the causative agent of melioidoisis, an emerging infectious disease of high mortality. Although the incidence of melioidoisis is more prevalent in the monsoonal wet season in Southeast Asia and Northern Australia, domestic gardens and farms also serve as a reservoir for *B. pseudomallei* in the dry season, in part due to irrigation and fertilizer use. We hypothesize that exogenous application of nitrate, a component of fertilizer and animal waste, inhibits and disperses *B. pseudomallei* biofilms by modulating the intracellular concentration of the secondary messenger c-di-GMP as well as by altering the expression of motility and capsular biosynthesis genes. In this study, we show that sodium nitrate inhibits biofilm formation *in vitro* and we evaluate the expression of the genes responsible for this phenotypic shift. We identified five mutants in genes that comprise key components of the de-nitrification pathway that no longer respond to nitrate inhibition. Mutations in the inner membrane-bound nitrate reductase, encoded by the *NarGHIJ* operon, as well as the nitrate transporter nrt formed biofilms in the presence of nitrate. In addition, colonies grown on media supplemented with sodium nitrate produced a distinct red metabolite and altered morphological phenotypes. Altogether, these results and observations serve as a basis for understanding the effects of exogenous nitrate on *B. pseudomallei* biofilm inhibition, dispersal, and the potential of anthropogenic disturbance to increase environmental pathogen distribution and niche expansion.

Graduate Student/ Microbiology, Immunology and Pathology

LIN28 regulates androgen receptor in the placenta

Erin S. McWhorter, Rachel C. West, Quinton A. Winger, and Gerrit J. Bouma

Preeclampsia (PE) and intrauterine growth restriction (IUGR) are significant causes of infant and maternal disease. In humans, impaired trophoblast differentiation and invasion into the maternal spiral arteries is thought to be an underlying cause associated with these placental disorders. LIN28 is important in maintaining pluripotency in stem cells, and our studies have shown a role for LIN28 in trophoblast cell differentiation. Lin28, through its actions on the small noncoding microRNA let-7, also regulates androgen receptor-dependent signaling in human prostate cancer. This is significant, as PE patients have significantly increased circulating serum androgens and increased placental expression of AR. Moreover, our preliminary studies have revealed that AR plays a role in placental angiogenesis, possibly through its interaction with VEGFA. Therefore it is possible that trophoblast differentiation and placental development involves a molecular interaction between LIN28 and AR. Based on these preliminary observations we hypothesize that LIN28 regulates AR through the let-7 family of microRNAs in trophoblast cells, leading to placental differentiation. Our goals are to determine the effect of LIN28 knockdown on let-7 microRNA and AR expression and trophoblast cell differentiation. Studies evaluating placentas in women with preeclampsia consistently show maternal and fetal vascular abnormalities, which may be related to impaired trophoblast differentiation. Therefore, understanding the regulation of human trophoblast differentiation and molecular events that control placental growth and development is crucial in understanding the underlying causes of disorders in placental diseases.

Resident/ Biomedical Sciences

Novel vaccination strategies for feline immunodeficiency virus

Craig Miller, Mauren Emanuelli, Elizabeth Fink, John Elder, and Susan VandeWoude

Feline immunodeficiency virus (FIV) is a naturally-occurring lentivirus of domestic cats that is similar to human immunodeficiency virus (HIV) and utilizes analogous modes of receptor-mediated entry. Recent studies have demonstrated that autoantibodies to the primary FIV receptor, CD134, are correlated with lower viral loads and improved health status in FIV-infected cats, and that autoantibodies to both CD134 and the viral surface glycoprotein (SU) are able to block FIV infection *ex vivo*; mediated through anti-CD134 binding and displacement of SU from the cell surface. These results highlight the potential for novel immunotherapies against both FIV and HIV infection which utilize anti-receptor antibodies to protect the host from viral spread and disease progression. To determine whether immunization with CD134 and FIV-SU complexes will induce a neutralizing antibody response, specific pathogen free (SPF) cats were vaccinated with soluble CD134, SU, or CD134-SU complexes prior to challenge with FIV_{PPR}. A sham-inoculated group served as a control. Vaccination with CD134 induced a strong anti-CD134 IgG antibody response in CD134 vaccinated cats, and a substantial anti-SU IgG response was observed in CD134-SU vaccinated cats. Virus inhibition experiments revealed that serum from CD134-SU vaccinated cats exhibited significant inhibitory effects during *in vitro* infection of Crandell Rees feline kidney (CRFK) cells. Unexpectedly, neither vaccination strategy provided protection from infection *in vivo*, and CD134-SU vaccinated cats demonstrated higher antibody titers to FIV_{PPR}-gag; indicating an enhanced FIV infection rate. Flow cytometry analysis revealed an increase in the mean proportion of B220 and CD4+ cells in CD134-SU vaccinated cats, and non-specific antibody production directed at vaccine construct by-products were detected by ELISA and microsphere immunoassays. Further analysis will seek to elucidate the interactions involving these anti-receptor antibody responses, with investigations into the relevance of increased target cell populations and non-specific antibody interference to better understand the mechanism for enhancement of infection.

Graduate Student/ Microbiology, Immunology and Pathology

Intestinal – Microbial Interactions in an Ex Vivo Slice Model

Luke A. Schwerdtfeger, Elizabeth P. Ryan, and Stuart A. Tobet

Evidence for signaling among gut neural and immune networks and the native gut microbiota is accumulating. In addition, commensal bacteria may affect systemic immune surveillance and central nervous system function. Signaling to these integrated networks appears to be a capacity of the native gut microbiota, but further evidence is needed, and teasing apart these interactions with cellular resolution requires a new model. To address this, we have developed an organotypic slice model that maintains mouse intestinal tissue for up to 6 days ex vivo in the presence of nicardipine. The model maintains all structural components of the gut, including the gut associated lymphoid tissue, muscular layers, submucosa and mucosa. Mucosal epithelial cells undergo cell proliferation and migration, based on the incorporation of the thymidine analog 5-Ethynyl-2'-deoxyuridine during DNA synthesis and subsequent changes in the position of labeled cells. Structure of enteric neurons are maintained, based on the live visualization of neurons with yellow fluorescent protein driven selectively under the control of the Thy-1 promoter. In addition, a subset of commensals are maintained, judged by labeling with a fluorescent gram-stain. To test the models efficacy for functional analysis, the impact of commensal bacteria on gut contractions was observed. After slicing, penicillin-streptomycin (PS) was used to kill roughly 50% of commensals in slices, and contraction rates were measured compared to non-PS treated slices that maintained a significantly greater bacterial load. PS treated (microbiome depleted) tissue showed significantly slower rates, contracting at 47% lower rates compared to their PS-free counterparts. This experiment demonstrates the competence of the model for studying gut-microbiota functional interactions, and sets the stage for use of cell-selective fluorescent transgenic mice for direct observation of the effects of chemical signaling in the gut over time.
Undergraduate Student/ Biomedical Sciences

Uncovering prion strain structural differences via examination with epitope-mapped antibodies and chaotropic agents

Vanessa Selwyn and Glenn Telling

Transmissible prion neurodegenerative diseases are invariably fatal and incurable. Prions bypass the central biological dogma; prions do not require DNA/RNA to replicate, they instead undergo pathological endogenous protein misfolding of the cellular prion protein by the pathogenic prion protein form. The pathogenic prion tertiary structure has yet to be identified and a further confounding issue to understanding prions is the existence of prion strains. Prion strains are defined as infectious prion protein particles with the same amino acid sequence that produce different neurodegenerative disorders. The lack of a detailed infectious prion structure creates a barrier in our understanding of prion strains and further prevents the development of effective treatments, specifically, because strain properties rely on physical tertiary conformations. The traditional methodology to examine prion strains has been bioassay; however, bioassay is costly for both time and financial resources. Cell culture models are the next frontier for examining prions; they are crucial and significant for addressing questions about prion structure and characterizing strains. The Cell-Based Conformational Stability Assay, which uses chaotropic agents to probe epitope-mapped regions of the prion protein, will allow us to create a map of specific regional differences between prion strains. Each epitope-mapped antibody used, thus far, has shown significant differences, which can be used to infer structural conformational information about the strains. Additionally, the 7-5 ELISA allows further information about the prion strain structure to be garnered. The 7-5 ELISA has shown significant differences ($p < 0.0001$) between prion strains (RML and mCWD) that have been previously undifferentiated molecularly. Together these complementary techniques could provide evidence for the basis of strain/species adaption, and ultimately the species barrier.
Graduate Student/ Microbiology, Immunology and Pathology

Associations between average daily gain and calf health, feeding and management practices in preweaned dairy heifer calves in the U.S.

Chelsey B. Shivley, Jason E. Lombard, Natalie J. Urie, and Charles P. Fossler

Preweaned dairy calves are traditionally fed lower amounts of milk compared to their beef counterparts. Feeding less milk is associated with lower average daily gains (ADG) and may also lead to increased morbidity and mortality. The objective of this study was to evaluate associations between ADG and calf health, feeding and management practices in preweaned dairy heifer calves in the U.S. This longitudinal study was conducted as part of the National Animal Health Monitoring System's (NAHMS) Dairy 2014 study and included 104 dairy operations in 13 states. During the preweaning period all health events were recorded and calf growth was assessed at 2-week intervals. Liquid diets were categorized by type (i.e., milk replacer, waste/whole milk, or combination) and by volume (i.e., > 5.8 kg/day or <= 5.8 kg/day). Proc Mixed in SAS was used to determine which diet, health and management practices were significantly associated with ADG ($p < 0.05$). This interim analysis is based on 1,541 calves from 97 operations (approximately 60% of the expected total). The average ADG was 0.7 kg/day, and the average age at weaning was 62.6 days. The preliminary model estimating ADG included disease event, liquid diet, season, and the interaction between diet and season. Calves without disease had a higher ADG than calves with one or more disease event. There was a complex interaction between diet, season, and ADG. Growth in preweaned heifer calves is complex and affected by multiple facets of calf management, which can be modified to improve calf growth and reduce disease occurrence.

Graduate Student/ Clinical Sciences

Association of genetic risk alleles and the loss of anergic, high affinity, insulin-specific B cells in type 1 diabetes

Mia J. Smith, Thomas A. Packard, Shannon K. O'Neill, Carole Dunand, Clayton Mathews, Patrick C. Wilson, Peter A. Gottlieb, and John C. Cambier

Given the efficacy of B cell depleting therapies in type 1 diabetes (T1D), as well as the observed necessity for anti-insulin B cells for disease development in NOD mice, we posit that insulin-binding B cells (IBCs) play a pathogenic role in development of T1D in humans. Moreover, we believe pathogenic IBCs are normally silenced by anergy, a type of B cell tolerance wherein autoreactive cells occupy peripheral lymphoid organs but are antigen unresponsive. Thus, we hypothesize there is a loss of anergic IBCs prior to development of T1D that contributes to disease. Using a magnetic particle enrichment scheme to enrich for IBCs, we explored the frequency, reactivity, and affinity of IBCs in the peripheral blood of subjects along the continuum of T1D development. We found that in healthy subjects, high affinity insulin-binding B cells (IBCs) occur exclusively in the anergic (B_{ND}) compartment. Consistent with a potential early role in autoimmunity, these high affinity IBCs are absent from the B_{ND} compartment of some first degree relatives (FDRs), and all pre-diabetic and new-onset T1D patients tested, but return to normal levels in individuals diabetic for >1 year. Loss of B_{ND} IBCs was found to be correlated with loss of the entire B_{ND} B cell compartment, suggesting a possible predisposing genetic defect. Hence, we analyzed HLA and non-HLA T1D associated risk alleles in B_{ND} sufficient and deficient FDRs and found that high risk HLA alleles, as well as a set of non-HLA risk alleles related to B and T cell development, are associated with a low B_{ND} phenotype. Hence, our results suggest a role for perturbation of B cell anergy in development of T1D, which may be dictated by high risk genotypes.

DVM/PhD Student/ Microbiology, Immunology and Pathology

Climate, lactation, and treatment factors influence shedding of virulent dairy Escherichia coli O157

Chloe Stenkamp-Strahm, Craig McConnel, Sangeeta Rao, Roberta Magnuson, Doreene R. Hyatt, and Lyndsey Linke

Problem addressed: Among the bacterial pathogens shed by cattle, *Escherichia coli* O157 ranks highest among those causing human illness. Although prevalence levels and risk factors for O157 shedding have been assessed in beef cattle, less is known about dairy cow colonization. Objective: The current study aimed to determine prevalence levels and risk factors for O157 atypical enteropathogenic *E.coli* (aEPEC) and enterohemorrhagic *E.coli* (EHEC) shedding in mature dairy cattle during early lactation. Methods and approach: Dairy cattle (n=939) within the first 21 days of lactation were sampled monthly over the course of one year, on three dry lot dairies surrounding Fort Collins, CO. During each visit multiple factors were measured (history of disease, pharmaceutical use, milk production, climate measures, etc.), and cattle feces were concurrently collected to assess presence of O157 and virulence genes. Logistic regression analysis was performed using O157 outcomes and potential risk factors. Results: An increase in humidity was positively associated with O157 aEPEC shedding, while having more fluid feces and a history of any disease or treatment showed a negative association. Meanwhile, an increase in temperature and recent antibiotic treatment was positively associated with O157 EHEC shedding, while a greater number of days in milk, higher hygiene score and cow contact were negatively associated with EHEC. Conclusions: These results may help guide mitigation strategies that reduce the spread of O157 to the human food chain.

DVM/PhD Student/ Clinical Sciences

The Lin28B-let-7-Hmga2 axis regulates human trophoblast cell differentiation

Rachel C. West, Erin S. McWhorter, Gerrit J. Bouma, and Quinton A. Winger

The let-7 family is a highly conserved family of miRNAs that work by inhibiting cell cycle regulators and oncogenes. Inhibition of the let-7 miRNAs by the RNA binding proteins Lin28A and Lin28B is necessary during early embryogenesis to ensure proper cell proliferation. As cells begin to differentiate and leave the cell cycle, Lin28 levels decrease, allowing let-7 miRNA levels to rise. The chromatin associated protein, Hmga2, is a downstream target of let-7 and is also important for rapid cell proliferation during early embryogenesis. As this pathway is essential for proper embryonic development, we believe that the Lin28-let-7-Hmga2 axis is also an important regulator for early placental development. Initial mRNA and protein analysis of human first trimester placental tissue revealed that Lin28B is more abundant than Lin28A, therefore we hypothesize that Lin28B is an important regulator in human placental development. We first generated an shRNA knockdown targeting Lin28B in the ACH3P cell line using a lentiviral approach. The knockdown was confirmed using quantitative PCR and western blotting. It was discovered that proliferation was significantly decreased in Lin28B knockdown cells compared to scramble control cells. Furthermore, the expression of hCG, a marker of the differentiated syncytiotrophoblast cell layer, was increased in the media of the knockdown cells. Analysis of let-7 miRNA levels showed that the let-7s a,e,f,g, and i were all upregulated compared with controls. These findings suggest that in the absence of Lin28B, let-7 levels increase and drive cytotrophoblast cells to a more differentiated state. These results indicate an important role for Lin28B in human placentation.

Graduate Student/ Biomedical Sciences

Tumor microenvironment: A critical determinant of cancer progression and therapeutic efficacy that can be maintained ex vivo

Stacy Willett, Chad Eitel, David Smith, Daniel Gustafson, Randy Bartels, and Stuart Tobet

Dissociated tumor cells and tissue explants or slices are common ways to work with neoplastic tissue outside of the body. In the former, the cells have little predictive value, and in the latter the quality/utility of slices has been mixed. To enhance the utility of dissociated tumor cells, we have injected them in a mouse to develop xenografted tumors that incorporate host cells into the tumor microenvironment. Some companies have recently utilized patient derived xenografts (PDX) for determining chemotherapeutic potential (http://www.oncotest.com), which is our long-term goal. Ex vivo cell line based xenograft slices are being used in a standardized slicing paradigm that has worked for several organ systems. To validate the slice paradigm, we used doxorubicin (Dox) and docetaxel (Doc) in a slice paradigm over 4 days. The current study established baseline characteristics and responses of ex vivo fluorescent MCF7 and MDA-MB231 xenograft tumor slices with live video fluorescent microscopy before and after 24h treatment with Doc or vehicle. Cell proliferation was assessed using ethynyl deoxyuridine (EdU) and death using ethidium homodimer (EtHD). Slices treated with Doc showed significant reduction in cell motility, decreased proliferation, and increased indications of cell death. Second harmonic generation (SHG) imaging has high specificity for collagen in a biological sample and may provide information regarding tumor progression or metastatic potential. SHG images indicated that the MCF7 and MB231 slices produced differential patterns of collagen matrix. Combined with our current slice paradigm, PDX experiments may provide quicker and more clinically accurate chemotherapeutic responses for future patient needs.

Graduate Student/ Biomedical Sciences

Effects of iatrogenic blood contamination on cerebrospinal fluid total nucleated cell count and protein concentration

Yenlie W. Zingale, Amy L. MacNeill, and Rebecca A. Packer

Purpose: The aim of this study was to determine the effect of iatrogenic blood contamination of canine cerebrospinal fluid (CSF) on total nucleated cell count (TNCC) and protein concentration. Materials/Methods: Records from the Colorado State University Veterinary Teaching Hospital were searched retrospectively between 2013 and 2015 for all dogs where CSF analysis was performed. TNCC, red blood cell (RBC) count, protein concentration, and cytologic interpretation were recorded for each case. Serial blood concentrations were added *in vitro* to normal canine CSF to predict TNCC and protein concentration. Results: Of the 439 samples reviewed, 40 samples had a cytologic diagnosis of blood contamination. RBC counts for all samples ranged from 0 to 200,000/uL. When a Spearman's rank order correlation was performed on all samples, there was no clinical correlation between TNCC and protein levels with iatrogenic blood contamination (R^2 values 0.2 and 0.57, respectively). When only those samples with a cytologic diagnosis of blood contamination were included, there was significant correlation between TNCC and RBC (R^2 value 0.7986). There was no correlation between increased RBC counts and protein concentrations in the CSF samples that were contaminated with blood (R^2 value of 0.3936). The *in vitro* analysis of normal CSF with serial blood concentrations was inconclusive and statistically underpowered. Conclusion: Erythrocyte counts in canine CSF as a result of blood contamination correlate well with nucleated cell counts, but correlate poorly with protein concentration. Additional cases are required for the *in vitro* component of the study to provide a clinical correlation predictive model.

Resident/ Microbiology, Immunology and Pathology

POSTER PRESENTATIONS

SALON III AND IV

1 | Indoor hockey officials' hearing threshold shifts and effect of helmet visor length on exposure to whistle noise

Karin L. Adams and William J. Brazile

Noise exposure and hearing thresholds of hockey officials in amateur and collegiate hockey leagues were measured to assess the impact of hockey game noise on hearing sensitivity. The effect of the hockey helmet visor length on the level of whistle noise to which hockey officials are exposed was evaluated to determine if visors may introduce a reflective plane for the whistle noise, resulting in increased noise exposure. Twenty-nine hockey officials participated in the study. Personal noise dosimetry was conducted to determine if officials were overexposed to noise. Pure-tone audiometry measured the hearing thresholds of officials before and after officiating games to determine if there was a 10 dB or greater decrease in hearing sensitivity. Audiometry was conducted in both ears at 500, 1000, 2000, 3000, 4000, 6000 and 8000 Hertz. Noise generated from whistle blowing was measured in the left ear of the Knowles Electronic Manikin for Acoustic Research for each of three helmet configurations: without visor, 2.75" visor, and 4.0" visor. Statistical analysis included the paired t-test. Mean personal noise exposure level was 92 dB, A-weighted (SD=2.2). Hearing threshold shifts of 10 dB or greater were observed in 86% of sampled officials, with statistically significant differences ($p < 0.05$) between pre- and post-game hearing thresholds observed in both ears at 2000, 3000 and 4000 Hz. Measured peak noise levels in the manikin ear were significantly different between the helmet configuration with the long (4.0") visor and the other helmet configurations ($p<0.05$). The results suggest that indoor hockey officials experience temporary hearing loss after officiating games. Further temporary threshold shift research may identify officials of other sports or at larger venues are at increased risk of noise-induced hearing loss. Manikin study results suggest that longer visors may act as a reflective plane for whistle noise and increase hockey official's noise exposure.

Graduate Student/ Environmental and Radiological Health Sciences

2 | CRISPR/Cas9-based Genome Editing to Investigate the Role of Lin28A and Lin28B in Regulation of Human Trophoblast Cell Differentiation

Asghar Ali, Kimberly M. Jeckel, Erin E. McWhorter, Rachel West, Gerrit J. Bouma, and Quinton A. Winger

Placenta development during pregnancy is critical for proper embryo development. It depends upon differentiation of trophoblast stem cells into specific subtypes. These development and differentiation steps are highly regulated in trophoblast cells. Lin28 is an RNA binding protein that has two homologues, Lin28A and Lin28B. It is highly expressed in undifferentiated cells and defines their stemness. Both Lin28A and Lin28B work alternatively in the cell, but the exact division of labor between them is not clear. Knocking down either one of Lin28 homologues by shRNA has been sufficient to identify their role in cell proliferation and differentiation. The studies attempting a double knockout of both Lin28 homologues have never been reported. We aimed to create a double knockout cell line using Clustered Regulatory Interspaced Short Palindromic Repeats (CRISPR) Cas9-based genome editing. The CRISPR-Cas9 system is a recent technique, a greatly expanding tool box used to modify mammalian genetics. In this system, single guide RNA (sgRNA) guides Cas-9 nuclease to matching double-stranded DNA. Target specificity of CRISPR-Cas9 system is very high due to a 20-base pair sequence at 5'-end of sgRNA. To create a double knockout (KO), we used two different vectors; lentiCRISPR v2 (plasmid #52961) and pLX-sgRNA (Plasmid #50662) from addgene. lentiCRISPRV2 is a puromycin resistant vector that expresses Cas-9 and sgRNA using lentiviral delivery system. While pLX-sgRNA is a blasticidin resistant vector that expresses only sgRNA through lentiviral delivery system. To create a double KO we first induced mutation in ACH3P cell line to KO Lin28A using lentiCRISPR v2. Then we infected this cell line with pLX-sgRNA vector expressing guide for Lin28B. This sgRNA, when expressed in Lin28A KO cell line, used already existing Cas-9 and mutated Lin28B alleles. The double KO cell line will be used to determine the role of Lin28A and Lin28B in trophoblast cell lines.

Graduate Student/ Biomedical Sciences

3 | TERRA in the Telomeric DNA Damage Response

Taghreed M. Alturki, Christopher B. Nelson, David G. Maranon, and Susan M. Bailey

Telomeres are nucleoprotein complexes that protect natural chromosomal termini and prevent their detection as DNA damage, thus they play critical roles in maintaining genomic stability. Telomeres are composed of tandem arrays of conserved repetitive sequence (TTAGGG in vertebrates), bound by a suite of proteins collectively termed "shelterin", which are essential for telomere length regulation and end-capping structure/function. As telomeres also possess an abundance of heterochromatic marks, they have long been regarded as silenced, non-transcribed features of the genome. Therefore, the finding of telomeric RNA (TElomere Repeat-containing RNA; TERRA) came as quite a surprise, one that opened many new avenues of investigation. TERRA is a long non-coding RNA (lncRNA) shown to serve a structural role at telomeres, as well as function in regulation of telomere length and telomerase activity (the specialized reverse transcriptase capable of elongating telomeres). Further, TERRA triggers telomeric recombination in tumors that maintain telomere length in a telomerase independent manner via the Alternative Lengthening of Telomeres (ALT) pathway. Emerging evidence also suggests that telomeric "DNA-TERRA hybrids" are indispensable for end protection and capping function; e.g., RNA interference mediated depletion of TERRA induced telomeric DNA damage responses and aberrations. Interestingly, it was recently reported that telomeric homologous recombination (HR) occurs in G0/G1 phase where RNA serves as the donor template (no sister chromatid available). Given the role of TERRA in facilitating telomeric recombination and in preventing inappropriate telomeric damage responses, *we hypothesized that TERRA plays a critical role in the repair of telomeric DNA damage by providing a template for telomeric recombination*. Here we present our findings utilizing a recombinant endonuclease to specifically cut telomeric DNA combined with RNA fluorescence in situ hybridization (FISH) to visualize TERRA localization and interrogate its role in the telomeric DNA damage response.

Graduate Student/ Environmental and Radiological Health Sciences

4 | Different factors contribute to increased expression of IGF2BP1 in human and canine osteosarcoma

Nouf M. Alyami, Brian T. Kalet, Liza E. Pfaff, Sarah Peck, and Dawn L. Duval

Purpose: Osteosarcoma is an aggressive malignant bone tumor that afflicts greater than 10,000 dogs, but only 400 adolescents yearly. Most dogs and approximately 25% of children eventually succumb to metastatic disease. Using microarray analysis, we identified elevated insulin-like growth factor II mRNA binding protein 1 (IGF2BP1) expression as a biomarker of poor prognosis in canine osteosarcoma. Gene amplification, hypo-methylation, increased transcription, and alterations in miRNA regulation directly or through 3'UTR shortening have all been hypothesized as mechanisms to increase IGF2BP1 expression in cancer. The current study explores these mechanisms in panels of human and canine osteosarcoma cell lines. Experimental design: We evaluated the expression and alternative polyadenylation of IGF2BP1 using RT-qPCR and western blot analysis. We measured miRNA expression in the cell lines and cloned fragments of the 3'UTR in a luciferase reporter construct to assess miRNA regulation of IGF2BP1. We assessed transcriptional activation of IGF2BP using luciferase reporters containing up to 2500 bp of 5'flanking sequence from the human and canine IGF2BP1 genes. Results: We found that loss of the distal 3'UTR correlated with increased IGF2BP1 expression in canine tumors and cell lines while significant loss of the 3"UTR was not observed in human cell lines. Consistent with this finding, reporter constructs containing fragments of the distal 3'UTR expressed significantly lower luciferase activity compared to control or the proximal 3'UTR fragment. IGF2BP1 promoter analysis suggested that the majority of regulatory elements were located within 800 bp of the start site of translation. Interestingly, one cell line with no IGF2BP1 expression exhibited significant promoter activity indicating that methylation may prevent transcription in this cell line. Conclusions: Overall, our data suggest that alternative polyadenylation is an important factor in the regulation of IGF2BP1 in canine cells, but that miRNA regulation and transcriptional activation may play a stronger role in human osteosarcomas.

Graduate Student/ Clinical Sciences

5 | PI3K-Akt signaling pathway association with oocyte competence

Gabriella M. Andrade, Gerrit J. Bouma, Juliano C. da Silveira, and Felipe Perecin

The ovarian follicle encloses oocyte in a microenvironment throughout its growth and acquisition of competence. Follicular cells are largely responsible for driving folliculogenesis and oogenesis, however its role in dictating oocyte competence remains elusive. There is increasing evidence for a dynamic interplay among follicular cells and oocytes since they are constantly exchanging "messages". MicroRNAs were identified as one of the molecular signals involved in intra-follicular communication and regulating cellular processes. To determine the cellular origin of miRNAs present within follicular environment we screened 351 miRNAs in granulosa cells (GCs) and cumulus-oocyte (COCs) complexes and in their secreted exosomes. We identified PI3K-Akt pathway as highly regulated by microRNAs exclusively identified in CGs or COCs, as well as in corresponding exosomes. To investigate if miRNAs can impact oocyte quality we dissected bovine ovarian follicles and recovered cumulus cells and oocytes. The oocytes were individually assigned for in vitro culture in order to track cumulus cells with oocyte competence as defined as their ability to reach the blastocyst stage. We determined levels of PI3K-Akt signaling pathway components in cumulus cells according to oocyte competence. Our study identified that this miRNA-modulated pathway is down regulated in lower quality oocytes. Using PI3K-Akt responsive genes we show decreased FOXO3a mRNA levels in follicular cells that are associated with lower quality oocyte group suggesting changes in cell progression and oxidative response. Together these results demonstrate that miRNA expression profile in follicular cells is useful to identify putative molecular pathways involved in oocyte competence. This principle was given proof by the determination of PI3K-Akt pathway components levels in cumulus cells from ovarian follicles carrying oocytes with distinct developmental competence.

Graduate Student/ Biomedical Sciences

6 | Communication among the three compartments of the equine stifle joint

Shelby Arrieta, Meredith Herdrich, and Valerie Moorman

Communication among the three compartments in the equine stifle has been previously documented, with the most common communication between the medial femorotibial and femoropatellar compartments. The objective of this study was to document whether there was one-way or two-way communication between the femorotibial and femoropatellar joint compartments. We injected a total of 50 mL (25mL contrast and 25 mL tap water with food coloring) into either the femoropatellar joint (green) or both of the femorotibial compartments (red in the medial and blue in the lateral) of equine cadaver stifles. Fourteen stifles were injected into the femoropatellar joint and four stifles had injections into the femorotibial joints. Once injected, radiographs were obtained pre and post joint flexion/extension to track the location/movement of the contrast within the joint. The stifle joints were then dissected to determine food coloring location and to identify any visible communications. Of the 14 stifles that were injected into the femoropatellar joint, 10 had visible communications. Two communicated with both femorotibial joints, one communicated with the lateral femorotibial joint, and seven communicated with the medial femorotibial joint. Six of these had visible communications but no contrast or food coloring had passed between the joint compartments. Of the 4 that were injected into both femorotibial compartments, 2 had visible communications and both were from the medial femorotibial joint to the femoropatellar joint. While joint communications are common in the stifle, contrast and food coloring did not always pass between compartments, especially from the femoropatellar to the femorotibial compartments.

DVM Student/ Clinical Sciences

7 | Effect of cryoprotectants and maturation status of oocytes on post-thaw cleavage and blastocyst rates

Eleonora A. Barbosa, Phillip H. Purdy, James K. Graham, and Jennifer P. Barfield

Cryopreservation of bovine oocytes results in low cleavage and blastocyst rates because the freezing procedures are not optimized. Our objective was to determine if an optimal cryoprotectant and maturation state (immature or mature) could be determined for cryopreserving oocytes. MATERIALS AND METHODS: Oocytes were aspirated, graded and placed in 7 treatment groups: (G1) control, unfrozen; (G2) immature, 1.5M ethylene glycol (EG); (G3) immature, 1.5M dimethyl sulfoxide (DMSO); (G4) immature, 1.5M EG and 1.5M DMSO; (G5) mature (22h), 1.5M EG; (G6) mature, 1.5M DMSO; and (G7) mature, 1.5M EG and 1.5M DMSO and then cryopreserved. Frozen-thawed oocytes were washed and used for IVF. Cleavage rates (CR) were determined at 72h post-fertilization and blastocyst rates (BR) were determined at day 7 of culture. Data were analyzed using Chi-square analysis. RESULTS: Cleavage rates for G1 were different from all treatments ($P < 0.0001$). No differences in CR were observed within the immature (G2, G3, G4) or mature treatments (G5, G6 G7; $P > 0.05$). Mature oocytes (G5, G6, G7) had higher CR compared to immature (G2, G3, G4; $P < 0.05$). The G1 (15%) and G5 (1%) treatments had different BR ($P < 0.001$) but were the only treatments to develop past cleavage. CONCLUSIONS: Use of single cryoprotectants, rather than combinations, and cryopreserving mature, rather than immature oocytes, results in greater cleavage and blastocyst rates. Additional studies are needed to optimize these techniques.

Graduate Student/ Biomedical Sciences

8 | Bartonella spp. PCR assay results using cerebrospinal fluid of naturally exposed dogs with central nervous system disease

Lisa R. Bartner, Adam Drury, Annie V. Chen, Arianne Morris, Melissa Brewer, Meri Hall, Michael R. Lappin, and Stephanie McGrath

The purpose of this study was to use polymerase chain reaction (PCR) to amplify Bartonella spp. DNA from cerebrospinal fluid (CSF) of naturally exposed dogs. CSF samples from 175 pure or mixed breed dogs were submitted to Colorado State University (CSU) from either Washington State University or the CSU Veterinary Teaching Hospital. Dogs with normal, focal, or multifocal neurologic examinations and CSF pleocytosis (total nucleated cell count >5 nucleated cells/µl and RBC <4,000 cells/µl) were included. The CSF samples were stored at -80C. They were then thawed and centrifuged at 10,000 X g for 15 minutes; the supernatant was removed and the pellet assayed in a previously published PCR assay that targets the 16S-23S rRNA intergenic region. All positive amplicons were sequenced to determine the infective *Bartonella* spp. A total of 67 dogs were included, none of which were positive for *Bartonella* spp. DNA in CSF. Of the other 108 CSF samples, one was positive for *B. henselae* DNA. The CSF from this dog contained 94 RBC/µl. As *Bartonella* spp. have an intra-erythrocytic phase, we speculate that minimal peripheral RBC contamination in the CSF of dogs with systemic *Bartonella* spp. infection may lead to positive *Bartonella* PCR assay results in the absence of CNS disease. Thus, positive PCR for *Bartonella* spp. DNA in the CSF must be interpreted in light of RBCs within the sample as well as inflammation or systemic infection. Failure to amplify *Bartonella* spp. DNA from the CSF of the 67 dogs with inflammatory disease suggests either the organism was not involved, was in CNS tissues but not CSF, or was present in quantities undetectable by this PCR assay. Since the combination of PCR and culture is the most sensitive way to detect *Bartonella* spp., the use of that technique should be considered in future studies.

Resident/ Clinical Sciences

9 | Crispr/Cas9 as a treatment for prion disease

Heather Bender, Amanda Hitpas, and Mark Zabel

Prion diseases are fatal neurodegenerative diseases that affect both wildlife and human populations, and are caused by the prion agent. Prions cause a normal cellular host protein, the prion protein (PrPC), to misfold into an abnormally conformed isomer, PrPRes, which causes massive neuronal degeneration. Despite years of research into anti-prion compounds, there is still no effective treatment for either wildlife or human populations affected by prion disease. Here we describe a Crispr/Cas9 system as a potential treatment for prion diseases. Crispr/Cas9 utilizes a single stranded gRNA and the Cas9 endonuclease to remove a piece of target DNA, which results in silencing of the target gene. We plan to use Crispr/Cas9 that is targeted towards the prnp gene of PrPC. We aim to treat mouse neuroblastoma cells (N2a cells) with Crispr/Cas9 plasmids coupled to a liposomal delivery system. Protein expression levels will be assessed by western blot and flow cytometry. We will then use the Crispr/Cas9 plasmids with the liposomal delivery system to treat mice with prion disease.

Graduate Student/ Microbiology, Immunology and Pathology

10 | Timing of superovulation and embryo collection in North American bison

Hayley M. Benham, Zella Brink, Will Falbo, Jennifer P. Barfield, Matthew McCollum, and Jack Rhyan

The goal of this study was to determine the best day to collect embryos after superovulation of bison. Five bison cows were synchronized and superovulated using two injections of follicle stimulating hormone (FSH) over a 48 h period (total of 400mg i.m.) and one injection of prostaglandin with the last FSH injection (25mg i.m.). After final injections, females were immediately moved to a pen with multiple bulls for natural breeding. Video recordings were captured for 4 days to determine when cows were bred. Reproductive tracts were collected from females 7 (n=3) or 8 (n=2) days after observed breeding. Embryos were recovered from the oviduct and uterus separately to determine the location of embryos 7 or 8 days after breeding. Embryos were evaluated for stage of development and quality before being frozen in straws using a slow freeze method. Superovulated females were bred 48-60 hours after the last FSH injection. Embryos were recovered from four of the five cows. Embryos collected 7 days post-breeding reached the morula stage, while embryos collected 8 days post-breeding reached the blastocyst stage of development. All embryos were found in the uterine horns, except in one female, which had two unfertilized ova in the oviducts 7 days post-breeding. The mean number of viable embryos, unfertilized ova, and degenerate embryos recovered from bison on day 7 and 8 were, 2±3.4, 3±1.7, 0.3±0.6 and 2±2.8, 0±0, 1±0, respectively. Hormonal stimulation during superovulation shortens the time from final prostaglandin injection to time of reproductive receptivity in bison to 48 hours, compared to 72 hours for non-superstimulated cattle and bison. This study suggests that superovulated bison should be bred 48-60 hours after administration of the final prostaglandin injection to allow for optimum fertilization rates. Determining when females enter estrus in bison could improve the success rates of embryo recovery.

Graduate Student/ Biomedical Sciences

11 | Innate immunity induced by BCG

Tom Bickett, Jolynn Troudt, Elizabeth Creissen, Amber Troy, and Angelo Izzo

In many countries, infants are vaccinated within the first weeks of life with the attenuated *Mycobacterium bovis* vaccine, Bacillus Calmette–Guérin (BCG) to protect against *Mycobacterium tuberculosis* infection. BCG was developed in the early 1900's, and first tested in 1921. Today, almost 100 years later, it remains the only option for vaccination against *M. tuberculosis* infection, which currently infects over 1/3 of the world's population and is responsible for millions of deaths annually. Unfortunately, BCG efficacy ranges anywhere from 0 to 80% and any protection established early in life wanes over time, yet it is given to more than 80% of infants born in the 157 countries that support the use of the vaccine. Differences in vaccination success can be dependent on geographic location, age when vaccinated, and the strain used, but little is known about the cause of the discrepancy in efficacy of the BCG vaccine. Many theories exist, but continued use of this vaccine is unavoidable as long as a more effective option has not been developed. Studies have shown that BCG vaccination can induce T and B cell responses, but it is unclear if this response correlates to protection against *M. tuberculosis* infection, as we and others have shown a disconnect between the magnitude of T cell responses and reduction in mycobacterial burden in animal models. BCG is also known to activate the innate immune system, allowing it to be used for protection against non-mycobacterial disease as well as against certain cancers. As the effect of BCG on the innate immune system is beneficial for other diseases, this study focuses on the early immune response shortly after BCG vaccination in the mouse model to help elucidate the mechanisms through which BCG establishes protection against *M. tuberculosis* and, eventually, could lead to the development of a more effective alternative.

Graduate Student/ Microbiology, Immunology and Pathology

12 | Canine Cutaneous Plasmacytosis: A Retrospective of 21 cases (2005-2015)

Brendan Boostrom, Anthony Moore, Carrie DeRegis, Cecilia Robat, Kim Freeman, and Doug Thamm

Background: Cutaneous plasmacytosis is a syndrome of multiple cutaneous plasma cell tumors, in the absence of multiple myeloma. While rare in both humans and dogs, treatment recommendations have historically been extrapolated from multiple myeloma protocols. A retrospective case series may better elucidate behavior, treatment response rates and prognosis of canine cutaneous plasmacytosis. Methods: In this retrospective study, the medical records of 21 dogs with cutaneous plasmacytosis, contributed from five oncology referral clinics, were reviewed. Diagnosis of plasmacytosis was based on histopathologic evaluation of at least one representative cutaneous/subcutaneous lesion in dogs with 3 or more lesions. Dogs with suspicion of multiple myeloma were excluded based on the presence of two or more of: bone marrow involvement, monoclonal gammopathy, lytic bone lesions or light chain proteinuria. Results: The median age of affected dogs was 8.5 years, with a median weight of 26.5 kg. 13/21 dogs were male. The most commonly affected breeds were the Golden (5/21) and Labrador retriever (3/21). 14 of 21 dogs had >10 lesions, with some having more than 100. Lesions were commonly described as round, raised, pink to red and variably alopecic or ulcerated, similar to solitary plasmacytomas. The most commonly used drug protocol was combined melphalan and prednisone, with an overall response rate of 73.7% (14/19 dogs). Single agent CCNU had a similar ORR of 71.4% (5/7 dogs). Single agent cyclophosphamide had an ORR of 40% (2/5 dogs). Median progression free interval following first treatment was 153 days. Median survival time from first treatment was 542 days. Conclusions and clinical importance: Alkylating agents were effective in inducing remission of cutaneous plasmacytosis, although melphalan and CCNU had higher response rates than cyclophosphamide. This is the first reported retrospective study of canine cutaneous plasmacytosis. A prospective study would be challenging given the rarity of cutaneous plasmacytosis.

Resident/ Clinical Sciences

13 | Public health nutrition for chronic disease control and prevention with rice bran and beans

Erica C. Borresen, Dustin G. Brown, Katie Schmitz, Melissa Wdowik, Sangeeta Rao, Tracy Nelson, Joanne O'Malia, Marlon Bazan, NaNet Puccetti, Gary Luckasen, Tiffany L. Weir, Regina J. Brown, and Elizabeth P. Ryan

Whole grains and dry beans demonstrate compelling chronic disease fighting properties, yet consumption of these staple foods remain extremely low. A growing community-academic partnership is conducting clinical trials for increased consumption in children to adults. Our main objectives are to 1) establish feasibility of increasing navy bean powder (NBP) and rice bran (RB) intake in children with elevated cholesterol levels and adults with a history of colorectal cancer, 2) examine changes in overall dietary intakes with the addition of RB and/or NBP, and 3) favorably modulate the blood and stool metabolome. Meals and snacks were developed for inclusion of NBP and/or RB in amounts that equate to roughly 5-10% of total dietary intake. Participants completed a pilot placebo-controlled, randomized, single-blinded dietary intervention trial. They consumed study meals daily for 4 weeks and recorded 3-day dietary food logs each week. Blood and stool samples were collected at three time points for blood and stool metabolome, and stool microbiome analyses. Adding NBP or RB into foods provided 4-9% daily caloric intake with 80-100% intervention compliance. Dietary intake data at baseline confirms a western dietary pattern including low fiber, high sodium, and high fat intake. This dietary intervention significantly increased total dietary fiber intakes at 4-weeks ($p < 0.05$). Adding NBP or RB into prepared meals represents an economically feasible and safe approach to achieve dietary intakes that may control or prevent chronic diseases. Our data suggest that NBP and RB are promising solutions that merit public health nutrition education and research attention.

Staff/ Environmental and Radiological Health Sciences

14 | Dual-energy x-ray absorptiometry scans of sedated and awake cats with the VetMousetrapTM device

Ariel Brody, Elissa K. Randall, Jeremiah Easley, and Jessica M. Quimby

The VetMousetrap™ is a device that allows for computed tomography imaging of non-sedated, non-anesthetized cats. Dual-energy x-ray absorptiometry (DEXA) is considered the reference method for assessing body composition in cats and could be useful for assessing nutrition of chronically ill delicate patients, but typically requires anesthesia for accuracy. The purpose of this study was to assess the efficacy of performing DEXA scans of awake cats with the use of the VetMousetrap™ device. Four healthy research cats with a range of body condition scores underwent a DEXA scan with or without dexmedetomidine sedation using the VetMousetrap™. As controls, a DEXA scan of just the sedated cat and just the VetMousetrap™ were also performed. The lean body mass and fat mass of the cats were compared between the three scans (awake with VetMousetrap™, sedated with VetMousetrap™, sedated without VetMousetrap™) using repeated measures ANOVA with Dunn's post-hoc analysis. The VetMousetrap™ device itself registers a reading, so the baseline device reading was subtracted from the readings obtained with the VetMousetrap™ before analysis. There was a statistically significant difference in lean body mass estimation using the VetMousetrap™ in awake cats in comparison to control readings when sedated without VetMousetrap™ ($p < 0.05$). There was no statistical difference in fat mass between the techniques but ability to determine significance is likely compromised by small sample size (type 2 error). DEXA scan in awake cats using VetMousetrap™ device significantly underestimated muscle mass. Patient position and inherent density of the device likely affect the readings. Additional studies are necessary to determine if analysis of a specific region of interest would be more accurate.

DVM Student/ Clinical Sciences

15 | Metabolomics Investigation of Xenobiotics and Metabolic Pathway Networks in Tumors, Adjacent Mucosa and Stool from Colorectal Cancer Patients

Dustin G. Brown and Elizabeth P. Ryan

Colorectal cancer (CRC) is the third leading cause of cancer-related death in the United States. Little is known regarding the profile of xenobiotics that may influence CRC progression across colonic tumor locations in people. We hypothesized that xenobiotic metabolites and pathways differ in both the presence and relative abundance in tumor tissue and adjacent mucosa when compared to detection in stool and across tumor locations. 33 tissue (16 tumor tissue and 17 patient-matched mucosa) and 13 stool samples were collected from CRC patients undergoing colonic resection and analyzed by Liquid Chromatrography-Mass Spectrometry and Gas Chromatography-Mass Spectrometry to determine global metabolite profiles (Metabolon, Inc). Normalized relative intensity values for xenobiotics detected were uploaded into MetaboAnalyst and Metabolon's Metabolync pathway analysis software to determine metabolic pathways affected. Matched-pairs and student's t-tests were used to evaluate differences between metabolite expression in tumors and mucosa and differences between tumor locations in stool and tumor tissue matrices. Of the 703 metabolites in this dataset, 86 were xenobiotics that included 6 benzoate, 14 chemical, 19 drug, 36 food/plant components, and 11 xanthine metabolites across all human tissue and stool samples analyzed. There were 15 xenobiotics specific to tissue, 50 unique to stool, and 21 detected in both. Of the shared metabolites, quinate had the greatest fold difference (2.86) between tumor tissue and mucosa. The following drugs (ofloxacin and probenecid), plant/food components (enterolactone and caffeate) and chemicals (tetraethylene glycol and brilliant blue FCF) were identified in stool with substantial variation in their relative abundance. Metabolic pathway analysis of xenobiotics further revealed modulation of caffeine metabolism with 5 shared metabolites across matrices affected, including paraxanthine, 1,7-dimethyluric acid, caffeine, 1,3,7-trimethyluric acid, and theobromine.The xenobiotic profiles identified using metabolomics showed novel relationships between sub-metabolic pathways shared between tumor tissue and mucosa as well as with distinctions in stool.

Staff/ Environmental and Radiological Health Sciences

16 | Cystolith dissolution in cats using a commercially available diet

Jamie M. Bunkers, Camille Torres, Elena T. Contreras, and Michael R. Lappin

The purpose of this study was to describe the clinical and laboratory findings in cats with radiopaque cystoliths fed the commercially available diet, Purina® Pro Plan® Veterinary Diets UR Urinary® St/Ox®. This diet has been formulated for the dissolution of struvite cystoliths and to lessen the recurrence of both struvite and oxalate cystoliths. Cats with clinical signs of lower urinary tract disease and cystoliths confirmed via radiographs were enrolled in this IACUC approved study. Complete blood cell count, serum biochemistry profile, abdominal ultrasound, and urinalysis with aerobic bacterial culture and antimicrobial sensitivity were performed on entry to and exit from the study. The cats were housed in a gang room, fed the study diet ad libitum, and assessed by abdominal radiographs weekly. Cats with cystoliths that resolved based on two sequential weekly radiographs and confirmatory ultrasound examination were considered diet successes. Cats with no change in cystolith size after four weeks underwent cystotomy for stone removal, aerobic culture and antimicrobial susceptibility testing, and analysis at the University of Minnesota Urolith Center. To date, five cats between the ages of four and eight years of age have completed the study. Total cystolith dissolution was achieved by Week 2 for three cats and two cats still had radiographic evidence of cystoliths by Week 4. One cat with persistent cystoliths had a single ammonium urate cystolith with a struvite nidus (normal pre and post-bile acids) and the other cat had multiple calcium oxalate cystoliths; cultures were negative for both cats. While larger case numbers are needed, the preliminary results suggest that feeding Purina Veterinary Diet UR Urinary® St/Ox® can successfully dissolve cystoliths that are likely struvite and may lessen risk of recurrence of struvite and calcium oxalate cystoliths.

DVM Student/ Clinical Sciences

17 | Investigation of whether Leptospira vaccinal antibodies react with Borrelia peptides used in a commercial assay

Amber Caress, Scott Moroff, and Michael Lappin

In small animal veterinary medicine, two common pathogenic spirochete genera are *Borrelia spp*. and *Leptospira spp*. The purpose of this study was to determine if Leptospira spp. antibodies induced by vaccination would cross-react with the *B. burgdorferi* antigens used in a commercially available assay. Staff and student owned dogs were recruited at a Veterinary Teaching Hospital in a *B. burgdorferi* non-endemic area. The dogs were randomized and administered one of four commercially available *Leptospira spp.* vaccines that contained serovars *Canicola*, *Gryppotyphosa*, *Icterohemorrhagiae*, and *Pomona*. Blood was collected prior to vaccine administration on Week 0 and Week 3 and then again on Weeks 4, 8, and 12. After confirming the maximal *Leptospira spp*. titers occurred on Week 4, an aliquot of this sera was shipped to Antech Diagnostics for analysis of *B. burgdorferi* antibodies against OspA, OspC, and OspF with the Accuplex 4 BioCD system. The Week 4 sera from all 31 dogs had an MAT titer of 1:100 for at least 1 Leptospira spp. serovar. MAT titers of 1:800 or greater were detected against multiple serovars in 27 dogs. None of the samples contained antibodies against the *B. burgdorferi* OspA, OspC, and OspF peptides used in the commercially available assay. In conclusion, the *B. burgdorferi* peptides used in the Accuplex 4 BioCD system do not recognize antibodies induced by the commercially available *Leptospira spp.* vaccines administered in this study. However, the results of this study may not be the same for all laboratories as specific sources of peptides vary.

DVM Student/ Clinical Sciences

18 | Targeting of the cellular exoribonuclease XRN1 appears to be a shared strategy among several families of RNA viruses

Phillida A. Charley, Stephanie L. Moon, John R. Anderson, Carol J. Wilusz, and Jeffrey Wilusz

The removal of unwanted RNA is critical for all eukaryotic cells and is tightly controlled by the RNA decay machinery. The major 5'-3' mRNA decay pathway is mediated by an exoribonuclease called XRN1. Additional evidence suggests that XRN1 may also be a key protein involved with networking mRNA decay with other aspects of gene expression. The prominent role of XRN1 in cellular gene expression, along with its cytoplasmic location, makes XRN1 an attractive and available target for inactivation by RNA viruses to enhance their replication. We have recently shown that members of the Flaviviridae (e.g. Dengue, West Nile, Hepatitis C and Bovine Viral Diarrhea viruses) use an RNA structure in their 5' or 3' untranslated region (UTR) to stall and repress XRN1, thus altering cellular RNA stability. We now hypothesize that members of the Arenaviridae (e.g. Junín virus), and Bunyaviridae (e.g. Rift Valley fever virus) may also have the ability to stall/repress XRN1 because of conserved, strong RNA structures present in the 3' UTRs of the RNAs made in their 'ambisense' coding strategy. In support of this hypothesis, XRN1 RNA decay assays performed using either recombinant XRN1 protein or cell-free extracts demonstrate decay intermediates consistent with XRN1 stalling on the 3' UTRs of multiple arenavirus RNAs as well as select bunyavirus transcripts. We are currently investigating the impact of XRN1 stalling on these viral 3' UTRs on arena/bunyaviral replication and cellular biology.

Graduate Student/ Microbiology, Immunology and Pathology

19 | Influence of bone protein vaccine on macrophage recruitment and healing following allograft reconstruction of a massive bone defect

Edward Cheng, Laura S. Chubb, Ruth J. Rose, Kaitlyn L. McNamara, Steven Dow, and Nicole P. Ehrhart

Critical size bone defects resulting from tumor resection or trauma remain a clinical challenge. Although allograft bone is often used for reconstruction, infection and poor incorporation often lead to construct failure. Studies have shown that mesenchymal stromal cells (MSCs) have anti-inflammatory and healing properties, and when combined with an allograft may improve outcomes. Macrophages play a key role in mediating inflammation and healing: type I macrophages (M1) are pro-inflammatory and type II macrophages (M2) are anti-inflammatory. We have shown that vaccination with donor bone proteins and/or administration of MSCs dampen(s) the recipient's immune response. We hypothesized that the vaccination of massive cortical allograft recipients with donor bone proteins would reduce the recipient's immune response to the allograft as demonstrated by an increased presence of M2 and by improved healing of allograft to host bone as compared to recipients that did not receive a bone vaccine. We also hypothesized that this effect would be greater when the bone vaccine was given with MSCs. To address this, we performed a study where a mid-femoral critical size defect was created in 20 mice and reconstructed with one of four treatments: autograft only, allograft only, allograft with bone protein vaccine, and allograft with bone protein vaccine and MSCs. Blinded qualitative histology was performed to assess healing at host-graft junctions and immunohistochemistry (IHC) was completed to quantify M1:M2 ratio. These results will be used to design a pivotal study to evaluate the benefits of vaccination with donor bone proteins to promote improved allograft outcomes.

DVM Student/ Clinical Sciences

20 | Opioids attenuate light-evoked firing of intrinsically photosensitive retinal ganglion cells via increasing a voltage-gated K+ current.

Allison M. Cleymaet, Mikhail Y. Lipin, and Jozsef Vigh

We have shown that intrinsically photosensitive retinal ganglion cells (ipRGCs) express μ-opioid receptors (MORs) and that MOR specific agonists strongly attenuate light-evoked firing of ipRGCs. The effect of MOR specific agonists on the underlying molecular events is unknown. It is known that an important inhibitory mechanism for opioid-induced anti-nociception is neuronal hyperpolarization via K+ channel opening. We hypothesized that MOR specific agonists attenuate ipRGC firing via increasing K+ current (I_K) near the membrane potential threshold of first spike firing. Solitary, cultured ipRGCs from mice with fluorescently labeled ipRGCs (Opn4::EGFP) were utilized. We dissected the effects of DAMGO (1 μM), a MOR specific agonist, on ramp-evoked firing and I_K of ipRGCs. Ramp-evoked spiking was recorded in control and with DAMGO. Parallel experiments were performed via pretreatment with MOR antagonist CTAP (5 μM). After pharmacologic isolation of I_K, leak subtracted, normalized ramp and step evoked current voltage characteristic curves corrected for liquid junction potential were generated. Students t-test was used for analysis. Data are reported as mean ± SEM. DAMGO increased the latency and current threshold of the 1st current ramp-evoked spike without altering the activation threshold of voltage-gated Na+ channels. However, DAMGO significantly reduced the activation threshold of I_K from -45.06±2.40mV to -52.74±1.71 mV ($n=8$, $p<0.001$). DAMGO-induced inhibition of ipRGC excitability is consistent with DAMGO-mediated increase in I_K at the activation threshold of voltage-gated Na^+ channels; the resulted increase in potassium conductance delays action potential generation by competing with the Na^+-mediated self-propelled depolarization. This mechanism underlies opioid-induced attenuation of light evoked firing of ipRGCs. As ipRGCs are exclusively responsible for the photoentrainment of the sleep-wake cycle and are key regulators of photophobia, further elucidation of opioid-induced inhibitory effects on ipRGCs may have significant impact on future therapeutic mediation of circadian rhythm pathology and photic-induced pain.

Resident/ Biomedical Sciences

21 | Mercury and selenium partitioning in Steller sea lion blood compartments

Kelly Cunningham, Judith M. Castellini, Todd M. O'Hara, and Lorrie D. Rea

Some Steller sea lions are exposed to high levels of mercury (Hg) as a result of their high trophic level. Selenium (Se), an essential trace element, has the ability to detoxify Hg directly by forming insoluble complexes in some tissues, while also important as an antioxidant and a cofactor in the glutathione peroxidase antioxidant pathway to protect against cellular oxidative stress. Thus, it is hypothesized that Se in Steller sea lion diets provides some protection from the effects of Hg toxicity. To further evaluate this, we measured Hg and Se concentrations and their molar ratio (TSe:THg) in three blood compartments (packed cells, plasma, and whole blood) to determine variations in compartmental distribution and potential for interactions. Selenium concentrations were measured using an Atomic Absorption (AA) Spectrometer with a Flow Injection for Atomic Spectroscopy (FIAS) System after two chemical digestions (nitric acid and hydrochloric acid). Mercury concentrations were measured in each compartment using a Direct Mercury Analyzer (Milestone DMA-80). Standard reference materials were used on both machines to calculate percent recoveries. Concentrations of Hg and Se in the plasma were significantly different than the concentrations of Hg and Se in the whole blood and packed cells, and the selenium to mercury molar concentration ratio (TSe:THg) in the plasma was significantly different than the ratio in the whole blood and packed cells (Kruskal Wallis ANOVA of $p<0.05$). We also compared TSe:THg among seven rookeries located in four different regions of Alaska, concluding that with the exception of two rookeries, TSe:THg varied among locations. In future studies we will use these ratios, along with population data from the different rookeries and regions, to conclude if, and to what degree, Se is protecting against the negative effects of Hg or may be at inadequate levels to perform some essential functions.

DVM Student/ Clinical Sciences

22 | Clinical data & prevalence of pathologic levels of copper in canine hepatic cytology

L. Eryn Del Monte, Emily Coffey, and A. Russell Moore

Reported prevalence of canine copper associated hepatopathy is increasing. Wright-Giemsa stained hepatic fine needle aspirates are poorly sensitive for hepatic copper. A cytologic copper grading (cCu) protocol which shows good correlation with quantitative copper (qCu) measurements has been established and suggests that cCu>5 indicates pathologic levels of hepatic copper. Knowledge of the prevalence of cCu>5 in routine canine liver cytology and associated clinical findings will help cytologists determine when to perform cytologic copper grading. The objective of this study was to determine the prevalence and associated clinical findings of cCu>5 in routine canine hepatic cytology samples. 163 archived canine liver aspirates submitted to the CSU Clinical Pathology Lab between 2011-2013 were randomly selected. Twenty cases with qCu and fifteen cases from a pilot study were also included. One slide from each case was rhodanine stained and copper graded. Clinical data, including the initial cytology report, were retrieved from the medical record. Nine cases with cCu>5 were found. In the randomly collected group, copper was not described in the initial cytology reports of any of the cases; prevalence of cCu>5 was 1.22% (2/163). ALT>180 IU/L and AST>90 IU/L were more common in the cases with cCu>5 than cases with cCu5 was 27.27% (6/22). Dogs with elevated ALT, AST, or a clinical suspicion of copper hepatopathy were found to be more likely to have an elevated cytologic copper grade.

DVM Student/ Microbiology, Immunology and Pathology

23 | Low pathogenicity avian influenza virus maternal antibody transfer among captive mallards (Anas platyrhynchos)

Katherine L. Dirsmith and Susan A. Shriner

Waterfowl have been found to be reservoirs of many haemagglutinin and neuraminidase subtypes of low pathogenicity avian influenza virus (LPAIV). Because infection may cause only mild pathology, LPAIV can be transported asymptomatically over large areas and affect many populations of wild birds. Maternal antibodies to LPAIV have been shown to be extremely important in chick survival early in life, as well as in population level disease dynamics. Multiple factors, including hen circulating LPAIV antibody concentration, are correlated with the concentration of circulating antibodies in chicks. In this study, we examined the transfer of maternal LPAIV antibodies in mallard (*Anas platyrhynchos*) chicks. Before chicks hatched, we monitored nest activity and determined hen-nest associations. After chicks hatched, we collected blood samples from chicks at regular intervals and tested these samples for LPAIV antibody presence by IDEXX ELISA. Our results indicate that approximately 23% of chicks remained positive, as indicated by ELISA S/N ratios of less than 0.7, for antibodies to LPAIV 14 days after hatching. Chicks belonging to approximately 30% of nests remained positive 14 days after hatching. Further investigation is warranted into how factors affecting maternal antibody transfer among mallards and other waterfowl species play a role in LPAIV epidemiology.

DVM Student/ Microbiology, Immunology and Pathology

24 | Correlation of Mycoplasma quantitative PCR to severity of conjunctivitis in cats

Alexis J. Dubin, Jennifer R. Hawley, Cynthia C. Powell, Michael R. Lappin, and Julia K. Veir

Mycoplasma species are one of the most common infectious causes of conjunctivitis in cats. *Mycoplasma felis* is commonly implicated as a primary pathogen, but other *Mycoplasma* species have also been detected in clinically ill cats. Findings from previous studies using conventional PCR (cPCR) to investigate the role of *Mycoplasma* species in causation of feline conjunctivitis have been mixed as *Mycoplasma* can be carried by apparently normal cats. Therefore, the purpose of this study was to determine if increasing severity of conjunctivitis in cats correlates with higher *Mycoplasma* species copy numbers using qPCR. A total of 77 conjunctival swabs collected from 29 shelter cats with conjunctivitis and confirmed to contain *Mycoplasma* species DNA using cPCR were selected for study. The severity of conjunctivitis at the time the samples were acquired was determined using a grading scheme from 0 - 9. The samples were evaluated using a previously validated qPCR to determine the *Mycoplasma* copy number. Statistical methods consisted of using the Spearman's rho test to determine if severity of conjunctivitis was correlated to qPCR *Mycoplasma* species copy number. The results revealed the severity of conjunctivitis significantly correlated to qPCR *Mycoplasma* copy number (Spearman's correlation coefficient -0.32, P=0.0042), however, the strength of this correlation was only mild to moderate. Based on the results of this study, future investigation of the impact of *Mycoplasma* species other than *M. felis* on the correlation of qPCR and severity of conjunctivitis in cats should be performed.

Resident/ Clinical Sciences

25 | In vitro comparison of three suture methods for closure of pelvic flexure enterotomy in normal horses

Jennifer D. Earnest, Ellison D. Aldrich, and Valerie J. Moorman

Pelvic flexure enterotomy is commonly performed during colic surgery in a variety of circumstances but results in prolonged surgical time. Increased surgical and anesthetic times, particularly in systemically ill horses, have been associated with higher morbidity; therefore, decreasing surgical time is of great benefit. Our objective was to evaluate a novel suture technique for a pelvic flexure enterotomy in which a two-layer closure was performed by beginning the second layer without cutting the suture at the end of the first layer. This novel suturing construct was compared to single layer and traditional two-layer hand sewn techniques. The pelvic flexures of 18 horses euthanized for reasons other than GI disease were harvested. Each pelvic flexure had one 10 cm enterotomy performed and 6 were randomly assigned to each group. All constructs were evaluated for suture time, reduction of luminal diameter via contrast radiographs, and bursting pressure. Data were analyzed using ANOVA with significance at $P < 0.05$. No significant differences in time to completion, reduction of luminal diameter, or bursting pressure were found between the traditional two layer closure and novel techniques. The single layer closer was found to be significantly faster to complete compared to both conventional two-layer closure ($P = 0.024$) and novel two-layer closure ($P = 0.030$). As a single layer closure can have a higher incidence of complications, a two-layer closure is usually preferred. These results suggest that the novel technique may be a suitable alternative technique to the traditional two layer closure.

DVM Student/ Clinical Sciences

26 | Genome mapping for loci that control differential strain susceptibility to lymphoid and non-lymphoid hematopoietic neoplasms in mice

Elijah Edmondson, D. Gatti, Christina Fallgren, Elvin Garcia, Debra Kamstock, O. Iancu, and Michael Weil

Neoplasms occur at significantly different incidences in mice based on constitutional genetic variations within strains. One example is thymic precursor T-cell lymphoblastic lymphoma, which occurs in essentially 100% of AKR/J mice by one year of age but has not been reported in C57BL6/J mice. To examine the genetic loci that are associated with susceptibility or resistance to specific tumor types, we utilize multi-parent outbreeding strategies that produce genetically unique mice and introduce allelic variants from multiple founder strains, high-density single nucleotide polymorphism (SNP) genotyping that adequately captures recombination events in large numbers of mice, and bioinformatic analytical strategies for mapping in genetically heterogeneous populations that utilizes founder haplotype information. 1,850 HS/Npt stock mice of both sexes were genotyped for 77,808 SNPs and exposed to 0.4 Gy of 240 MeV/n ^{28}Si ions or 600 MeV/n ^{56}Fe ions, 3 Gy of ^{137}Cs γ-rays, or sham irradiated. Genome reconstructions, which provide the basis for genome-wide SNP imputation, were completed for each mouse using algorithms for probabilistic assembly of founder haplotypes. Polygenic covariance among related individuals was corrected for during quantitative trait loci (QTL) mapping using a kinship term and significance thresholds were determined with permutation tests. Out of 1850 mice, 612 lymphomas were identified over the 800 day study. Lymphomas were sub-typed using tissue microarrays for B220 and CD3, histomorphology, and anatomic distribution. Lymphoid and non-lymphoid hematopoietic neoplasms were found to be highly heritable and clustered within families.

Postdoctoral Fellow/ Environmental and Radiological Health Sciences

27 | Patterns of PD-L1 Expression by Canine Tumors and Association with T Cell and Myeloid Cell Infiltrates

Erica A. Faulhaber, Jonathan Coy, Daniel Regan, Molly Schlichenmayer, Genevieve Hartley, Amanda Guth, Robyn Elmslie, and Steven Dow

In human medicine, tumor expression of PD-L1 is associated with prognosis and the density of T cell infiltrates in these tumors can predict responses to PD-1/PD-L1 immunotherapy. This information is currently not available for our veterinary patients, therefore the purpose of this study was to measure tumor PD-L1 expression and the density of T cell and macrophage infiltrates in canine tumors and determined whether there was an association between the three. Tumor tissues from 31 dogs were immunostained for PD-L1 expression, using a canine PD-L1 specific mAb. Tissues were also immunostained with antibodies to CD11b and CD3 to enumerate macrophages and T cells respectively. Immunofluorescence images were obtained using a confocal microscope and ImageJ software was used for image analysis. PD-L1 expression was observed in 32% of the tumors. The expression was variable within histotypes, with the highest expression seen in more biologically aggressive tumors such as malignant melanoma, histiocytic sarcoma and metastatic mammary carcinoma. We conclude that there is a considerable degree of variability in PD-L1 expression among canine tumors. Although, PD-L1 expression appears to be increased in the tumors with more inflammation, this correlation is not statistically significant. These findings suggest that PD-1 or PD-L1 blockade is an attractive target for tumor immunotherapy in dogs.

Resident/ Clinical Sciences

28 | Effector MAIT cells in Johne's Disease Cattle

Darcy M. Fletcher, Torsten M. Eckstein, and Diane J. Ordway

Johne's disease, caused by Mycobacterium avium subspecies paratuberculosis, is a chronic infectious disease of the intestine in ruminants divided into four stages: (1) silent stage, (2) subclinical stage, (3) clinical stage, (4) advanced clinical stage. The majority of studies have assessed the immune response in the last two stages of disease, but have not evaluated these responses during the beginning stages. It remains unknown how during early infection the host controls the infection in the micro-environment of the intestine. One of the potential protective mechanisms is the MR1-restricted MAIT system, a phenotype which becomes depleted from the blood over time. Our aim was to study this phenomena to better understand what causes the decline of MR1-restricted MAIT cells. Most of the resources were developed for the human model and no cross-reactivities with other animal models were yet reported. Here we present our findings on the cross-reactivities with the bovine system as well as percentages of MAIT cells in the peripheral blood of healthy and clinical cattle infected with Johne's disease.

Graduate Student/ Microbiology, Immunology and Pathology

29 | Time Motion Evaluation of Repeated Lumbosacral Flexion in Dairy Workers

Rebecca H. Foos and John C. Rosecrance

Low back pain has been consistently rated high among dairy parlor workers due the extended and physically rigorous shifts the industry demands. Dairy milking parlor design has two main purposes: to ensure worker safety by maintaining separation from the cow, and to ensure access for udder preparation and milking. These factors together require a certain reach distance for the worker to accomplish the requisite tasks. This forward reach forces the majority of workers to flex at the lumbosacral portion of the spine, a motion that when repeated increases the risk for chronic low back pain and eventually musculoskeletal disorders. This study aims to quantify the amount of forward lumbosacral flexion experienced during a full 12 hour work shift in modern dairy milking parlors. Three different dairy parlor configurations were filmed over a 48 hour period, and duration of lumbosacral flexion past 15 degrees quantified by visual analysis for each worker. Worker anthropometric data was regressed with the determined duration of flexion, together with peak flexion values observed during normative working tasks. This study served as an introduction to low back ergonomic investigation within the dairy worker population. Future research will incorporate inclinometry to quantify exact flexion values paired with heart rate data representing metabolic demand within the various task cycles.

Graduate Student/ Environmental and Radiological Health Sciences

30 | Optimizing Digital Droplet PCR to quantify mRNA expression levels in naïve and Mtb infected mouse models

Joylynn B. Gallegos, Jennifer S. Arab, Carol J. Wilusz, and Mercedes Gonzalez-Juarrero.

Mycobacterium tuberculosis (Mtb) is an infectious bacterium causing Tuberculosis (TB) disease, with multi-drug-resistant (MDR)-TB being resistant to the most powerful anti-TB drugs. Due to MDR-TB, development of new drugs to combat TB is important. siRNA duplexes to knockdown gene expression are used toward target sequences determined to be important towards progression of tuberculosis. Knocking down gene expression to see effect on TB progression can be used to develop new drug treatments. However, our method of quantifying changes in gene expression during pulmonary chronic Mtb infection using Quantitative Reverse Transcription PCR (qRT-PCR) was not effective in detecting low copy number transcripts. IL-10 and STAT3 are targeted, due to the importance of these inhibitory molecules in the pathogenesis of chronic pulmonary TB. Since IL-10 and STAT3 mRNA have low levels of expression, and Digital Droplet PCR (ddPCR) technology core has been introduced to Colorado State University, ddPCR is utilized for detection of our low level targets. To optimize ddPCR to quantify IL-10 and STAT3 mRNA expression levels, RNA from naïve and Mtb infected mouse models were used. Lungs from naïve and Mtb infected Balb/c, C57BL/6, and Vert-X mice are harvested and extracted using Trizol/chloroform extraction. cDNA is synthesized via reverse transcription and used as a PCR template. ddPCR is ran using a BioRad Evagreen ddPCR supermix and primers specific for STAT3 or IL-10, with the reaction partitioned into ~20,000 droplets using microfluidics prior to thermal cycling. Amplification is assessed by measuring fluorescence in each droplet using the QX200 droplet reader. DNA copy number can then be inferred from the number of positive droplets using Poisson distribution. Our results show that ddPCR can effectively quantify expression of STAT3 in RNA obtained from naïve and Mtb infected lung mouse samples. We are currently optimizing ddPCR to quantify expression of IL-10 and other transcripts.

Graduate Student/ Microbiology, Immunology and Pathology

31 | Fgfr1 and Fgfr2 protein expression in canine osteosarcoma

Teresa Garcia, Deanna Dailey, J. Brad Charles, and E.J. Ehrhart

Appendicular osteosarcoma (OSA) is the most common primary tumor of bone in domestic dogs. OSA carries a poor prognosis; with standard of care, the median survival time is 284 days. Because of the multifaceted etiology of some cancers, supplemental treatments targeting oncogene products have improved disease outcome in some types of human cancer, and show potential for similar results in dogs. Fibroblast growth factor receptor (FGFR) subtypes 1 and 2 have been implicated as oncogenes in cancers including canine soft tissue sarcomas. In canine OSA, preliminary RT-qPCR analysis revealed FGFR1 and FGFR2 transcripts to be significantly elevated as compared to normal bone. The current study examined protein expression of FGFR1 and FGFR2 in OSA tumors from 25 dogs, 14 with disease free interval (DFI) greater than 300 days and 11 with DFI less than 100 days following amputation and chemotherapy. Two different scoring methods were used to assess staining intensity for 5 high powered fields per sample. We confirmed the expression of FGFR1 and FGFR2 protein in 20/20 of canine OSAs via immunohistochemistry. There was a significant increase in staining intensity score for FGFR2 in dogs with DFI less than 100 as compared to dogs with a DFI of greater than 300 days, and there was no significant difference in staining intensity score for FGFR1 between these cohorts. These results support a potential role for FGFR1 and FGFR2 in the progression of OSA. Future studies will utilize OSA cell lines expressing FGFR1 and FGFR2 as models to examine the efficacy of FGFR-directed targeted therapies.

DVM Student/ Microbiology, Immunology and Pathology

32 | Comparison of blood progesterone values obtained from an in-house one hour enzyme linked fluorescent immunoassay (ELFA) or radioimmuno assay (RIA)

Kristina E. Glapa, Ryan A. Ferris, Brittany Bayer, and Pat M. McCue

Determination of blood progesterone concentration is routinely performed during equine reproduction case management. The goal of this project was to compare an ELFA progesterone assay (Mini Vidas) to a previously validated progesterone RIA. Material and Methods. Experiment 1: Serum and plasma (sodium heparin and EDTA) were collected from eighteen mares. Samples were centrifuged and serum/plasma removed divided into aliquots and frozen. Experiment 2: A total of 238 blood EDTA plasma samples were collected from throughout the estrus cycle. Samples were centrifuged, plasma removed, divided into aliquots and frozen. The same two technicians performed progesterone analysis (Mini Vidas) in accordance with manufacturers instructions. Briefly 250 µl of equine plasma was diluted in 250 µl of serum free buffer, 200 µl of the diluted equine plasma was deposited in the test cartridge, and the progesterone program was selected on the machine. Paired frozen plasma aliquots were submitted for determination of progesterone by RIA. Results: Experiment 1. EDTA plasma was found to be more accurate as compared to serum and heparinized plasma. Similar results between the Mini Vidas and RIA were obtained across a wide spectrum of progesterone values. The inter- and intra-assay coefficient of variation was 4% and 9% respectively. Discussion. The Mini Vidas one hour progesterone assay is able to return similar results to a previously validated RIA. Clinically this allows practitioners to make an informed clinical decision based on rapid and accurate progesterone values.

Undergraduate Student/ Clinical Sciences

33 | Sorting of equine sperm using a microfluidic device as a method of sperm selection for IVF and ICSI

Raul Gonzalez-Castro and Elaine M. Carnevale

Microfluidic technology can be used for sperm separation. Microfluidic devices generate a fluid flow to sort sperm from a media reservoir into a collection chamber, avoiding centrifugation, which can diminish the risk of ROS exposure and DNA damage. In the human and mouse, the use of microfluidic devices resulted in the selection of sperm with improved motility, morphology and DNA integrity for IVF, intrauterine insemination and ICSI. We hypothesized that equine sperm can be separated using a microfluidic sorting device (FERTILE PLUS™, DxNow, MA) to improve the sperm quality for ICSI. The aim of our research was to evaluate sperm parameters in frozen-thawed samples of equine semen before and after sorting using this device. Frozen-thawed semen samples (n=10) from research stallions (n=3) with good quality and clinical samples (n=11) from private stallions (n=7) with variable quality were sorted and analyzed. Sperm analyses included motility, morphology, live-dead sperm, membrane integrity (HOS, hypo-osmotic swelling test), and DNA fragmentation (Sperm chromatin dispersion). Two sample t-tests were used to compare sperm parameters. The use of the sorting device improved all sperm parameters in research samples (motility: 37.2±13.0% and 62.2±15.6%, P=0.002; normal morphology: 60.1±12.2% and 75.5±9.7%, P=0.006; live sperm: 55.8±16.0% and 73.6±12.9%, P=0.03; HOS: 33.7±7.2% and 48±9.7%, P=0.001; DNA fragmentation: 12.3±4.4% and 5.6±4.4%, P=0.004). Also, improvements were noted in clinical samples with increased motility (22.0±13.0% and 57.0±11.6%, P=0.0009), normal morphology (58.4±9.6% and 74.0±10.3%, P=0.005), percentage of live sperm (55.5±11.2% and 68.3±14.2%, P=0.04), and decreased DNA fragmentation (22.3±14.7% and 8.2±8.3%, P=0.004); no effect was observed on HOS (21.2±6.0% and 24.9±11.5%, P=0.19). Our results demonstrate that use of the FERTILE PLUS™ resulted in a sperm subpopulation with improved quality parameters. Separation of sperm using a microfluidic device has the potential to select sperm with desirable characteristics for equine assisted reproductive techniques.

Graduate Student/ Biomedical Sciences

34 | Intracellular lipid content impacts Dengue particle infectivity

Rebekah Gullberg, J. Jordan Steel, Richard J. Kuhn, and Rushika Perera

Positive strand RNA viruses interact with cellular phospholipid membranes and their biosynthesis machinery at multiple stages of their life cycle. These interaction points represent novel therapeutic targets for viruses such as Dengue virus (DENV), a +ssRNA virus in the *Flaviviridae* family, which causes an estimated 400 million infections per year and has no approved antivirals. DENV proteins in particular have been shown to interact with fatty acid synthase, an enzyme that generates the fatty acid tails that are the building blocks of phospholipids. Intriguingly, not all fatty acids behave the same in a phospholipid membrane. In fact, unsaturated fatty acids can lead to curvature and enhance fluidity of the membrane. The rate-limiting enzyme in mono-unsaturated fatty acid (MUFA) biosynthesis, stearoyl coA desaturase-1 (SCD1), is conserved across multiple genera and is under considerable investigation for its role in diseases such cancer, diabetes and metabolic syndrome. Interestingly we have found that knockdown and pharmacological inhibition of SCD1 leads to a decrease in DENV replication. Furthermore, we have found that DENV specifically upregulates SCD1 expression in infected cells. DENV buds from the endoplasmic reticulum (ER) where it gains its lipid envelop. This is also the site of MUFA synthesis by SCD1. Remarkably, we have found that inhibition of SCD generates a less infectious virus particle suggesting that the lipid content of the ER is altered, incorporated into the virion and affects its interaction with a new cell. We hypothesize that DENV alters the lipid content of the ER through interactions with SCD1 to generate an infectious particle. Therefore, targeting this enzyme represents a novel therapeutic approach.

Graduate Student/ Microbiology, Immunology and Pathology

35 | A methyl-specific RNA-binding protein is highly expressed in induced pluripotent stem cells

Adam Heck, Aimee Jalkanen, Jeff Wilusz, and Carol Wilusz

Pluripotent stem cells possess the ability to differentiate into every cell type in the body, allowing them to play a pivotal role in driving early growth and development. Achieving differentiation down a specific pathway requires a coordinated change in gene expression that involves both transcription and mRNA decay. One poorly understood mechanism for regulating mRNA decay involves methylation of adenosines in the RNA molecule. This modification influences RNA structure and association of RNA-binding proteins (RBPs)/ miRNAs and has recently been linked with establishment and maintenance of pluripotency making it a high priority for intensive investigation. YTHDF2 is an RBP that associates specifically with methylated RNAs to induce their decay, implicating it as a factor capable of remodeling the transcriptome. We have determined that the YTHDF2 protein is up-regulated in induced pluripotent stem cells (iPS) as compared to fully differentiated human foreskin fibroblasts (HFF) despite similar mRNA abundances in both cell lines. This led us to conclude YTHDF2 is regulated at the translational or post-translational level. Based on the recently established connections between pluripotency and RNA methylation and the differential regulation of YTHDF2 expression we uncovered, we propose that down-regulation of YTHDF2 may be required during differentiation to slow decay and stabilize mRNAs encoding lineage specific factors.

Graduate Student/ Microbiology, Immunology and Pathology

36 | Seasonal cold exposure modulates metabolic phenotype and mitochondrial function in obese golden-mantled ground squirrels

Ashley B. Heim, Melanie A. Lashbrook, Susana A. Rosales, Jordan B. Wakefield, Gregory L. Florant, and Adam J. Chicco

Golden-mantled ground squirrels (Callospermophilus lateralis; GMGS) develop seasonal obesity in early autumn, which provides essential fuel stores for successful hibernation through winter. The present study evaluated the effect of seasonal cold exposure on body composition, glucose tolerance and tissue mitochondrial function in obese GMGS prior to hibernation. We hypothesized that cold exposure compliments seasonal adiposity by altering metabolic and mitochondrial parameters that further optimize physiological preparation for hibernation. Adult GMGS were allowed to acclimate to a 22°C environment for at least 4 weeks before being sacrificed as lean summer controls (Lean) or randomized to continue for 4-6 more weeks at 22°C (Ob-warm) or 15°C to simulate the seasonal decrease in temperature normally encountered by GMGS (Ob-cold). Blood glucose disposal was much more rapid in Lean compared to Ob groups, and greater in Ob-cold vs. Ob-warm animals, indicating an expected decrease in glucose tolerance with obesity that was attenuated by cold exposure. Liver mitochondria from obese animals exhibited greater maximal ADP-dependent (OXPHOS) respiration vs. Lean with both carbohydrate (pyruvate) and lipid (palmitoylcarnitine) substrates. Cold exposure attenuated the increase in lipid OXPHOS in obese animals, but had no effects on carbohydrate OXPHOS capacity. Conversely, in cardiac mitochondria, maximal and carbohydrate OXPHOS capacity was similar between Lean and Ob-warm groups, but significantly elevated in Ob-cold animals. These adaptations might serve to optimize systemic preparation for prolonged hypometabolism and reliance on lipid stores during hibernation, and provide insight into the metabolic effects of physiologic cold exposure with potential relevance to obesity-related metabolic disorders.

Graduate Student/ Biomedical Sciences

37 | A high fat diet versus regular chow drives an inflammatory cytokine profile in fat depots in obese guinea pigs

Emily Hein, Lauren Radakovich, and Kelly Santangelo

In the U.S., obesity affects 34.9% of adults aged 20 years or older. Western high fat diets (HFD) contribute to the growing trend of obesity, highlighting the importance of understanding the influence of diet composition on systemic health. Adipose tissue not only serves as a storage depot but is also an active, cytokine-secreting endocrine organ. Preliminary studies in our laboratory provide evidence that, when equated for body weight, obese guinea pigs fed HFD experience greater systemic inflammation than obese animals fed regular chow (RC). Based on these findings, we aimed to investigate the effects of diet-induced obesity on the expression of specific inflammatory cytokines from visceral fat depots, with the hypothesis that those eating a HFD will express higher levels of inflammatory cytokines. 2 month-old Hartley guinea pigs were fed ad libitum HFD (n=6) or RC (n=6) for 2 months. Total RNA was extracted from gonadal and retroperitoneal fat depots, and fold changes were calculated using the comparative CT method (relative to the GAPDH) following real time quantitative RT-PCR. In both fat pads, animals consuming HFD had significantly decreased expression of interleukin (IL)-10, an anti-inflammatory cytokine, and increased expression of IL-1beta, a pro-inflammatory cytokine. These findings suggest the presence of active inflammation in adipose and a dampening of negative feedback on this inflammation. The results imply that diet composition, and not strictly calorie consumption, may influence the degree of local inflammation in visceral fat depots, providing rationale for continued studies to investigate the underlying mechanism(s) for these differences.

DVM Student/ Microbiology, Immunology and Pathology

38 | Localization of bacteria during cases of equine bacterial endometritis

Margo Hennet, Ryan Ferris, Grace Borlee, Pat McCue, and Brad Borlee

Bacterial endometritis is a significant cause of reduced pregnancy rates in the equine breeding industry. The goal of this project is to determine the location of bacteria during infections and define the best fixative for evaluation of endometrial samples in routine practice. A model of equine bacterial infections was utilized, in which mares (n=6) were placed on progesterone for 5 days followed by bacterial inoculation. Bacterial inoculation consisted of 10^{6-8} CFU of 3 clinical Pseudomonas aeruginosa isolates genetically modified to constitutively express a luciferase gene. Mares remained on progesterone for 5 days post inoculation, at which point the mares were euthanized and the reproductive tracts were removed. The uteri were imaged with an IVIS imager to localize the presence of the tagged bacteria. The uteri were subsequently washed to remove non-adherent cells and reimaged. Endometrial samples were collected for histopathology from areas with and without bacteria (as determined by luminescence). Non-adherent bacteria and adherent were detected at the base of and extending into both uterine horns. The adherent material could be visualized on the surface of the endometrium in Bouin's fixed tissue (100%) but was not present in buffered Formalin fixed tissue (0%) (4 samples per mare evaluated from 6 mares). Clinically this information suggests to practitioners that routine sampling to detect bacteria in cases of bacterial endometritis should be taken at the uterine bifurcation or proximally, and that Bouin's solution is the preferred method for detecting adherent material.

DVM Student/ Clinical Sciences

39 | Pharmacokinetics of intravenous and subcutaneous dolasetron and pharmacodynamics of subcutaneous dolasetron in purpose-bred cats

Andrea Herndon, Liberty Sieberg, Leigh Davis, Amber Caress, Ryan J. Hansen, Luke Wittenburg, and Daniel L. Gustafson and Jessica M. Quimby

Dolasetron is a 5-HT_3 receptor antagonist anti-emetic, dosed at 0.5-1 mg/kg intravenous (IV) or subcutaneous (SQ) daily. Pharmacokinetic (PK) and pharmacodynamic (PD) studies in cats have not been performed and this was the purpose of this study. PK study: Five cats received 0.8mg/kg SQ and IV dolasetron in a cross-over manner. Serum samples were obtained via jugular catheter at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36 and 48 hours after dolasetron administration. Dolasetron and active metabolite hydrodolasetron were measured using liquid chromatography/tandem mass spectrometry. Non-compartmental pharmacokinetic analysis was performed. PD study: Subcutaneous dolasetron (0.8mg/kg or 1.0mg/kg) or placebo was administered 30 minutes before intramuscular administration of 0.44 mg/kg xylazine in a randomized crossover manner. Number of emetic events, time to onset of vomiting and visual nausea score were scored by blinded observer. PK: Dolasetron was metabolized to hydrodolasetron with only two cats in each administration route having measurable concentrations beyond 2 hours, limiting assessment of dolasetron PK. There was no significant difference in hydrodolasetron exposure between administration routes. Pharmacokinetic parameters are summarized below. Mean maximum hydrodolasetron serum concentration (ng/ml) 140.6±106.0 IV, 78.3±26.1 SQ; mean time to maximum concentration (hr) 0.4±0.1 IV, 0.63±0.3 SQ; Mean halflife (hr) 4.2±1.8 IV, 4.0±1.0 SQ; area under the curve (hr*ng/mL) 314.0±130.9 IV, 429.4±165.4 SQ. PD: When dolasetron was administered prior to xylazine, there was no significant difference in number of emetic events, time to onset of vomiting or visual nausea score compared to placebo. Dolasetron did not maintain serum concentrations for 24 hours or adequately control xylazine-induced vomiting. Additional studies are needed to determine if a higher dose is efficacious.

DVM Student/ Clinical Sciences

40 | Preliminary investigation of overgrown hooves in Colorado cervids

Dana Hill, Eric Bergman, Lisa Wolfe, Sushan Han, and Karen Fox

For a number of years, Colorado Parks and Wildlife has received reports of hoof overgrowth in free-ranging deer, elk, and moose. To gain a preliminary understanding of factors that may be involved in hoof overgrowth in Colorado cervids, we solicited reports of affected animals to estimate overall disease prevalence and distribution, and when available we examined affected hooves to generate hypotheses regarding pathogenesis of the disease. For the initial phase of this study we took a species-specific approach, focusing our efforts on moose. Factors known to affect hoof wall growth in wild and captive moose include conformational abnormalities, metabolic disturbances, trace mineral imbalances, environmental/substrate factors, toxicities, and infectious diseases causing vasculitis and/or hyperkeratosis. We established baseline levels of hepatic trace minerals (n=72) including copper, manganese, molybdenum, and selenium, and analyzed seroprevalence of epizootic hemorrhagic disease virus (n=57), bluetongue virus (n=57), bovine viral diarrhea virus (n=57), and ovine herpesvirus-2 (n=68) in apparently healthy, free-ranging, hunted moose from CO. We used semi-nested PCR for the detection of active and/or latent ovine herpesvirus-2 (malignant catarrhal fever) in archived retropharyngeal lymph nodes from hunter-killed moose. Hooves from affected and unaffected moose were examined histologically to evaluate hoof architecture at the cellular level and determine possible mechanisms for the pathogenesis of overgrown hooves. These preliminary data will be used to interpret mineral levels, serology, PCR, and histopathology findings from moose with overgrown hooves and determine likely contributing factors to this unusual disease.

DVM Student/ Microbiology, Immunology and Pathology

41 | The predictive value of in vivo drug assays against Mycobacterium abscessus

Emily L. Hill, Kimberly Arnett, Barb Andre, Mary Anne DeGroote, Anne J. Lenaerts, and Diane J. Ordway

Purpose: *Mycobacterium abscessus* infections are associated with higher fatality rates than other rapidly growing Mycobacteria (RGM). These emerging pathogens follow in the footsteps of their family member *Mycobacterium tuberculosis* (Mtb). The aim of this study is to determine how well *in vivo* assays of novel compounds against *Mycobacterium abscessus* in a murine model predict clinical outcomes in human patients infected with *M. abscessus*. A predictive value quantifies how accurately the murine model predicts the outcome of clinical treatment of patients. The positive predictive value (PPV) is a ratio of true positive (TP) outcomes in mice to the sum of true positive and false positive (FP) outcomes in mice, where true and false positives are determined by the analogous outcome in human trials. Our approach to track the PPV of *Mycobacterium abscessus* infected Severe Combined Immunodeficiency (SCID) and Granulocyte Macrophage Colony Stimulating Factor knockout (GM-CSF -/-) compound treated mice is based on clinical practices used to evaluate compound efficacy in *M. abscessus* infected patients. Materials / Methods: SCID mice were infected intravenously with *M. abscessus*. Bacterial burden in the lung, liver, and spleen, organ histology, and animal survival curves were collected from ethically euthanized mice. Results: *M. abscessus* infected GM-CSF -/- mice begin to succumb to the infection around 40 days after infection while SCID mice survive into chronic disease. Conclusion: *M. abscessus* infected SCID and GM-CSF -/- mice experience a progressive chronic infection resulting in pulmonary pathology similar to that present in patients infected with *M. abscessus*. Future studies will focus on infection of SCID and GM-CSF -/- mice and tracking bacterial burden, organ histology, and animal survival after standard drug treatment to determine a PPV of our model.

Graduate Student/ Microbiology, Immunology and Pathology

42 | The Use of Equine Bone-Marrow Derived Stem Cells as a Potential Treatment Against Preformed Biofilms

Marta K. Hura, Ryan Ferris, and Brad Borlee

This study aimed to determine if equine bone-marrow derived mesenchymal stem cells are capable of disrupting a biofilm or killing the bacteria within the biofilm in vitro. Bacterial isolates utilized in the study were recovered from equine clinical cases of bacterial endometritis. Methods included using minimum biofilm eradication concentration assays to provide a consistent biofilm matrix to challenge. Variables evaluated included stem cell numbers, fresh vs frozen stem cells, and stem cells in in combination with non-lethal concentrations of antibiotics. Endpoints to assess an effect on the biofilm included determination of biofilm biomass through crystal violet staining and bacterial viability via determination of CFUs. Frozen equine mesenchymal stem cells at a concentration of 160,000 per mL were unable to disrupt a preformed biofilm (control 2.43+/-0.87 as compared to stem cell treatment 1.30 +/- 0.31 at OD600), or reduce the number of colony forming units (control log 8.41 +/-0.22 as compared to stem cell treatment log 8.37 +/-0.20 per mL). Additional variables were evaluated. Future work may include cell migration assays to determine if white blood cells are stimulated to hone to the stem cells during bacterial challenge and in vivo studies in clinical cases of infectious endometritis. Implications of this study are to identify new avenues in the treatment of difficult/non-resolving cases of equine infectious endometritis.

DVM Student/ Clinical Sciences

43 | Post-transcriptional mechanisms coordinate expression of zinc finger protein mRNAs

Aimee L. Jalkanen, Caleb M. Schmidt, Jeffrey Wilusz, and Carol J. Wilusz

The C2H2 zinc finger proteins (ZNFs) are a vast and rapidly evolving family of transcription factors important for development, differentiation, and tumor suppression. Global analysis of mRNA decay rates in human induced pluripotent stem (iPS) cells and genetically matched human foreskin fibroblasts (HFF) revealed that mRNAs encoding C2H2-ZNFs were significantly more stable in iPS cells than in the fully differentiated fibroblasts. Given that over 100 C2H2-ZNF mRNAs were affected, coordinated changes in their expression potentially have wide-ranging impacts on pluripotency and differentiation. Therefore, characterization of the mechanisms, sequences, and factors involved in C2H2-ZNF mRNA metabolism is a high priority. Consistent with previous reports, we find that in HeLa, iPS, and HFF cells, a significant population of multiple C2H2-ZNF mRNAs have unusually short poly(A) tails (often less than 20 nt). Since the removal of the poly(A) tail generally leads to rapid mRNA decay, most mRNAs accumulate with poly(A) tails ranging from ~50 to 300 nt. In contrast, the C2H2 ZNF mRNAs that have short poly(A) tails may be able to resist canonical mRNA decay pathways. Furthermore, we observe that C2H2-ZNF mRNAs were significantly more abundant in the nucleus compared to control mRNAs in HeLa, iPS, and HFF cells. The nuclear retention of C2H2-ZNF mRNAs is another potential mechanism for coordinately regulating gene expression that may be related to the short poly(A) tail of these mRNAs. Analysis of C2H2-ZNF mRNA reporter constructs suggests that sequences in the open reading frame (ORF) and the 3' end are involved in regulating poly(A) tail length and nuclear localization. Previous reports have shown that C2H2-ZNF ORFs contain multiple microRNA binding sites. However, knockdown of Dicer, the key protein involved in microRNA processing, does not alter C2H2-ZNF mRNA short poly(A) tail length or nuclear localization suggesting that microRNAs do not influence these characteristics of C2H2-ZNF mRNAs.

Postdoctoral Fellow/ Microbiology, Immunology and Pathology

44 | Evaluation of antineoplastic effects of JQ1 against a panel of canine lymphoma-derived cell lines

Mark Jeon, Barbara Rose, and Douglas Thamm

The *c-myc* oncogene is known to be a contributing factor in the pathogenesis of human cancer. However, in canine cancer, it is unclear whether MYC is shown to play a part in its pathogenesis. Studies have shown that the amplification of the MYC protein influences the progression of cell cycling and cell division correlating with a poor prognosis for survival. JQ1, a bromodomain inhibitor, is noted to downregulate c-myc transcription by competitively inhibiting bromodomains and BET family of bromodomain proteins. This results in antiproliferative effects, cell cycle arrest, and cell senescence based on studies with human B-cell lymphoma. We hypothesized that MYC is one of the main contributors in the pathogenesis of canine lymphoma and JQ1 would be capable of suppressing canine lymphoma growth. To address this hypothesis, we tested four canine and two human lymphoma-derived cell lines with incremental concentrations of JQ1 and used manual cell counting, flow cytometry, and western blot analysis to measure growth inhibition, apoptosis/cell cycle, and MYC protein expression respectively. JQ1 at nanomolar and micromolar concentrations effectively inhibited the proliferation of canine lymphoma cells. Western blot analysis confirmed that the MYC oncoprotein is present and JQ1 inhibited its expression. With the information gathered, c-myc targeting is a step closer to a therapeutic treatment for cancer. These results suggest that canine lymphoma may be a faithful model for the study of pharmacologic bromodomain inhibitors such as JQ1.

DVM Student/ Clinical Sciences

45 | Coagulopathy in Prairie Rattlesnake (Crotalus viridis) envenomation: with carbon monoxide releasing molecule - 2 increases clot strength and attenuates fibrinolysis

Tyler E. Johnson and Christine Olver

Each year 9% of the hundreds of dogs envenomated by rattlesnakes in Colorado develop coagulopathy. Treatment with anti-venin is largely successful; however some treated patients have persistent bleeding, suggesting hypocoagulation or hyperfibrinolysis. Carbon Monoxide Releasing Molecule-2 (CORM-2), an emerging therapeutic in human medicine, has shown potential to ameliorate the coagulopathy secondary to venom exposure in vitro. There are no publications evaluating the coagulation and fibrinolysis effects of CORM-2 in canine patient population. The effects of C. viridis venom on the viscoelastic properties of coagulation and fibrinolysis in pooled canine patient plasma, with and without CORM-2,was determined using thromboelastography (TEG). The following treatments were used to evaluate the effects of venom with and without CORM-2: 1. Coagulation only (Tissue Factor (TF) activated) + DMSO (vehicle for CORM-2) alone; 2. Coagulation and fibrinolysis (TF and tissue plasminogen activator (tPA)) + DMSO alone; 3. Coagulation (TF activated) + DMSO + venom; 4. Coagulation and Fibrinolysis (TF and tPA activation) + DMSO + Venom; 5. Coagulation (TF activated) + venom + CORM -2, and 6. Coagulation and Fibrinolysis (TF and tPA activation) + venom + CORM-2. CORM -2 was incubated with plasma 5 minutes before addition of venom, and venom was added 5 minutes prior to preforming TEG. Each condition was repeated 6 times. One way ANOVA was used to determine treatment differences in clot strength (MA), time to clot formation (R), speed of clot formation (K), and time to clot lysis (CLT). Our findings thus far indicate that CORM exposure prior to venom incubation causes enhanced clot strength, decreased time to clot formation, and resistance to fibrinolysis. Further analysis of in vitro CORM-2 effects on individual normal canine plasma and the plasma of naturally envenomated patients are required to further evaluate CORM-2 as a possible adjunct therapy for rattlesnake envenomation.

DVM Student/ Microbiology, Immunology and Pathology

46 | DNA damage response signaling inhibition differentially affects canine osteosarcoma cell radiosensitivity

Mariel K. Knox, Jac A. Nickoloff, and Christopher P. Allen

Osteosarcoma is the most common bone tumor in dogs and is proposed as a model for human osteosarcoma. Traditional treatment for canine appendicular osteosarcoma is amputation and chemotherapy. Advances in canine radiotherapy treatment are being pursued as alternatives to traditional treatment. The DNA damage response (DDR) is a coordinated response to genotoxic stress such as ionizing radiation (IR) and chemotherapy as well as environmental insults and cellular stresses. Several DDR proteins are being investigated as targets for cancer therapy in humans. DDR responses are generally highly conserved through evolution, yet little is known about the canine DDR. We hypothesized that combining radiotherapy with targeted DDR inhibition may increase the radiotherapy efficacy for canine osteosarcoma. To test this hypothesis, we conducted clonogenic survival assays combining IR with drugs that inhibit key DDR protein kinases, DNA-PKcs, ATM, ATR, Chk1 and Chk1-2 across three canine osteosarcoma cell lines (D17, McKinley, and Moresco). ATR inhibition significantly increased radiosensitivity in all 3 cell lines. ATM inhibition produced cell line-dependent radiosensitization. Surprisingly, Chk1, Chk1-Chk2 and DNA-PKcs inhibition did not increase radiosensitivity in any cell line. These results suggest that specific defects in the DDR pathways of these cell lines may exist. This may account for the lack of radiosensitization. Alternatively, these compounds may differentially inhibit human and canine DDR proteins. Further investigation of DDR pathways in canine cancer is necessary to determine if targeted inhibition could enhance the efficacy of radiotherapy and refine the role of the dog as a model for human cancers.

DVM Student/ Environmental and Radiological Health Sciences

47 | Effects of Low-level Brodifacoum Exposure on the Feline Immune Response

Jennifer H. Kopanke, Katherine E. Horak, Esther Musselman, Kristine Bennett, Sue VandeWoude, and Sarah E. Bevins

Anticoagulant rodenticides have recently been suggested as a potential inciting factor in the development of notoedric mange in bobcats and other wild felids that may regularly consume rodents. To date, however, these studies have failed to provide direct evidence of a causative association between anticoagulant rodenticide exposure and immune suppression in non-target species. Therefore, the present study sought to determine whether chronic, low-level exposure to brodifacoum resulted in alterations in the direct and recall immune response in felines. Age-matched, specific pathogen free (SPF) domestic cats were exposed to either 0.05 mg/kg brodifacoum or sham bait on a weekly basis for 6 weeks to model environmentally realistic exposure scenarios. Complete blood counts (CBC) and prothrombin time (PT) were monitored bi-weekly as measures of immune function and coagulation. Cats were vaccinated and boosted with 50 µg of two irrelevant antigens (ovalbumin and keyhole limpet hemocyanin (KLH)) at different points during the course of the experiment to assess the recall and direct immune responses, respectively. Measures of immune response included delayed-type hypersensitivity tests (DTH) and cell proliferation assays for ovalbumin and KLH. Ongoing tests include ELISA analysis for ovalbumin- and KLH-specific antibodies, as well as cytokine induction following exposure to vaccine antigens. To date, no coagulopathies have developed, and no differences have been measured in the DTH response between the brodifacoum and control group. This study indicates that cats maybe more resistant to clinical effects of brodifacoum exposure than other species. Completion of this investigation will assess gross impacts of environmentally realistic brodifacoum exposure on humoral and cell mediated immunity against foreign antigen exposures in domestic cats.

Resident/ Microbiology, Immunology and Pathology

48 | PharmCat: a physiologic-based pharmacokinetic (PBPK) model to study virtual drug dosing in cats

Renee C. Lake, Ryan J Hansen, Paul J. Lunghofer, and Daniel L. Gustafson

Cats have known genetic abnormalities in UGT1A6 and ABCG2, leading to alterations in glucuronidation and drug transport that have often resulted in severe drug toxicities. Thus, predicting drug disposition in cats via extrapolation from canine-based pharmacokinetic models for drugs metabolized via such pathways is rarely appropriate. The purpose of this research is to develop a physiologic-based pharmacokinetic (PBPK) model of the cat to better simulate drug disposition by virtue of modeling differences with regard to metabolic pathway efficiencies. Ondansetron was selected for modeling given its frequent use in cats as well as the availability of in vivo pharmacokinetic data for model validation. Experimental determination of ondansetron tissue:blood partitioning was measured from 14 hour time course incubations of feline liver, kidney, GI, heart, and muscle tissues (n=3) for 2 ondansetron concentrations (100, 500 ng/ml). Analysis of final tissue and PBS drug concentration was performed using an LC/MS/MS method and coefficients were calculated as the ratio of [tissue]/[PBS]. Matlab Simbiology was used to construct and simulate a 6 compartment PBPK model for 3 different dosing routes (IV, subcutaneous, and oral dosing). Results for mean tissue:blood partitioning coefficients were as follows: liver (0.43), kidney (0.55), heart (0.63), muscle (0.34), duodenum (0.49), jejunum (0.44), ileum (0.35). Model simulations from initial parameter estimation exercises currently show an 18.77% (percent error) difference between in vivo and in silico AUC values for the IV model, indicating a need for further parameter optimization to properly reflect serum concentrations for later time points (4 hr+). Optimization of subcutaneous and oral dosing routes is pending finalization of the IV model. The development of a feline PBPK model is a first step for estimating tissue drug distribution in cats, and represents a potential clinical tool that can be leveraged in the development of optimized clinical protocols for treating feline disease.

Graduate Student/ Clinical Sciences

49 | Guinea Pig Bone Marrow Derived Macrophages Alter Glucose Metabolism Under Mycobacterium tuberculosis Infection

Natalie A. Lakey, David F. Ackart, Brendan K. Podell, Adam J. Chicco, and Randall J. Basaraba

Elevated blood glucose levels (hyperglycemia) and impaired glucose tolerance are associated with a number of chronic inflammatory diseases including Mycobacterium tuberculosis (Mtb) infection. As has been demonstrated in PET/CT images of late-stage tuberculosis patients, this hyperglycemic state may be due to an increase in glucose demand within Mtb lesions which contain infected immune cells. To investigate, we assessed glucose uptake and metabolism of guinea pig bone marrow derived macrophages under Mtb infection or hyperglycemia using a glucose oxidase assay and high resolution respirometry. Mtb infected macrophages demonstrated (1) an increase in glucose uptake and (2) an increase in rate of extracellular acidification while showing (3) a decrease in rate of oxygen consumption as compared to uninfected macrophages. However macrophages under hyperglycemic conditions demonstrated the opposite trend as compared to those maintained under normal glucose conditions. We conclude that under infected conditions macrophages transition from oxidative phosphorylation to a glycolytic state, while under hyperglycemic conditions macrophages continue to use oxidative phosphorylation to meet their energy demands.

Graduate Student/ Microbiology, Immunology and Pathology

50 | Increased mucosal immunogenicity of L. acidophilus expressing HIV MPER and utilizing adjuvants IL-1β or FliC

Jonathan S. LeCureux and Gregg A. Dean

Background: Mucosal vaccination using HIV-1 antigen-expressing commensal probiotic bacteria is an attractive strategy that is inexpensive, orally delivered and could induce mucosal immunity. We have previously shown that a highly conserved HIV gp41 epitope known to induce neutralizing antibody from the membrane-proximal external region can be expressed within the ubiquitously expressed surface layer protein of *Lactobacillus acidophilus* (SlpA-MPER). Mice immunized with SlpA-MPER produced anti-MPER serum IgG and mucosal IgA. However, antibody levels were relatively low after multiple boosts. To remedy this we utilized two different adjuvant strategies: SlpA-MPER secreting IL-1β and SlpA-MPER expressing Salmonella Flagellin C (FliC) on the bacterial surface. Methods: Balb/c mice were dosed with SlpA-MPER with and without adjuvant to identify adjuvant effects. In addition, CD40L-deficient mice were also dosed to determine whether antibody responses were T cell-dependent or independent. Mice received a prime dose and five boosts and then were sacrificed at 12 weeks. Serum was analyzed by ELISA for MPER-specific serum IgG and vaginal, cecal and fecal IgA. Single cell suspensions of the spleen, mesenteric lymph node, Peyer's patches, female reproductive tract, and large intestine were subjected to MPER-specific IgA ELISpot. Results: Balb/c mice treated with SlpA-MPER + IL-1β or SlpA-MPER + FliC had higher levels of IgG and IgA, as well as MPER-specific B cells, compared with SlpA-MPER-only treatment. CD40L-deficient animals had no MPER-specific B cells and very low levels of detectible antibody. Both the SlpA-MPER + IL1b and SlpA-MPER + FliC strains provided an adjuvant effect for the MPER-LA mucosal vaccine. The lack of a detectible immune response in CD40L-deficient mice indicates the vaccine acts through a T cell-dependent pathway.

Graduate Student/ Microbiology, Immunology and Pathology

51 | Thermal Imaging as an Alternative to PIT Tagging for Monitoring Body Temperature and Clinical Disease Progression in Mice

Erin S. Lee and Lon V. Kendall

Body temperature is thought to be an important parameter to track when monitoring clinical disease progression in conjunction with clinical signs, such as sick rodent posture and other animal behaviors. Passive Integrated Transponder (PIT) tags have been used to monitor mouse body temperature with relatively little stress on the animal. We hypothesized that a thermal imaging camera may be used to obtain body temperatures from mice in manner that is less obtrusive than PIT tags with comparable results. PIT tags were implanted subcutaneously into 2 groups of mice. One group was then infected with *Burkholderia spp*, and one group remained uninfected. Mice were assessed for clinical behavior and body temperature the day prior to infection and daily for 7 days post infection. Clinical behavior was scored on a scale of 0-4 (0- normal; 1- questionable illness; 2- mild but definitive illness; 3- moderate illness; 4- severe illness). Body temperatures were taken with both the PIT tag, as well as thermal images of the eye, base of the ear, and flank. Although thermal imaging temperatures were lower than PIT tag temperature, they trended similarly with eye temperature being the closest to PIT temperatures followed by ear and flank. Increased clinical behavior scores correlated well with lower body temperatures from the PIT tag and thermal imaging. This indicates that a thorough clinical observation scoring system may be a more efficient and effective to track disease progression in mice than monitoring body temperature.

Resident/ Microbiology, Immunology and Pathology

52 | The value of diversity: A genomic analysis of historical and contemporary blue tongue virus isolates

Justin S. Lee, Mark Stenglein, Florante Delacruz, Jim MacLachlan, and Christie Mayo

Bluetongue virus (BTV) is the cause of bluetongue, an economically important, emerging arbo-viral disease of ruminants. The emergence of virulent BTV strains is occurring with increasing frequency in many parts of the world. The introduction of virulent BTV into North America would be catastrophic to the livestock industry in the US. Characterization of the contribution of viral diversity to the distribution, virulence, and transmission of BTV is currently lacking. Such information is critical to the creation of accurate and ecologically sound predictive models and, ultimately, to the formulation of effective control strategies. The overall goal of this project is to better characterize the epidemiology of BTV infection in the western US, with a specific emphasis on accurately describing the molecular evolution of archived field strains of the virus collected over the past 60 years. We hypothesize that full-genome sequences will reveal higher levels of genetic diversity and more clearly elucidate mechanisms of evolution than widely used serology- and PCR-based assays currently allow. To accomplish this, we optimized sample processing and library preparation protocols and used them to sequence over 100 BTV genomes with the Illumina MiSeq and NextSeq sequencing platforms. The resulting genomic sequences were used to construct phylogenetic trees to evaluate spatio-temporal patterns of genetic diversity, and were screened for reassortment and recombination. Preliminary results reveal high levels of genetic diversity across all 10 genome segments. Analyses to unravel the relative contribution of mutation, recombination, and reassortment to this diversity are ongoing. We also plan to compare genetic characteristics of viral isolates to the epidemiologic features of its associated infection of livestock.

Postdoctoral Fellow/ Microbiology, Immunology and Pathology

53 | Evaluation of the equine mental foramen nerve block: foramina anatomic positioning and cadaveric evaluation of needle placement and injectate distribution

Craig S. Lesser, Luke Bass, Benjamin Prytherch, and Jennifer E. Rawlinson

Equine veterinarians performing advanced dental therapies require effective regional anesthesia. A multimodal approach to pain control is necessary to address patient comfort and ensure practitioner efficiency and safety particularly as extractions and advanced treatments are increasingly performed under standing sedation. The mental foramen block is reported to anesthetize the teeth, soft tissue, and bone of the rostral mandible although no evidence-based reports are available regarding foramen anatomy and/or block technique, efficacy, duration, and dosage. This study evaluated 42 helical computed tomographic scans of the equine mandible to investigate the position and location reliability of the mental foramen. Injection technique, anesthetic volume, and injectate distribution were evaluated in 9 longitudinally sectioned cadaveric heads. Two injection techniques were performed, and two volumes (3 and 5 mls) of injectate, a 0.2% New Methylene Blue and iohexol mixture, were randomly injected into specimen mental foramina. Radiography and anatomic dissection were used to determine needle location, the pattern and distribution of injectate, and circumferential inferior alveolar nerve staining. Tests of statistical significance were performed to detect possible associations among variables. Horizontal positioning of the mental foramen was negatively correlated to age (located more rostral with increasing age) and significantly impacted by breed (located more rostral in larger breeds). Injection technique significantly influenced needle placement. Needle placement was significantly associated with the pattern of injectate distribution. Patterning varied from injection site bolusing to contrast threading extending beyond the mandibular foramen. There was no significant impact of injection volume or technique upon circumferential staining of the nerve. Study results demonstrated variability in precise positioning of the mental foramen and distribution of the simulated injectate. Results suggest further investigation into the efficacy of the mental nerve block is warranted to improve the reliability of regional anesthesia to the rostral mandible.

DVM Student/ Clinical Sciences

54 | Fertility may depend on conceptus-derived signals in lactating Holstein cows

Bethany E. Liebig, Milton G. Thomas, Kevin D. McSweeney, Hana Van Campen, Jeanette Bishop, and Thomas R. Hansen

Infertility and early embryonic mortality (EM) are sources of major economic loss to the dairy industry. Interferon tau (IFNT) is a cytokine that is released by the bovine trophoblast and induces transcription of genes (i.e., ISG15) critical to embryo survival and maternal recognition of pregnancy. We hypothesized that selection of cows based on daughter pregnancy rate (DPR) and services per conception (SPC) would result in more viable embryos with greater IFNT production. To test this hypothesis, Holstein cows assigned to high fertile pregnant (HP), low fertile pregnant (LP) or high fertile nonpregnant (NP) groups (n=7 each) based on both DPR (Clarifide®; Zoetis) and SPC and were artificially inseminated. Embryos were collected on day 16 and typed as viable or EM based on morphology and length. Data were analyzed using protected ($P < 0.05$) t-test. Serum progesterone, days in milk, number of lactations and calving date were not different between groups. The DPR was negatively correlated with SPC. The HP cows had more viable (n=5) embryos than the LP cows (n=3). Viable HP embryos were longer than LP embryos. IFNT concentrations in uterine flushing (UF) were: 1) greater in HP compared to LP cows, 2) positively correlated with DPR and 3) negatively correlated with SPC. ISG15 mRNA concentrations determined by qRT-PCR were upregulated in endometrial and peripheral blood mononuclear cells from HP compared to LP and NP cows. In conclusion, selection of dairy cows combining DPR and SPC may improve fertility through increased production and action of IFNT.

Graduate Student/ Biomedical Sciences

55 | Prevalence of select infectious disease agents in client owned cats in Moscow, Russia

Katherine MacMillan, Arianne Miller, Melissa Brewer, Jennifer Hawley, and Michael R. Lappin

Client-owned cats in Moscow, Russia can be exposed to infectious agents with some developing clinical problems noted in cats of other countries. However, limited data are available concerning prevalence rates for this area. The overall objective of this study was to determine the estimated prevalence of select feline infectious disease agents in a convenience sample population of cats from Moscow, Russia. Using previously validated assays, sera were assayed for *Toxoplasma gondii* IgG (Toxo), *Bartonella spp*. IgG (Bart), FeLV antigen (Ag), and FIV antibody (Ab). Total DNA was extracted from blood samples and DNA of *Anaplasma* spp., *Bartonella* spp., *Ehrlichia* spp., and haemoplasmas were amplified by previously validated PCR assays with *Mycoplasma hemofelis* (Mhf) amplicons confirmed by sequencing. None of the cats were positive for DNA of Anaplasma spp. or Ehrlichia spp. but many of the cats showed evidence of exposure or current infection with one of more of the other agents. Bartonella spp. antibodies were most common in serum and *'Candidatus* M. hemominutum (Mhm) DNA was most common in blood. *Toxoplasma gondii* IgG and *Bartonella* spp. IgG titers ranged from 1:64 to 1:2048. Of the 81 cat samples evaluated in all assays, 57.8% of the cats had evidence of infection or exposure to one or more of the select agents. The results from this sample population suggest cats in Moscow are exposed to infectious agents and they should be on appropriate clinical differential lists, processed foods should be fed, ectoparasite control should be maintained, and feline-feline contact should be avoided when possible.

DVM Student/ Clinical Sciences

56 | Robust expression of early innate immunity genes in dromedary camel plasmacytoid dendritic cells in response to MERS-CoV

Ashley Malmlov, Vincent Munster, Sandra Quackenbush, Corey Campbell, and Tony Schountz

Middle Eastern respiratory syndrome coronavirus (MERS-CoV) has caused more than 1000 confirmed cases of disease with a 36% fatality rate. In vitro studies demonstrated that MERS-CoV is sensitive to type I interferons (IFN) and the virus down regulates the IFN response in human respiratory cells, suggesting that innate immunity plays a critical role in the outcome of infection. Plasmacytoid dendritic cells (pDC) produce high amounts of IFN in response to viral infection and are important in controlling viral infections. Human DCs secrete large amounts of IFN when cultured with MERS-CoV. Furthermore, while studies are conflicting, MERS-CoV does not appear to replicate in human DCs. Dromedary camels (*Camelus dromedarius*) are thought to be reservoir hosts of MERS-CoV. A large number of domesticated camels in the Middle East have antibody to the virus, suggesting a potential for spillover to humans. Experimental infection of camels with MERS-CoV showed that camels were susceptible to MERS-CoV infection with subclinical to mild disease that is confined to the upper respiratory tract, including regional lymph nodes. We sought to characterize the innate immune response to MERS-CoV in camel pDCs to delineate differences between humans and camels. Flt3L-derived pDCs were established from camel bone marrow and peripheral blood monocytes and cultured with MERS-CoV. No virus replication occurred. Cells remained healthy upon microscopic examination. Many genes involved in viral sensing were elevated after 8 hours of exposure to MERS-CoV, including TLR7, STAT1, MDA5, RIG-I, IKKE and TBK1. Expression of genes late in the IFN signaling pathway appeared less sensitive to MERS-CoV, or viral accessory proteins inhibit their expression. TNF expression was substantially elevated at 2 and 8 hours; however, it had subsided by 24 hours. These data indicate that camel pDCs are responsive to MERS-CoV, are not productively infected, and may control virus early after cellular entry.

Graduate Student/ Microbiology, Immunology and Pathology

57 | Coxiella burnetii DNA not identified in fleas from domestic cats in Australia and the United States

Alison C. Manchester, Jennifer Hawley, Julia Beatty, Vanessa Barrs, and Michael R. Lappin

Coxiella burnetii is a rickettsial pathogen with serious zoonotic implications (Q fever) transmitted by direct contact and vectored by ticks. Cats infected with *C. burnetii* have been implicated in human infections in Australia and the United States but minimal information exists concerning routes of feline infection. Recently, *C. burnetii* DNA was isolated from *Ctenocephalides felis* collected from wildlife in Cyprus; this flea commonly infests cats. The purpose of this study was to evaluate groups of fleas from cats in the Australia and United States for the presence of *C. burnetii* DNA using a previously optimized PCR assay. The DNA samples utilized in the study had been extracted from fleas infesting 96 cats in previously published studies and maintained at -80°C until the present study. The fleas were collected from cats in eastern Australia (86) and the southern United States (4 Alabama, 6 Florida) and pooled in groups of a maximum of 5 fleas before DNA extraction. A previously reported conventional PCR assay utilizing primers pairs targeting the IS-1111 (IS-5, IS-9, IS-14, IS-20) insertion sequences transposase elements in the *C. burnetii* genome was used. If positive amplicon was detected, genetic sequencing would be performed to confirm *C. burnetii* presence. The detection sensitivity of the assay was shown to be 3.14 ng total *C. burnetii* genomic DNA per PCR reaction utilized in all primer sets. While all positive and negative controls performed as expected, none of the 96 pooled flea extracts were positive for *C. burnetii* DNA. Coxiella burnetii DNA was not recovered from fleas collected from cats in eastern Australia or the southern United States. The results suggest that fleas infesting domestic cats in these regions are not important vectors for *C. burnetii* and are unlikely to play a role in the transmission of the organism to humans with Q fever.

Resident/ Clinical Sciences

58 | [18F]-FDG positron emission tomography – an innovative technique for the diagnosis of canine lameness

Kelly A. Mann

Positron emission tomography imaging with fluorine-18-fluorodeoxyglucose ([18F]-FDG) is widely known for its use in the diagnosis and tracking of primary and metastatic tumors via uptake and retention of the radiopharmaceutical by hypermetabolic cells. [18F]-FDG is also used to study the normal physiology of glucose uptake, metabolism, and muscle activity during and after exercise. A pilot study adding PET imaging to the diagnostic evaluation of canine patients undergoing computed tomography (CT) for mild or intermittent fore- and hind-limb lameness is ongoing. Dogs with an observable (grade II) lameness that have undergone routine radiography and complete physical examination by board-certified veterinary surgeons and sports medicine and rehabilitation specialists are enrolled. Each patient undergoes force plate analysis and leash-walking for 15 minutes prior to premedication and induction of general anesthesia for the PET-CT exam. [18F]-FDG is injected intravenously and a whole body PET exam is conducted after one hour of radionuclide uptake time. Standard, whole body pre- and post-contrast CT exams and a focused, bone-detail CT scan of the fore- or hind-limb areas of interest are obtained concurrently. Abnormal PET-CT findings are further investigated with additional diagnostic imaging or at surgery (e.g., ultrasound, MRI, arthroscopy). This poster presentation describes a recommended imaging protocol and discusses the strengths and limitations of molecular imaging in the clinical evaluation of canine patients with musculoskeletal disease. [18F]-FDG PET-CT adds valuable physiologic and anatomic information to the diagnostic evaluation of patients presenting with indistinct or intermittent clinical signs of musculoskeletal inflammation or injury. The PET acquisition protocol and radiopharmaceutical parameters affect the physiologic information gleaned from the exam.

Postdoctoral Fellow/ Environmental and Radiological Health Sciences

59 | Transposon mutagenesis of potential cyclic-di-GMP metabolic genes in Burkholderia pseudomallei to characterize their function in biofilm formation and motility

Kevin Martin, Brooke Plumley, Grace Borlee, and Brad Borlee

Burkholderia pseudomallei is an emerging pathogen and a potential biothreat with no preventive measures and limited therapeutic options. The goal of our research is to investigate the basic underlying signaling that controls biofilm formation and pathogenesis in *B. pseudomallei*, which is an environmental pathogen that can opportunistically infect humans and cause melioidosis, an often fatal disease. Cyclic di-GMP (c-di-GMP) is a universal secondary messenger in bacteria that regulates biofilm formation, virulence, and motility, which are just a few of the behaviors regulated by c-di-GMP. In bacterial pathogens, elevated levels of c-di-GMP generally result in increased biofilm production. Conversely, low cellular levels of c-di-GMP regulate acute virulence factors. This molecule has been well studied in many bacterial species, but not in *B. pseudomallei*. We have identified 24 genes implicated in c-di-GMP metabolism. These genes fall into five distinct classes; diguanylate cyclases (DGC), phosphodiesterases (PDE), hybrid DGC or PDE or both, hydrolases (HD-like), and c-di-GMP receptors (PilZ). We have identified and characterized transposon mutants in all 24 genes implicated in cyclic-di-GMP metabolism and regulation in *B. pseudomallei* 1026b. Transposon mutants of Bp1026b_I2284 (hybrid) and Bp1026b_I2285 (HD-like) were significantly reduced in swimming, while Bp1026b_II2523 (DGC) had an increase in swimming as compared to wild-type 1026b. Interestingly, the Bp1026b_II2523 transposon mutant also exhibited several temperature-dependent phenotypes with respect to biofilm and exopolysaccharide production. Statistical significance was found using a paired student's t-test with the Bonferroni correction to account for multiple comparisons.

Staff/ Microbiology, Immunology and Pathology

60 | Telomere length and telomerase activity as biomarkers in astronauts

Miles J. McKenna, Lynn Taylor, Kerry George, and Susan M. Bailey

The ends of human chromosomes are capped by telomeres, tandem arrays of repetitive DNA sequence and associated proteins, critical features of genomic stability as they protect chromosomal termini from degradation and prevent them from triggering improper DNA damage responses. Telomerase, the enzyme capable of maintaining telomere length via de novo addition of telomeric repeats, is sufficiently active to do so only in germ, stem, and cancer cells. Research supports telomere maintenance as a key integrating component for the cumulative effects of genetic, environmental, and lifestyle factors on aging and aging related diseases; therefore, the rate at which telomeres shorten provides an informative biomarker. Further, telomere dysfunction and/or decreased telomere length can be linked to age-related degenerative pathologies, ranging from reduced immune function, loss of fertility, cardiovascular disease, and cancer. In addition to a variety of lifestyle factors that negatively impact telomere length, (e.g., physiological and psychological stressors, inflammation and infection), ionizing radiation (IR) exposure also influences telomere length and levels of telomerase activity. We hypothesize that for astronauts, telomere maintenance represents a particularly relevant biomarker, as it reflects the combined exposures and experiences encountered during a mission. The goal of this study is to identify and define risks related to telomere shortening and changes in telomerase activity associated with spaceflight. We are assessing telomere lengths and telomerase activity in blood samples from twin and unrelated astronauts (and age-matched controls), pre-flight, in-flight, and post-flight. Additionally, chromosome aberrations are being analyzed using directional genomic hybridization (dGH) in order to evaluate inversions – a feature not previously included in NASA biodosimetric efforts. Results will begin to define the influence of IR exposure and lifestyle stressors of relevance to astronauts, to changes in telomere length and telomerase activity, as well as suggest potential interventions for further study.

Graduate Student/ Environmental and Radiological Health Sciences

61 | Differentiation of canine induced pluripotent stem cells into neural progenitor cells

Kaitlyn L. McNamara, Lyndah Chow, William Wheat, Saiphone Webb, Peter Koch, and Steven Dow

Background and Rationale: New advances in stem cell technology, including induced pluripotent stem cells (iPSC), offers new hope for patients with neurological disease and spinal cord injuries. Therefore, we evaluated the ability of canine iPSC to be differentiated into neural progenitor cells (NPC) in vitro as a precursor to clinical trials in dogs with spinal cord injury. Approach: iPSC were generated from canine fibroblasts and characterized based on phenotype, gene expression analysis, lineage differentiation, and teratoma formation. Canine iPSC were then induced to differentiate into NPC by culture in defined medium supplemented with specific growth factors. NPC were characterized by phenotype, flow cytometry, immunofluorescence, and gene expression analysis. Results: Canine iPSC could be readily induced to differentiate into NPC following 2-3 weeks in culture. Specific culture conditions led to enrichment of NPC for cells with characteristics of oligodendrocytes, astrocytes and neurons. NPC did not form teratomas in mice, whereas the parental iPSC cells did. Conclusions/Implications. Canine iPSC can be induced to form NPC in vitro by altering cell culture conditions, cell substrate, and addition of specific growth factors. These studies provide evidence that iPSC technology can be used to generate NPC for use in neural regeneration in dogs with neurological injuries.

DVM Student/ Microbiology, Immunology and Pathology

62 | Developing 3D tractography maps for identification of parafascicular corridors to improve surgical access to deep brain tumors in dogs

Emily K. McNeilly, L. Ray Whalen, and Rebecca A. Packer

The purpose of this study is to create a database of white matter tract pathways of the canine brain to establish standard parafascicular corridors for surgical access to deep brain tumors. Magnetic resonance (MR) imaging data from dogs admitted to the CSU Veterinary Teaching Hospital Neurology service, undergoing brain MR imaging in which no structural brain lesions were identified, were used for analysis of fiber tractography. Fiber tractography maps were created for each of three categories of cephalic index. The cephalic index was used to categorize all dogs into three skull types: dolichocephalic, mesaticephalic and brachycephalic, following previously published methodology. Diffusion tensor data and 3D anatomic MR scans were acquired and used to construct the 3D fiber tractography maps for each dog using the BrightMatter Plan software package (Synaptive Medical). For comparison of MR images with gross morphology, five canine cadaver brains were dissected for anatomical visualization of white matter tracts. These tracts were then visually catalogued with corresponding color overlay. Within each category of skull type, data from all modalities were used to produce detailed anatomical maps of canine white matter tractography, and the functional importance of each tract was described. This comprehensive database provides a baseline from which to evaluate deviations from normal with presence of tumor or disease, and provides clinically-relevant information as to the location of parafascicular corridors through which deep brain tumors can be safely accessed for biopsy or resection.

DVM Student/ Clinical Sciences

63 | Assessment of digital venograms in 24 polo horses

Nigel P. Miller, Britta S. Leise, Luke D. Bass, Valerie J. Moorman, Sammy Pittman, Amy Rucker, and Ric F. Redden

Digital venography in horses without known digital pathology demonstrates slight variations in the vascular pattern of the equine foot. Obtaining a full series of images, including early and late lateral views and weight-bearing and non-weight bearing images are necessary when assessing the vascular pattern in all horses. Introduction: Venography is frequently used to assess vascular perfusion in the hoof. However interpretation of the venogram remains subjective. The purpose of this study was to evaluate venographic technique in athletic horses and to describe variations in the pattern. Methods: Horses were evaluated for lameness and survey radiographs were obtained for each foot. Venograms were performed in both front feet of 25 horses. The amount of contrast and time required for infusion was recorded for each foot. Survey radiographs were evaluated for bone and hoof angle, palmar angle and sole depth. Venograms were evaluated for quality of perfusion throughout the digital vasculature. Results: A total of 44 venograms in 23 horses were successfully performed. Amount of contrast infused in each foot averaged 22.9 mls. All radiographic images were obtained within an average of 97 seconds post-infusion. Horses with increases sole depth had improved detail of the terminal papillae. Fourteen feet had decrease contrast in the dorsal lamellar vessels on the early weight-bearing view, but all were contrast returned when viewed on the unweighted lateral. Discussion: Numerous variations in the venographic pattern can be seen and is related to hoof conformation and weight-bearing; however, full assessments can be given if multiple views are obtained.

DVM Student/ Clinical Sciences

64 | Ectoparasites and vector-borne pathogens of dogs in Baja California Sur

Cody N. Minor, Dana C. Hill, Danielle M. Straatmann, and Michael R. Lappin

Several cases of human clinical illness in Baja California Sur, México, have been suspected to be associated with rickettsial disease agents. This study's objective is to determine the prevalence of select vectors and vector-borne disease agents carried by dogs of the region. Samples of ectoparasites and blood from 67 dogs were collected. Sera were assayed for antigen of *Dirofilaria immitis* and antibodies against *Ehrlichia canis/E. ewingii*, *Anaplasma phagocytophilum/A. platys*, and *Borrelia burgdorferi* using a commercial kit (SNAP® 4DXPlus®, IDEXX Laboratories). Sera were also assayed for antibodies against *Rickettsia* species by indirect fluorescent antibody (IFA) testing (*R. rickettsii* antigen). Total DNA was extracted from blood and evaluated with conventional polymerase chain reaction (PCR) assays to amplify DNA of *Anaplasma* species, *Babesia* species, *Bartonella* species, *Ehrlichia* species, *Rickettsia* species, and hemotropic *Mycoplasma* species (Hemoplasmas). Positive amplicons were sequenced to ascertain the species. Ectoparasites *Rhipicephalus sanguineus* (33 dogs; 49.3%), *Ctenocephalides felis* (11 dogs; 16.4%), and *Pulex irritans/simulans* (four dogs; 6.0%) were identified. *Dirofilaria immitis* antigen (one dog; 1.5%) and antibodies against *A. phagocytophilum/A. platys* (10 dogs; 14.9%) and *E. canis/E. ewingii* (31 dogs; 46.3%) were detected. Two samples had probable antibodies against *R. rickettsii* through IFA testing and are being confirmed. Evaluation of DNA from 41 dogs has been completed to date; *E. canis* was amplified from eight dogs (19.5%), *A. platys* from six dogs (14.6%), *Babesia canis vogeli* from three dogs (7.3%), and *Mycoplasma haemocanis* from three dogs (7.3%). Coinfections were confirmed in four dogs (9.8%) and consisted of *D. immitis/E. canis* (one dog), *E. canis/A. platys* (one dog), *E. canis/M. haemocanis* (one dog), and *E. canis/A. platys/M. haemocanis* (one dog). The detected vector-borne agents likely reflect common exposure to *R. sanguineus*, as this tick vectors each of the PCR-confirmed agents. Further PCR assays of ectoparasites and remaining blood samples are planned.

Postdoctoral Fellow/ Clinical Sciences

65 | Public Health Needs Assessment and Proposed WaSH Solutions: Leadership Training in the Tro Pang Cho Commune, Cambodia

Tavia Mirassou-Wolf, Kari Grady Grossman, Marueen DeCoursey, and Elizabeth P. Ryan

The purpose of this collaborative public health research project is to improve the health status of community members living in the Tro Pang Cho Commune of the Kampong Speu Province in Cambodia. The two main objectives of this research are to: 1.) Develop a mentoring program in collaboration with students from Colorado State University (CSU), the Colorado School of Public Health (CSPH), and local Cambodian scholars trained through scholarships provided by Sustainable Schools International (SSI), 2.) Develop a community service public health project that will be implemented by SSI supported nursing student graduates. An emerging partnership between CSU, CSPH, and SSI will assist in the establishment of basic public health infrastructure and health education that will help in lowering the prevalence of diarrhea and improving the overall health outcomes of the commune. Remediation of these health concerns will be addressed through a community service project involving local Cambodian nursing students.

Graduate Student/ Environmental and Radiological Health Sciences

66 | Relationship of Hepatic Copper Concentrations and Histological Changes in the Dog

Dan Moezzi, Barbara Powers, and David Twedt

Abnormal hepatocyte copper (Cu) concentrations are associated with oxidative stress resulting in cell death and subsequent necroinflammatory hepatic changes. The accumulation of hepatic Cu with inflammatory liver changes in the dog has been linked to certain breeds, due to suspected excessive dietary copper intake and the result of cholestatic disorders. We hypothesized that Cu concentrations would be higher in dogs having necroinflammatory liver biopsies compared to those with non-inflammatory hepatopathies or "other changes" that did not fulfill the criteria of inflammatory or non-inflammatory. A second aim was to determine if the level of hepatic Cu concentrations are related to the extent of necroinflammatory changes. We examined the CSU Diagnostic Laboratory records of 675 samples obtained in 2014 having both liver histopathology and hepatic Cu quantitation. Normal hepatic Cu concentrations are reported to range from 200-400 µg/g dry weight. Inflammatory groups were characterized by being lymphoplasmocytic or suppurative and grouped as being mild, moderate or severe. Non-inflammatory samples were characterized by hepatocellular cell swelling, hyperplasia, or lipidosis. A third "other changes" group was characterized either as nonspecific reactive hepatopathies, fibrosis or hepatic neoplasia. The mean hepatic Cu concentrations (in parts per million) were higher in the inflammatory groups; mild (553), moderate (1013) and severe (1143) compared to the non-inflammatory (353) and other group (471). These preliminary findings suggest that the highest hepatic copper concentrations are associated with inflammatory liver disease and that the severity of inflammatory changes tends to increase with hepatic Cu concentrations.

DVM Student/ Clinical Sciences

67 | Biomarkers for antimicrobial resistance: A pilot study identifying novel antimicrobial drug resistance markers for developing clinically-applicable detection and therapeutic tools

Sean E. Montgomery, Lyndsey M. Linke, Roberta J. Magnuson, Lisa M. Wolfe, Corey D. Broeckling, Jessica E. Prenni, Doreene R. Hyatt, Meagan E. Chriswell, Clarissa C. Freemyer, Paige J. Tenneson, and Sangeeta Rao

Antimicrobial resistance (AMR) is an increasing threat to both human and animal public health. When attempting to treat AMR bacterial infections, clinicians are forced to provide reactionary therapies to combat AMR infections, resulting in high treatment costs, prolonged treatment length, and an increased risk for bacterial spread. However, assays capable of rapidly identifying phenotypic expressions of AMR infections would lead to more efficacious therapies and better AMR surveillance. *Salmonella typhimurium* is a ubiquitous pathogen found in both humans and animals. Our previous research with *S. typhimurium* isolates has shown a relationship between class I integrons, also known as mobile genetic elements, and AMR. We have hypothesized that exposure to antimicrobials is not only associated with these genetic elements, but modifies metabolomic and proteomic expression. The purpose of this study was to characterize metabolomic and proteomic biomarkers unique to AMR *S. typhimurium* and investigate the diversity of these markers among established genetic patterns of resistance. Multi-drug resistant isolates containing similar integron profiles were grown in a liquid nutrient broth both with and without the ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline) drug panel. These isolates were subjected to protein and metabolite extraction, followed by non-targeted proteomic analysis via liquid chromatography coupled with tandem mass spectrometry (MS/MS), ultra-performance liquid chromatography-mass spectrometry (UPLC-MS), and gas chromatography-mass spectrometry (GC-MS). Proteins were annotated using the Uniprot database and metabolites annotated after screening against several spectral libraries and the Golm database. Results indicate distinct biomarker patterns in both protein and metabolite expression levels between isolates grown with and without ACSSuT drugs compared statistically using t-test. The results of this pilot will provide a better understanding of biomolecule expression key to antibiotic resistance. Future studies with greater sample sizes may serve as a means of developing novel AMR surveillance and diagnostic tools to best mitigate AMR infections.

Graduate Student/ Clinical Sciences

68 | A canine model for studying the role of the cellular prion protein in cancer cells

Sophia A. Moore, Mark Zabel, Wilfred Goldmann, David J. Argyle, and Lisa Y. Pang

The cellular prion protein PrP^c is a neuronal protein best known for its association with neurodegeneration. However, recently it was shown that PrP^c is up-regulated in many human cancers. Different functions for PrP^c in cancer have been proposed including an anti-apoptotic role; in general the role of PrP^c in cancer is not well understood. Our initial studies confirmed that PrP^c is expressed in a panel of canine cancer cell lines with unique patterns of expression and cleavage. Flow cytometry revealed that PrP^c is expressed on the cell surface of these cancer cells. Following the hypothesis that PrP^c may have a role in the stress response and survival of these cancer cells, we performed time course experiments using doxorubicin and ionizing radiation. Western blot analysis allowed visualization of the PrP^c full-length and C1 fragment. These experiments indicate that, in some of the cancer cell lines tested, either the PrP^c full-length, C1 or both protein levels are elevated in response to treatment and that the ratio of full-length PrPc to C1 changed, as did the glycosylation of PrP^c. To test whether the PrP^c full-length and C1 half-life were altered in response to doxorubicin-induced stress, protein synthesis was inhibited with cyclohexamide. Initial results appear to indicate stabilization of the full-length PrP^c and a highly stable C1 fragment. Future work will include apoptotic assays and tissue microarrays to investigate the use of PrP^c as a prognostic marker for cancer patients.

DVM Student/ Microbiology, Immunology and Pathology

69 | Prediction of pregnancy survival after embryo transfer based on initial ultrasound pregnancy examination

Jennifer K. Morrissey, Ryan A. Ferris, and Patrick M. McCue

Embryo transfer involves recovery of an embryo from a donor mare 7 or 8 days after ovulation and transfer of the embryo into the uterus of a recipient mare. Ultrasound examinations are subsequently performed periodically on the recipient mare to confirm pregnancy status and monitor embryonic development. The goal of this study was to determine if embryo size during the early post-transfer ultrasound examinations could predict future pregnancy survival. A retrospective study was performed using reproductive records over four breeding seasons (2012 to 2015). Data were included if pregnancy status and diameter of the embryonic vesicle were recorded at days 12, 14 and 16 (embryo age) and if pregnancy status was confirmed at day 25. A p value of less than 0.05 was considered statistically significant and data are presented as the mean ± sem. Data from a total of 108 pregnant recipient mares met the study criteria and were used for subsequent analysis. A significant difference ($p < 0.05$) was noted in mean embryonic vesicle size at days 12, 14 and 16 between mares that remained pregnant and mares that subsequently lost their pregnancy by day 25 of pregnancy. This suggests that ultrasound evaluation of embryo diameter on days 12 to 16 of gestation can be used to predict future survival of that pregnancy. The clinical relevance for veterinarians and horse owners is that ultrasonographic monitoring of early embryonic development in pregnant mares is useful in prediction of pregnancy outcome and consequently provides valuable information for future reproductive management decisions.

Resident/ Clinical Sciences

70 | Rice bran in the presence and absence of probiotics differentially alters the porcine large intestinal and serum metabolome for enhanced protection against human rotavirus infection

Nora Jean Nealon, Dustin G. Brown, Lijuan Yuan, and Elizabeth P. Ryan

Globally, human rotavirus (HRV) is a leading cause of severe diarrhea in children, and it is responsible for 500,000 deaths annually. Since vaccines have variable efficacy, novel strategies need to be implemented to treat HRV. One solution involves consuming rice bran, which has demonstrated roles in promoting intestinal health, in part via its metabolism by probiotic gut bacteria. Past studies with porcine models demonstrated that gnotobiotic neonatal pigs colonized with combinations of *L. rhamnosus* GG (LGG) and *E. coli Nissle* (EN) and fed Calrose rice bran as 10% of their daily caloric intake have an enhanced ability to resist infection by HRV compared to treatments receiving only the probiotic or rice bran individually. Although results reported that combinations of rice bran and probiotic did not reduce viral shedding, diarrhea was completely eliminated in these animals. Current investigations are evaluating the potential metabolites involved in this protective effect using large intestinal contents (LIC) and serum samples collected from these subjects. Metabolites were analyzed using a global, non-targeted metabolomics approach via gas chromatography-mass spectrometry. Preliminary results report decreased histamine in the LIC of HRV-challenged piglets receiving combination treatments when compared to analogous rice bran or probiotic-only groups. Furthermore, the LIC of the combination group had lower detectable levels of n-6 polyunsaturated fatty acids (PUFA). Histamine and n-6 PUFA's are associated with pro-inflammatory pathways, suggesting that one way probiotics and rice bran work synergistically to protect against HRV is through reducing inflammation in the gastrointestinal tract. Other metabolites differ between treatment groups and are currently being evaluated. Collectively, current results suggest that combinations of rice bran and probiotics enhance gut protection against HRV, and this effect may be mediated through metabolites produced during probiotic fermentation of rice bran.

DVM Student/ Environmental and Radiological Health Sciences

71 | Recognition and processing of telomeric double strand breaks in human cells

Christopher B. Nelson, Lynn E. Taylor, and Susan M. Bailey

The majority of DNA double strand breaks (DSBs) are repaired with high fidelity within the first few hours of their occurrence via non-homologous end joining (NHEJ), or (to a much lesser extent) homologous recombinational repair (HRR). However, some DSBs are susceptible to misrepair – or are difficult (if not impossible) – to repair at all. Both misrepaired and unrepaired DSBs have the potential to contribute to genomic instability, or conversely to trigger cell death or senescence, potent drivers of carcinogenesis or degeneration of human tissues with age, respectively. Telomeres, the ends of eukaryotic chromosomes, must avoid recognition as DSBs to prevent inappropriate processing by the cellular repair machinery. As such, end-specific binding proteins and higher-order telomere structure serve to inhibit conventional DNA damage responses, suggesting that telomeres may be vulnerable to misrepair or non-repair of damage. Utilizing a recombinant endonuclease that specifically cuts telomeric DNA (EN-T), we are exploring telomere DSB recognition and processing in immortalized normal human fibroblasts (BJ1 hTERT). The response to telomeric DSBs appears unusual in that recruitment of 53BP1 to the break site, which promotes NHEJ by preventing exonuclease activity, was undetectable; leading us to hypothesize that telomeric breaks may undergo extensive resection, which is the first step of HRR. Indeed, fluorescence in situ hybridization (FISH) in cells expressing EN-T revealed an abundance of single stranded ssDNA at telomeres, an observation corroborated by co-localization of phosphorylated-RPA foci. However, co-localization of RAD51 (recombination) foci at telomeres was not observed in EN-T expressing cells, suggesting that the ssDNA overhangs may not be resolved by HRR. However, these studies were conducted in asynchronous cell populations, making it difficult to assess recombination (which occurs primarily in S/G2). We will report our ongoing efforts to define cell cycle dependency and preferred repair pathway choice for telomeric DSBs.

Graduate Student/ Environmental and Radiological Health Sciences

72 | Detection of Prions on Plants Collected from Rocky Mountain National Park

Aimee Ortega, Jan Leach, and Mark Zabel

Chronic Wasting Disease (CWD) affects cervids such as elk, deer, and moose and since its discovery in 1967 has become endemic in certain areas. Prevalence in captive herds have reached as high as 90%, and by measuring a large herd within Rocky Mountain National Park (ROMO) we have found that most recent estimates reach up to 19%. CWD is one of many transmissible spongiform encephalopathies which occur due to the accumulation of an abnormally folded, proteinase K resistant, form of the normal cellular prion protein PrP^{C}. This abnormally folded form, PrP^{CWD}, seeds conversion of PrP^{C} into PrP^{CWD} and eventually forms amyloid fibrils. Spread of CWD occurs through horizontal, vertical, and indirect/environmental routes. PrP^{CWD} has been found in both soil and water. Additionally, PrP^{CWD} is very resistant to degradation which makes it stable in the environment for long periods of time. A study has shown that the abnormal prion protein can remain viable in the environment for as long as 16 years. Wanting to explore environmental transmission of CWD we surveyed three sites within Rocky Mountain National Park and collected a total of 32 plants. Plants were collected from both outside and inside exclosures that serve to keep wildlife out and allow for restoration and regrowth of the flora. Plant samples were assayed via the Protein Misfolding Cyclic Amplification assay for detection of PrP^{CWD}. Here we show novel evidence of PrP^{CWD} on the surface of a number of plants collected.

Graduate Student/ Microbiology, Immunology and Pathology

73 | Desmin immunostaining does not differentiate mesothelial hyperplasia from malignant mesothelioma in dogs

Stephen Pannone, Aaron Holling, and Kelly Santangelo

Purpose: The differentiation between mesothelial hyperplasia and malignant mesothelioma in dogs can be challenging when the histologic diagnosis is unclear. Studies in human medicine have shown that positive immunostaining of desmin is an effective indicator of mesothelial hyperplasia and can be used to rule out malignant mesothelioma. The aim of this study was to evaluate the efficacy of desmin immunostaining for inclusion on a panel of antibodies to be used to differentiate ambiguous histologic and/or cytologic cases of mesothelial hyperplasia versus malignant mesothelioma in canine tissue. Based on work published in people, our hypothesis was that positive desmin immunostaining would be consistent with a diagnosis of mesothelial hyperplasia while the loss of desmin positivity would be indicative of malignant mesothelioma. Materials/Methods: Archived tissue from canine cases with defined diagnoses of mesothelial hyperplasia or mesothelioma were collected (n=23 per group). Immunohistochemical staining was performed with desmin using standard techniques and appropriate negative and positive controls. Slides were evaluated by a board-certified pathologist to determine the presence of positive and negative staining throughout the tissue samples. Results: All cases with a diagnosis of mesothelial hyperplasia were positive for desmin in expected cells. Of the 23 cases of malignant mesothelioma, only two neoplasms were completely negative for desmin immunostaining. The remaining desmin-positive cases fell into two categories: diffuse strong immunostaining throughout the tumor; or variable staining that consisted of weakly to markedly positive neoplastic cells admixed with negative cells. Conclusions: Desmin immunostaining was not able to reliably distinguish between mesothelial hyperplasia and malignant mesothelioma in canine patients. Further research is required to identify an antibody that can be used as an immunohistochemical or immunocytochemical marker in the differentiation of these two entities.

DVM Student/ Microbiology, Immunology and Pathology

74 | Experimental infection of horses and sheep with MERS coronavirus

Stephanie M. Porter, Danielle R. Adney, and Richard A. Bowen

Since the discovery of Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012, there have been over 1300 confirmed human infections. MERS-CoV, which causes severe respiratory tract disease in people, can be transmitted from human-to-human, though many cases in the Middle East have been associated with animal-to-human transmission. Dromedary camels are likely a reservoir, as well as important in virus transmission; however, the role of other animals as reservoirs still remains unclear. We were interested in the pathogenesis of MERS-CoV infection in horses and sheep, two species prevalent in the Middle East. An equine cell line has been shown to be permissive to MERS-CoV, exhibiting cytopathic effects and producing infectious virus. While serological testing of both horses and sheep from the Middle East has failed to reveal antibodies for MERS-CoV, one recent study showed that serum from six sheep did react with MERS-CoV antigen. This experimental evidence indicates that these species may be susceptible to MERS-CoV infection, but in vivo infection has not yet been investigated. We inoculated 4 horses and 3 sheep with a human MERS-CoV isolate, then collected nasal swabs and serum to track viral shedding and antibody production. Overt disease was not seen in any animal. Only minor virus shedding was detected in the horses, while sheep shed low levels of virus and produced neutralizing antibodies. This suggests that neither horses nor sheep likely play a significant role in MERS-CoV transmission.

DVM Student/ Biomedical Sciences

75 | Feline leukemia virus distribution in a privately held colony of domestic cat-leopard cat hybrids

Jordan Powers, Melody Roelke, Elliott Chiu, and Sue VandeWoude

Feline leukemia virus (FeLV) is a retrovirus which causes disease in domestic cats, especially in feral or social populations. Exogenous FeLV (ExFeLV) is a horizontally transmitted virus that causes high morbidity and mortality in a significant percentage of infected animals. Endogenous FeLV (EnFeLV) is a germ-line proviral sequence that is only found in the Felis species most closely related to the domestic cat (Felis catus) and does not produce an active infection in the host. Other felidae, such as the Leopard Cat (Prionailurus bengalensis) do not have EnFeLV proviral sequences. The goal of this project was to determine prevalence of FeLV in a colony of privately held domestic cat-leopard cat hybrids with an endemic ExFeLV infection (N=64). The contribution of domestic versus nondomestic genotypes as well as coinfection with feline gamma herpesvirus (GHV) and feline foamy virus (FFV) were evaluated as parameters relating to FeLV infection and disease. Thirty-three of 64 cats (51%) of cats were positive for circulating ExFeLV antigen. A subset of 32 animals were tested for FFV and GHV using qPCR. Twenty cats out of the 32 tested positive for FFV (62%) and zero out of 32 cats tested positive for GHV. There was no significant influence of FFV on ExFeLV infection rate. EnFeLV and ExFeLV quantitation is being conducted to relate these parameters to disease state. Information gained from this study will benefit understanding between EnFeLV and ExFeLV interactions in both domestic and nondomestic cats.

Undergraduate Student/ Microbiology, Immunology and Pathology

76 | In vitro effects of PI3K/mTOR inhibition in canine hemangiosarcoma

Alex A. Pyuen and Douglas H. Thamm

While extremely rare in humans, hemangiosarcoma (HSA) accounts for nearly 2% of canine neoplasia, and is characterized by both aggressive local growth/invasion and a high rate of metastasis. Both canine and human HSA exhibit sustained aberrant PI3K/Akt/mTOR pathway signaling. The purpose of this study was to examine the in vitro effects of a novel dual PI3K/mTOR inhibitor, VDC-597, in canine HSA cells. Three canine HSA cell lines (DEN-HSA, CIN-HSA, and SB-HSA) were employed in multiple in vitro assays. Western analysis evaluated activation (phosphorylation) of key downstream pro-survival proteins in the PI3K/mTOR pathway. Changes in tumor cell growth/apoptosis were assessed using bioreductive assays (Alamar Blue) and in vitro live-cell imaging (IncuCyte), in the presence and absence of doxorubicin, a standard-of-care cytotoxic drug for canine and human sarcomas, as well as in the presence and absence of U-0126, an inhibitor of the MEK pathway. Migration was assessed using Boyden chamber and scratch assays, via live-cell imaging (IncuCyte). Matrigel invasion was assessed using traditional Boyden chambers. Finally, ELISA was utilized to quantify relative expression of vascular endothelial growth factor (VEGF). VDC-597 suppressed activation of Akt and 4eBP1 in canine HSA cells in a dose- and time-dependent fashion, with an IC50 of approximately 0.3 uM, a concentration predicted to be clinically achievable based on preliminary early-phase canine and human studies. VDC-597 dose-dependently reduced proliferation, migration, invasion, and VEGF expression in HSA, while promoting tumor cell apoptosis. VDC-597 demonstrated additive antiproliferative effects when combined with doxorubicin and U-0126. Together, these results suggest that inhibitors of the PI3K/Akt/mTOR pathway may act against multiple components of the neoplastic process, including proliferation/apoptosis, chemosensitivity, invasion/migration and angiogenesis, and justify the evaluation of PI3K/mTOR inhibitors in canine, and eventually human, HSA. Experiments to examine the effect of VDC-597 in reducing tumor burden and metastasis in a rodent model are ongoing.

Graduate Student/ Clinical Sciences

77 | Sensory substitution via electro-tactile stimulation of the tongue

Josiah Racchini, Joel Moritz Jr., John Williams, and Leslie M. Stone-Roy

Hearing loss or impairment affects more than 30 million Americans. Although many people have had success using cochlear implants to convey lost sensory information, factors such as cost, injury, and surgery success can prevent patients from utilizing cochlear implants and similar devices. Our research seeks to develop a sensory substitution device that is inexpensive, convenient, and usable by patients with all kinds of auditory impairments or injuries. Custom made printed circuit boards containing electrode arrays stimulate the tongue using proprietary software to stimulate electrodes in specific patterns. Electrical stimulation activates nerve fibers in the tongue, causing a light buzzing sensation. The tongue was chosen as the site of stimulation because of its sheltered nature and the conductivity of saliva, providing a more conductive surface than the skin of the hands. Other authors conducting sensory substitution experiments used similar prosthetic devices that have successfully communicated sensory information to experimental participants. Our current research focuses on determining the 2 point discrimination distances and the pattern of perceived intensity differences across the surface of the tongue. For these studies, we divided people into groups based on their ability to perceive propylthiouracil (PROP), a bitter compound correlated with tongue sensitivity to taste and mechanical stimulation. Our studies indicate that there are differences across the tongue for sensitivity to electrical stimulation and minimum 2 point discrimination distances. The relationship between papillae density, PROP sensitivity and electrotactile stimulation appears complex and is still under investigation. Results from the current studies were used to help develop an improved mouthpiece for the electrotactile device and future work will focus on using this improved device to investigate the ability of people to learn vocabulary words encoded with different lingual stimulation patterns.

Undergraduate Student/ Biomedical Sciences

78 | Evidence of inflammaging and gastrointestinal bleeding in old dogs

Lauren B. Radakovich, Christine S. Olver, and Kelly S. Santangelo

Effects of aging on hematologic and biochemical variables are well-described in humans. Anemia of the elderly is attributed to iron deficiency, anemia of chronic disease, chronic kidney disease, myelodysplasia, or idiopathic causes. Limited studies have examined these variables in aging dogs, but they have typically examined single breeds in research settings. The objective of this study was to identify differences in complete blood count (CBC) and biochemistry values in adult and aged dogs of many breeds. Dogs presenting for wellness examinations and minor dental/elective surgeries that were otherwise clinically healthy were retrospectively identified. Dogs were categorized by age: adult (1-7.9 years), senior (8-11.9 years), and geriatric (12+ years). CBC and biochemistry data were collated. Asian breeds, greyhounds, and dogs with data indicating overt underlying disease were excluded. Data was not normally distributed; Kruskal-Wallis tests were used to compare groups with statistical significance set at $P < 0.05$. Hematocrit, mean cell volume (MCV), and serum iron decreased with age, indicating possible iron-restricted erythropoiesis (IRE), due to iron deficiency or low-grade chronic inflammation. Total protein, globulins, and platelet counts increased with age while albumin decreased, suggesting low-grade inflammation. Blood urea nitrogen was increased in older dogs without a concurrent increase in creatinine, possibly indicating gastrointestinal bleeding or azotemia. Clinically healthy, aging dogs may have altered physiologies compared to younger adult animals, including conceivable evidence of IRE, inflammation, and potential gastrointestinal bleeding, indicating a similar trend to that of elderly humans. Future studies will examine markers of iron metabolism and inflammation in aging dogs.

Resident/ Microbiology, Immunology and Pathology

79 | The mini-pig as a neonatal TB vaccine efficacy animal model

Laylaa Ramos, Jennifer Arab, Andres Oregon-Henao, Andrea Sanchez Hidalgo, Angelo Izzo, Richard Bowen, and Mercedes Gonzalez-Juarrero

Progress towards decreasing the prevalence of Tuberculosis (TB) has been hampered by vaccine development. Vaccines show promise in adult animal models yet fail to protect infants from TB disease in clinical trials. In this study, we developed the mini-pig as a neonatal animal model for TB vaccines. We showed mini-pigs could be infected with *Mycobacterium tuberculosis* by monitoring for clinical signs and TB lesions in tissue. Further, neonatal mini-piglets were vaccinated with Bacillus Calmette-Guerin (BCG) to monitor the immune response until adulthood. Our findings suggest mini-pigs have the potential of serving as an effective neonatal animal model for TB vaccines.

Graduate Student/ Microbiology, Immunology and Pathology

80 | Unique features of immunoglobulin gene use and mutation status in Boxers with chronic lymphocytic leukemia

Emily D. Rout, Robert C. Burnett, Stacey A. George, Courtney R. Abbott, Janna A. Yoshimoto, and Anne C. Avery

Canine B cell chronic lymphocytic leukemia (B-CLL) is common in dogs and shares many features with human B-CLL. In human B-CLL, immunoglobulin (Ig) gene use and mutation status are important markers for disease behavior. Patients with mutated Ig genes (having greater than 2% mutations compared to germline sequence) have an indolent disease course and a median survival time of 25 years, while patients with unmutated genes (less than 2% mutations) have an aggressive disease course and median survival time of 9 years. Our objective was to characterize Ig gene use and mutation status in dogs with B-CLL, which had not previously been described. We sequenced the immunoglobulin heavy chain variable region (VH) genes from neoplastic peripheral blood B cells in 50 dogs with B-CLL. Twelve VH genes were used in patients, many of which are commonly used in normal dogs. Two interesting findings were observed in Boxers with B-CLL. First, Boxers preferentially used a particular VH gene, with 55% (11/20) of Boxers using the VH41 gene. In contrast, VH41 was only used in 9% of non-Boxer breeds with B-CLL. Second, Boxers with B-CLL preferentially used unmutated VH genes (90% of cases), whereas non-Boxer breeds with B-CLL preferentially used mutated VH genes. When Boxers were excluded from the analysis, 63% of B-CLL patients used mutated VH genes, which is similar to the proportion of mutated and unmutated cases in human B-CLL. These data indicate that Boxers may be a useful model for investigating why a particular VH gene is preferentially used and as a model for studying B-CLL associated with unmutated VH genes. This study lays the foundation to study B-CLL pathogenesis, correlate Ig gene use and mutation status with outcome in dogs, and further establish canine patients as good models for human B-CLL.

Resident/ Microbiology, Immunology and Pathology

81 | Reference ranges in blood hematology in the Alaskan sled dog

Vanessa J. Schilling, John Harley, and Todd O'Hara

The domestic dog has been advocated as a medical model and sentinel for human health as part of a One Health initiative. Blood hematology tests in dogs are necessary to asses both the health of the dog for veterinary services as well as for scientific research. Most veterinarians use established reference ranges to determine whether the values of these tests fall within normal limits. However, previous studies have found that some breeds of dogs have parameters with distributions that differ from established reference ranges. Alaskan sled dogs have been bred for many years to compete in long distance endurance races and to survive extreme weather conditions. Since sled dogs are such unique and exceptional athletes, we decided to compare the blood hematology test results of sled dogs to established reference ranges provided for domestic dogs. Blood was collected from each sled dog by cephalic or jugular stick. CBC and CHEM panels were performed by the University of Alaska Fairbanks veterinary service. Using the statistical program R, we created density plots for each of the hematologic test results and found evidence to suggest that albumin, γ–glutamyltransferase, red cell distribution width, hemoglobin, globulin, lymphocyte count, and white blood cell count values fell partially, or in the case of RDW, mostly outside of the established reference ranges. We determined that further investigation is required to establish specific reference ranges for the Alaskan sled dog.

DVM Student/ Clinical Sciences

82 | The Effect of C-DIM12 on Mitigating the Development of Inflammation in Cultured Equine Chondrocytes and Synoviocytes

John A. Schwartz, Mary Afzali, William Hanneman, C.W. McIlwraith, and Laurie R. Goodrich

Purpose: *Para*-phenyl-substituted-diindolylmethane (C-DIM) compounds, specifically C-DIM12: 1,1-bis(3'-indolyl)-1-(p-chlorophenyl)methane, has been shown to display anti-inflammatory properties when used to treat neurodegeneration. The goal of this experiment was to test the hypothesis that CDIM-12 would exert anti-inflammatory effects on equine chondrocytes and synoviocytes when stimulated with interleukin-1β (IL-1β). Methods: Chondrocytes and synoviocytes were seeded in 24-well plates in triplicate. Tolfenamic Acid (TA), a class of NSAIDs, served as the positive control. Each cell type were divided into six experimental groups: Cells Alone, Cells+C-DIM12 (10uM/ml), Cells+TA (10uM/ml), Cells+IL-1β (5ng/ml), Cells+IL-1β+TA, and Cells+IL-1β+C-DIM12. At the 24-hour time point, media was collected to determine IL-1β concentration using ELISA analysis. Results: For both cell types, a significant difference was found between the group means as determined by a one-way ANOVA ($p < 0.001$). IL-1β was not detected in Cells Alone, Cells+C-DIM12, and Cells+TA. For chondrocytes, the mean IL-1β concentrations (pg/ml) for the Cells+IL-1β, Cells+IL-1β +TA, and Cells+IL-1β +C-DIM12 groups were 9896.34, 3245.73, and 2799.68, respectively. For the synoviocytes, the mean IL-1β concentrations were 32856.33, 4520.05, and 5625.96, respectively. For chondrocytes, a Tukey's Post-Hoc test showed there was a significant difference between Cells+IL-1β and cells treated with C-DIM12 ($p < 0.001$) and between the Cells+IL-1β and cells treated with TA ($p < 0.001$). There was no significant difference between cells treated with C-DIM12 and cells treated with TA ($p = 0.338$). For synoviocytes, the Post-Hoc test showed there was a significant difference between Cells+IL-1β and cells treated with C-DIM12 ($p < 0.001$) and between Cells+IL-1β and cells treated with TA ($p < 0.001$). No significant difference was found between cells treated with C-DIM12 and cells treated with TA ($p = 1.00$). Conclusions: Because C-DIM12 has shown the same anti-inflammatory effect on chondrocytes and synoviocytes as TA, the compound could one day be used as an alternative to NSAIDs in treating osteoarthritis.

Staff/ Clinical Sciences

83 | Repeat Ivermectin Mass Drug Administrations for the control of Malaria (RIMDAMAL): a randomized pilot safety and efficacy study

Jonathan A. Seaman, Haoues Alout, Abdul Gafar V. Coulidaty, Windtaré Roland Bougma, Nöel Rouamba, Roch K. Dabiré, and Brian D. Foy

RIMDAMAL is a, pilot, cluster-randomized control safety/efficacy study designed to test the hypothesis that repeated ivermectin mass drug administrations (MDA) to Burkinabé villagers throughout the rainy season is safe and can reduce malaria episodes in children by controlling malaria transmission. The hypothesis is based on data showing high mortality of *Anopheles* mosquito vectors that bite ivermectin-treated people, and subsequent reduction of parasite transmission in treated villages. The trial utilizes existing structures for the control of neglected tropical diseases (NTDs) in southwest Burkina Faso and will examine if integrated control of malaria and NTDs is possible with MDA. The control villages received a single MDA of ivermectin plus albendazole (active comparator) at the beginning of the rainy season for normal public health efforts to control NTDs; the experimental villages received the same intervention, but then received 5 more MDA of ivermectin alone, every three weeks, through the end of the rainy season. Eight villages located near the town of Diebougou agreed to participate and were randomly allocated between the two arms in May, 2015. Informed consent of 2,662 village participants took place between June-July and the first MDA occurred between July 17-July 19. The primary outcome of malaria episode incidence (defined as >38°C temperature or history of fever in the last 24 hours and a positive rapid diagnostic test) in children ≤ 5 years of age was assessed weekly through active case surveillance in the villages by study nurses, during which they also recorded any adverse events through passive monitoring of all enrolled participants. Data through the middle of the trial shows a 16% reduction in the percentage of child malaria episodes in the treatment arm relative to the control arm and an analysis of the complete dataset is underway. Secondary parasitological and entomological outcomes are also being assessed.

Staff/ Microbiology, Immunology and Pathology

84 | Inflammatory response to advanced glycation end products in the murine subcutaneous air pouch model

Robert T. Sedam, David F. Ackart, Brendan K. Podell, Christian Melander, Roberta Melander, and Randall J. Basaraba

Glycation is a pathologic process consisting of random binding of sugar moieties to proteins, lipids, and complex carbohydrates. Advanced glycation end products (AGEs) are stable, pro-inflammatory macromolecules that accumulate as part of normal aging. There are no FDA approved drugs that target AGEs, nor a reliable model to study anti-AGE compounds in vivo. The subcutaneous air pouch model in mice was used as an in vivo model to study AGE-induced inflammation, and the efficacy of a novel class of 2-Aminoimidazole (2-AI) based small molecules. Sterile air was injected subcutaneously in BALB/C mice on days 0 (5mL) and 4 (2-3mL). Bovine serum albumin (BSA) was incubated with methylglyoxyl (MG) for 7 days and AGE formation and protein concentration confirmed by fluorescence (370/440nm; 335/385nm) and Bradford assay. BSA-MG was then treated for 7 days with a 2-AI compound (BSA-MGTx). On day 6, mice were randomly assigned to treatment groups: 1XPBS (negative control, 1mL), BSA (106ug), BSA-MG (106ug), BSA-MGTx (0.5mL), and 1XPBSTx (0.5mL treatment control), MG alone (0.5mL, negative control) or carrageenan (positive control, 1%). The 4mm X 3mm pouch histologically consists of 1-3 layers of mesenchymal cells with early collagen deposition confirmed by Masson's trichrome staining. Between all groups, the pouch was structurally similar; however, perivascular inflammation and subcutaneous congestion was present amongst all treated groups at variable degrees of severity; with the most severe being BSA-MG and Carrageenan. This model will prove useful in investigating the pathogenesis of AGE-mediated inflammation and the in vivo effectiveness of 2-AI compounds.

DVM Student/ Microbiology, Immunology and Pathology

85 | Emerging Roles of Synaptotagmin: Modeling Neurogenic Disease in Drosophila

Mallory Shields, Matthew Bowers, Maddi Bollig, Rita Horvath, Alysia Mortimer, Roger Whittaker, and Noreen Reist

Synaptotagmin, a synaptic vesicle protein, is widely known as the fast, synchronous Ca++ sensor that mediates neurotransmitter release. It's C2B Ca++ binding domain has been extensively analyzed for its essential role in triggering synaptic vesicle fusion in many animal models. Due to its essential nature, many synaptotagmin mutations result in early lethality when expressed in the null background (sytnull/sytnull) in animal models. However, when expressed in a heterozygous background (sytWT/sytnull), some of these same mutations impair synaptic transmission but still support viability. Recently, whole-exome sequencing has identified mutations in synaptotagmin that are associated with human disease. In two families, multigenerational dominant deficits (sytm/sytWT) have been linked to single adjacent point mutations in synaptotagmin's C2B domain. These dominant mutations are located in a highly conserved sequence within the Ca++ binding pocket. Patients with either mutation present with symptoms similar to Lambert-Eaton Myasthenic Syndrome (LEMS): including decreased compound muscle action potential amplitude accompanied by synaptic facilitation, as well as muscle wasting and weakness. With a view to identifying the molecular mechanisms underlying the human phenotype, we have generated a homologous point mutation in the C2B domain of Drosophila synaptotagmin. By expressing the mutant transgenic protein in a synaptotagmin heterozygous background (sytWT/sytnull;P[sytm]/+), we obtained synaptotagmin expression approximately equivalent to that seen in homozygotes (sytWT/sytWT). Thus our expression system should approximate that seen in the human patients. Initial results indicate that we can successfully mimic several of the symptoms seen in the affected family.

Graduate Student/ Biomedical Sciences

86 | Evaluation of doctor of veterinary medicine program curricula on animal welfare, animal behavior, and animal ethics courses

Chelsey B. Shivley, Franklyn B. Garry, Lori R. Kogan, and Temple Grandin

The objective of this study was to evaluate educational programs offered on animal behavior, animal ethics, and animal welfare among AVMA Council on Education (COE)-accredited colleges and schools of veterinary medicine. A questionnaire was e-mailed to Associate Deans of Academic Affairs at all COE-accredited colleges and schools of veterinary medicine for the survey. The curricula for all COE-accredited colleges and schools of veterinary medicine in the United States were obtained and evaluated for courses on animal behavior, animal ethics, and animal welfare. The sample included 49 COE-accredited colleges and schools of veterinary medicine for the survey, and 30 colleges and schools of veterinary medicine in the United States for the curriculum evaluation. Survey results from 18 institutions found that 10 offered a formal animal welfare course, 9 offered an animal behavior course, with another 5 offering a combined animal welfare, behavior, and ethics course, and 8 offered a formal animal ethics course, with differences found between international and U.S. schools. Evaluation of posted curricula of 30 U.S. schools found that 6 offered a course on animal welfare, 22 offered a course on animal behavior, and 18 offered a course on animal ethics. There is a need for more colleges to have formal education in animal welfare, behavior, and ethics so veterinarians can be advocates for animals and assist clients with behavioral challenges.

Graduate Student/ Clinical Sciences

87 | Serial evaluation of thromboelastography and platelet aggregometry in healthy dogs

Sarah B. Shropshire, Christine S. Olver, and Michael R. Lappin

Thromboelastography (TEG) and platelet aggregometry are assays that help to evaluate the viscoelastic properties of blood clotting and platelet function, respectively. If interindividual variability (CV_G) is high, serial evaluation within an individual rather than single measurements may provide greater sensitivity to detect clinically significant changes. If the intraindividual variability (CV_I) is greater than the CV_G however, then serial evaluation would not offer increased sensitivity. The purpose of this study was to compare CV_G and CV_I over time for TEG and platelet aggregometry variables in healthy dogs. Tissue factor activated TEG was performed in six specific pathogen free (SPF) sex and age-matched Beagles at three time points where the variables reaction time (R), clotting time (K), rate of clot formation (α), and maximum amplitude (MA) were recorded. Adenosine diphosphate (ADP)-induced and arachidonic acid (AA)-induced platelet aggregometry in addition to platelet count, hematocrit, and fibrinogen were performed concurrently. APTT, OSPT, antithrombin activity, and D-dimer concentrations were measured on the first day of the study. A one-way random effects model was used to analyze the following variables; R, K, α, MA, area under the curve for ADP (AUCADP), and area under the curve for AA (AUCAA). Variance components were created from the random effects model to calculate coefficients of variation for CV_G and CV_I. The CV_I was lower than the CV_G over time for MA, AUCADP, and AUCAA however the CV_I was higher than the CV_G for the TEG variables R, K, and α. Based on the results of this study, serial measurements for ADP and AA-induced platelet aggregometry and the TEG variable MA may provide a more sensitive method to detect relevant changes when monitoring patients. However, due to the high CV_I for the TEG variables R, K, and α, serial measurements may not be more beneficial than a single measurement.

Graduate Student/ Clinical Sciences

88 | In vitro evaluation of the effects of pre-conditioning on female canine adipose-derived mesenchymal stem cell cytokine production

Stephanie M. Smith, Rebecca M. Timmons, Craig B. Webb, and Tracy L. Webb

The use of mesenchymal stem cell (MSC) therapy to treat clinical diseases, such as chronic enteropathy, may be optimized through selection and pre-conditioning methods that would enhance their immunomodulatory functions. We investigated the effects of several pre-conditioning methods on cytokine production of female canine adipose-derived MSC in vitro to optimize the clinical effect of MSC application. The passage 3 canine MSC were generated from cryopreserved subcutaneous adipose tissue from 3 adult female dogs. MSC were subjected to 6 different pre-conditioning agents/conditions for 24 hours: control, poly I:C, canine IFNγ, TGFβ, serum-free media, and a mixed hypoxic environment. Supernatants from the MSC were harvested after 1, 6, 12, 18, and 24 hours, aliquoted, and frozen for batch analysis. Levels of IL-8, IL-10, TGFβ, VEGF, and MCP-1 present in the supernatants were determined by ELISA. Passage 3 female adipose-derived canine MSC, IL-10 and TGFβ were not released in measurable levels within a 24 hour period. MCP-1 (mean = 10.2ng/ml), VEGF (mean = 2.9ng/ml), and IL-8 (mean = 40.9pg/ml) were constitutively produced at the 24 hour time point. All of the pre-conditioning strategies except TGFβ altered cytokine production in the MSC from the control levels at 24 hours: poly I:C increased IL-8 (mean = 1810.4pg/ml) and MCP-1 (mean = 14.1ng/ml) secretion; IFNγ increased MCP-1 (mean = 12.9ng/ml) secretion and decreased VEGF (mean = 1.6ng/ml) and IL-8 (mean = 18.6pg/ml) secretion; serum-free decreased IL-8 (below the level of detection), MCP-1 (mean = 0.1ng/ml), and VEGF (mean = 0.3ng/ml) secretion; and the mixed hypoxic environments inhibited MCP-1 (mean = 0.9ng/ml) and VEGF (mean = 0.2ng/ml) secretion. All changes in cytokine secretion were evident at the 6 hour time point with most at maximal levels by the 12 hour time point suggesting that 12 hour culture periods are sufficient to induce changes in MSC cytokine secretion.

DVM Student/ Clinical Sciences

89 | The neural regulation of feeding: anorexigenic circuits

Arik Smith and Shane Hentges

Proopiomelanocortin (POMC) neurons, within the base of the hypothalamus, play a critical role in controlling food intake. Activating these neurons inhibits feeding and promotes weight loss, which makes them particularly interesting to human and veterinary medicine. Gap junctions, specialized cell-to-cell contacts that allow direct intercellular communication, sharpen neuronal activity through synchronizing populations. Due to the importance of collective POMC neuronal activity in metabolic regulation, we hypothesized that these cells express gap junctions to like cells, allowing a fast and unified activation. Our preliminary results, using electrophysiology with dye coupling, indicate that, on average, each POMC cell couples though gap junctions to 4 other non-POMC cells, the phenotype of which remains unidentified at the current time.

DVM Student/ Biomedical Sciences

90 | Detection of tuberculosis antibodies and clinical signs in lions in Kruger National Park, South Africa

Eliza Stout, Michele Miller, Peter Buss, Jenny Hofmeyr, Konstantin Lyashchenko, Paul van Helden, and Francisco Olea-Popelka

Tuberculosis has been of increasing concern in free-ranging lions in Kruger National Park since its initial discovery. Understanding the impact of tuberculosis relies on accurate methods of detecting infection and clinical disease. Our study evaluates associations between serological test results and the presence of tuberculosis related clinical signs. A total of 312 serological samples were collected in Kruger National Park from 230 free-ranging lions and 25 necropsied lions. Sera were tested using the ElephantTB STAT-PAK (Chembio Diagnostic Systems, Inc.). At time of sampling, veterinarians conducted a physical exam on each lion and recorded the presence of hygromas, skin nodules, and enlarged lymph nodes. Overall, 13.78% of samples were positive on the STAT-PAK test. There was a significant association between the presence of a hygroma and the risk of a positive STAT-PAK result. 31.43% of samples obtained from lions with a hygroma tested positive to STAT-PAK versus only 11.55% of samples collected from lions without hygromas (p=0.001). A borderline significant association was observed between skin nodules and a positive STAT-PAK result with 25.93% of lions with skin nodules testing positive versus 12.63% of lion samples without skin nodules (p=0.055). A trend was observed between enlarged lymph nodes and the risk of a positive STAT-PAK; whereas 18.10% of samples obtained from lions with enlarged lymph nodes tested positive, only 11.59% of samples from lions without enlarged lymph nodes tested positive (p=0.115). These results provide information that can be used in conjunction with other screening and diagnostic tools (i.e. mycobacterial culture) to evaluate the tuberculosis status of free-ranging lions.

DVM Student/ Clinical Sciences

91 | Comparison of qPCR and ELISA for assessment of Felis catus gammaherpesvirus 1 infection of domestic cats

Kathryn Stutzman-Rodriguez, Ryan Troyer, Joel Rovnak, and Sue VandeWoude

Felis catus gammaherpesvirus 1 (FcaGHV1) is a newly described virus that infects domestic cats. To determine FcaGHV1 antigens, we developed an immunofluorescent antibody assay by transfecting recombinant plasmids of FcaGHV1 genes and incubating these cells with sera fromFcaGHV1-positive cats. Based on the immunofluorescent assay, ORF52 and ORF38 were selected and their recombinant antigens were used to develop two, indirect FcaGHV1-ELISAs.ELISAs were used to screen sera from free-range cats previously tested by qPCR for FcaGHV1 in blood-cell DNA. Combined result of both ELISAs indicated 32% (n=133) FcaGHV1prevalence, compared to previously published 16% (n=135) qPCR-evaluated prevalence. Nineteen of twenty qPCR-positive cats were also seropositive. Risk factors identified in previous publications were confirmed by ELISA: geographic location, male sex, and adult age. This FcaGHV1-ELISA may provide a more sensitive assessment of virus exposure than qPCR and could aid in determining association of FcaGHV1 with disease and transmission route.

DVM Student/ Microbiology, Immunology and Pathology

92 | Induction of Cytotoxic and Genotoxic Responses by Novel Glycerol Glucoside

Cathy Su, Junko Maeda, Yasushi Aizawa, Takamitsu A. Kato

Glycerol is widely used in pharmaceutical formulations and industrial uses. Glyceryl glucoside (GG, α-D-glucosyglycerol) is a natural glycerol derivative found in alcoholic drinks. Recently, GG is used as an alternative for glycerol in cosmetic products. However, the safety of using GG is still unclear. By using Chinese Hamster Ovary cells (CHO), the study examined cytotoxic and genotoxic responses induced by dimethyl sulfoxide (DMSO), glycerol and GG to determine the safety of GG for cosmetic products. So far, we have completed cytotoxic analysis with three compounds and genotoxic analysis with glycerol and GG. In cytotoxic studies, DMSO displayed the highest cytotoxicity above 3% in cell doubling time delay among three chemicals. For acute cytotoxicity with trypan blue dye exclusion assay, GG showed stronger cell killing effect within 24 hours above 4%. Continuous cytotoxicity with colony formation assay for 7 days, DMSO showed significant reduction in clonogenic ability above 2%.In genotoxicity studies, CHO treated with glycerol at 2% concentration induced a three times higher frequency in sister chromatid exchange (SCE) than background levels. GG did not induce a significant amount of SCE compared to background levels. Micronuclei formation was equally observed in 2% and above concentrations of glycerol and GG. Our data showed that glycerol and GG have similar cytotoxicity effects to CHO, while glycerol induced genotoxic responses in the same concentration. Therefore, we conclude that GG may be a safer alternative compound for glycerol.

Staff/ Environmental and Radiological Health Sciences

93 | Effect of serum and N-acetyl-L-cysteine on chondrogenesis of equine bone marrow-derived mesenchymal stem cells

Suwimol Tangtrongsup and John D. Kisiday

Reactive oxygen species (ROS) are thought to play an important role in mesenchymal stem cells (MSCs) chondrogenesis. We investigated oxidative stress during MSCs chondrogenesis by suppressing the production of ROS using serum and the antioxidant N-Acetyl-L-cysteine (NAC).MSCs were encapsulated in agarose and cultured in serum-free chondrogenic medium for up to 15 days. Experimental conditions included 5% serum and/or 5mM NAC. Samples were stained for ROS production over time. Total glutathione (GSH) levels were quantified on days 1 and 6. Cell viability was evaluated on days 7 and 15. After 15 days, total accumulated glycosaminoglycan (GAG) and hydroxyproline were quantified. Data were analyzed by ANOVA and least squares means. In serum-free culture, ROS generation increased with time, reaching a maximum on day 6. After 8 hours of culture serum did not affect ROS production, while NAC suppressed ROS production. After 3 days of culture, serum suppressed ROS production relative to serum-free cultures. NAC induced ROS production in serum-free culture, but did not have an effect in serum. Total glutathione decreased 90% with time in culture, with minor differences among conditions. In serum-free and serum cultures, GAG accumulation or cell viability were not different, while hydroxyproline accumulation was higher in serum. NAC decreased GAG accumulation in both serum-free and serum culture, and decrease cell viability and hydroxyproline in serum-free culture. In serum-free medium, increasing production of ROS may be due to decreases in total GSH. NAC did not consistently suppress ROS; additional information on whether NAC acts as an anti- or pro-oxidant is needed to potentially determine how NAC interferes with chondrogenesis. Serum may be a more effective modulator of oxidative stress if suppression of ROS on day 3 persists over time. If so, serum cultures would demonstrate that the production of ROS may be reduced without suppressing chondrogenesis.

Graduate Student/ Clinical Sciences

94 | Effects of venom from the Prairie Rattlesnake (Crotalus viridis) and the Western Diamondback Rattlesnake (Crotalus atrox) on coagulation and fibrinolysis in equine plasma: in vitro evaluation using thromboelastography

Brianne Taylor, Stephen Mackessy, and Christine Olver

Rattlesnake venom, particularly that of the Prairie Rattlesnake (*Crotalus viridis*) in Colorado, affects both humans and companion animals. While the effects of rattlesnake venom have been characterized in the plasma of humans and dogs, the changes in coagulation and fibrinolysis in equine plasma is not well documented. In a pilot study done by the primary investigator, thromboelastography (TEG) evaluation has shown hypocoagulation in dog plasma treated with *C. viridis* venom. There are no existing publications of TEG evaluation on equine plasma treated with rattlesnake venom. This study will better understanding of the hemostatic effects of both *C. viridis* and *C. atrox* (Western Diamondback Rattlesnake) venom using thromboelastographic analysis of equine plasma. Plasma was obtained from 10 random horses. Thromboelastography without venom resulted in expected waveforms indicative of normal clot formation. The original venom concentration was 6 ug/mL, the same concentration used in a pilot study done on canine plasma with *C. viridis* venom. This concentration resulted in hypocoagulation in canine plasma, with a maximum amplitude waveform of half normal coagulation. However, addition of either *C. viridis* or *C. atrox* at this concentration yielded no clot formation as evidenced by the lack of TEG waveform. Furthermore, 1:2 (3µg/mL), 1:5 (1.2µg/mL), 1:10 (0.6µg/mL), and 1:20 (0.3µg/mL) dilutions of the 6µg/mL stock solution also failed to form a clot (the TEG waveform was a flat line). This strongly suggests that equine plasma is highly sensitive to rattlesnake venom at the molecular level, much more so than canine plasma.

DVM Student/ Microbiology, Immunology and Pathology

95 | The effect of micropatterned surfaces on biofilm formation in dogs with indwelling urinary catheters

Emma Thomas, Lauren Sullivan, Ayla Preston, Ethan Mann, and Shravanthi Reddy

Purpose: Catheter-associated urinary tract infection (CAUTI) is the most common hospital-acquired infection, resulting in significant morbidity within the intensive care unit (ICU). Biofilm formation along the urinary catheter sheath significantly increases the risk of CAUTI. This study compares the ability for a novel micropatterned urinary catheter surface to reduce biofilm formation when compared to a smooth silicone surface in dogs requiring indwelling catheterization within an ICU setting. Materials/Methods: Hospitalized dogs requiring indwelling urinary catheterization for a minimum of 48 hours were included and subsequently randomized to receive a micropatterned or smooth silicone urinary catheter. Urinalysis was performed at baseline and immediately following catheter removal. Catheters were immediately proceed for scanning confocal microscopy. Biofilm formation was assessed pictorally using 8mm punch biopsies from three locations along the urinary catheter (urinary bladder, urethra and meatus). Results: Catheters were evaluated from a total of 10 dogs, with six dogs receiving the micropatterned catheter and four dogs receiving the smooth silicone catheter. Duration of catheterization did not differ between groups (4.3 + 3.9 vs 3.0 + 1.4 days, P=0.54). Qualitative evaluation using scanning confocal microscopy revealed greater biofilm on the smooth silicone catheters compared to the micropatterned catheters, with the meatus collecting more biomass compared to the other two locations. Respective to each location, overall biofilm accumulation was reduced when the micropattern was present. Conclusions: In dogs hospitalized within an ICU and requiring indwelling urinary catheterization, a novel micropatterned surface appears to reduce biofilm formation . Further prospective studies are warranted to determine if micropatterned urinary catheters will reduce the incidence of CAUTI and their associated complications within an ICU setting.

DVM Student/ Clinical Sciences

96 | Establishment of minimum inhibitory concentrations (MIC) for ciprofloxacin for bacterial organisms cultured from the mare reproductive tract

Margo R. Hannet, David A. Trundell, Ryan A. Ferris, Bradley A. Borlee, and Patrick M. McCue

Bacterial endometritis is a major cause of reduced fertility in the mare. Recent reports indicated that certain strains of Gram negative bacteria (i.e. *Escherichia coli*) associated with bacterial endometritis can be resistant to commonly used intrauterine antibiotics (i.e. β-lactams and aminoglycosides). Ciprofloxacin, a fluoroquinolone antibiotic, has good in vitro antimicrobial activity against most Gram negative bacterial species. A pharmacokinetic study showed that intrauterine infusion of 600 mg of ciprofloxacin results in uterine lumen and endometrial tissue concentrations of ciprofloxacin of greater than 90 µg/ml and 65 µg/g, respectively, over a 24 hour period. In addition, no significant adverse effects of intrauterine treatment were observed. The goal of the present study was to determine minimum inhibitory concentrations (MIC) and MIC_{90} for ciprofloxacin for the most common bacterial species associated with infectious endometritis in the mare. Bacterial organisms (identified by MALDI-TOF and PCR) were cultured from the reproductive tracts of mares, and isolates frozen at -80oC. MIC and MIC90 for ciprofloxacin were determined for 10 isolates each of *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. MICs were established using the standardized CLSI protocol. MIC90 was defined as the MIC value at which ≥ 90 % of isolates within a population tested were inhibited. The MIC (± SE) and MIC90 for *Escherichia coli* were 0.025 ± 0.002 µg/mL and 0.031 µg/mL; *Klebsiella pneumoniae* 0.393 µg/mL ± 0.072 µg/mL and 1.0 µg/mL; and *Pseudomonas aeruginosa* 0.735 ± 0.160 µg/mL and 2.0 µg/mL. In conclusion, intrauterine infusion of ciprofloxacin may be an appropriate option for treatment of infectious endometritis associated with susceptible bacterial organisms in the mare.

Graduate Student/ Clinical Sciences

97 | Age-Dependent Shal Channel Stability in Neurons

Maximiliano J. Vallejos, Timothy H. Vernier, and Susan L. Tsunoda

Ageing typically relates to a decline in cognitive abilities and coordinated behaviors. These changes are likely due to age-related changes in neuronal signaling. Ion channels, which underlie much of neuronal signaling, have been described to undergo age-dependent changes in aggregation and distribution. Little is known about the ageing effects on ion channel stability. Here, we show an age-dependent decrease in Shal/Kv4 ion channel in *Drosophila*. Shal is a voltage-gated potassium channel involved in locomotion, learning and memory. We report that natural age-dependent decline in locomotion is improved by *Shal* overexpression. To investigate the mechanisms underlying the age-dependent decline in Shal protein, we quantitated *Shal* mRNA in young/old flies. *Shal* mRNA levels remain unaffected, suggesting that transcription is not involved in declining Shal protein. To test if the decline involves loss of a scaffolding protein, we examined SIDL (Shal-interactor of di-leucine), a novel protein identified to interact with a highly conserved C-terminal di-leucine motif required for somato-dendritic targeting. We found that *SIDL* transcript is significantly decreased with age. To test whether SIDL is necessary for Shal stability, we used an RNAi-SIDL construct resulting in 60% and 25% *SIDL* mRNA knockdown at larval and adult stages, respectively. This knockdown resulted in a 40% decrease of Shal protein levels in adults, suggesting that SIDL is required for Shal stability *in vivo*. Interestingly, RNAi-SIDL expressed during development resulted in decreased *Drosophila* viability. Our results suggest that loss of SIDL during ageing leads to a decline in Shal, and deterioration in behaviors such as locomotion.

Graduate Student/ Biomedical Sciences

98 | Creating awareness of tuberculosis caused by Mycobacterium bovis

Angela T. Varnum, Adrian Muwonge, Alejandro Perera, Paula I. Fujiwara, and Francisco J. Olea-Popelka

Mycobacterium tuberculosis is recognized as the primary causal agent of human TB throughout the world. However, due to the absence of routine surveillance data, the role of *Mycobacterium bovis*, the causal agent of zoonotic TB (ZTB) in humans, is uncertain. ZTB patients face important challenges because *M. bovis* is naturally resistant to pyrazinamide, ZTB is associated with extra-pulmonary TB, and the risk for ZTB increases in rural areas in developing regions of the world where bovine TB is endemic and people live in direct and indirect contact with infected animals or animal products (i.e. consuming unpasteurized milk). Increasing ZTB awareness especially among health and research institutions is crucial for alleviating disease. We assembled a multi-institutional team including medical and veterinary experts from the Union, WHO, CDC, and the World Organisation for Animal Health. We submitted a manuscript to The Lancet Infectious Diseases Journal calling key stakeholders to action worldwide. Resulting from the production of strategic health communication information, ZTB is now included in the most recent Global Plan to 'END TB'. Furthermore, we have secured spots in world-renowned medical and veterinary conferences to discuss challenges posed by ZTB. Finally, we have started field projects in Uganda and Pakistan to evaluate new diagnostic tools for ZTB in humans. Given the adoption of the 'END TB' strategy, understanding and thus preventing and effectively treating ZTB is crucial to achieving WHO's goal of ending the global TB epidemic by 2035.

DVM Student/ Clinical Sciences

99 | Reversal of phenotypic resistance of Anti-mycobacterial Compounds against Non tuberculosis mycobacteria

Deepshikha Verma, Randall Basaraba, Christian Melander, Mary Jackson, and Diane Ordway

Pulmonary infections due to non-tuberculous mycobacteria (NTM) are an emerging problem worldwide and pose serious health problems in both immunocompetent and immunocompromised individuals. NTM's are ubiquitous in environment and are associated with the biofilm formation that may contribute to their antibiotic resistance. The spread of antibiotic resistance has emerged as a major challenge when dealing with human health concerns. Evidences of role of bacterial efflux pumps for the antimicrobial resistance in the biofilm structure are widely cited in several microorganisms. We performed *in vitro* and *in vivo* assays using wild type and mutant (mmpL46 transporter gene family involved in Lipid transportation) of *M. massiliense* to determine the effect of mutation on the biofilm formation with and without small molecule biofilm inhibitors. Our preliminary results demonstrate *in vitro* minimum inhibitory concentration (MIC) obtained using the microbroth dilution method combined with *in vivo* animal infection supports the hypothesis that, inhibition of biofilm results in reversal of phenotypic drug resistance to standard anti-mycobacterial treatment. In conclusion, our studies provide evidence to support the notion that biofilm formation during NTMs infection impedes drug efficacy which can be reversed through the use of small molecule inhibitors to restore the efficacy of chemotherapy against NTM's.

Postdoctoral Fellow/ Microbiology, Immunology and Pathology

100 | Myxoma virus causes cytopathic effects in canine osteosarcoma cells

Karyn M. Wesley, Katelyn M. Polemi, and Amy L. MacNeill

A variety of modalities are available for canine cancer treatment, yet the long-term prognosis for many cancer types remains grave. The median survival time for a canine patient with osteosarcoma (OSA) treated via amputation and chemotherapy is one year. This necessitates the continued exploration of improved, novel therapy options, including oncolytic viral therapy. Studies were performed to evaluate the oncolytic effects of myxoma virus (MYXV) on canine OSA cells grown in primary culture. The cultured cells were obtained following surgical excision of a chondroblastic OSA tumor, removed from the distal tibia of a canine patient. The cells were allowed to replicate in primary culture. Cytochemical assays confirmed that the cultured cells have characteristics of canine osteoblasts. Cells were then inoculated with a known amount of MYXV to determine if the OSA cells were susceptible to infection. Cytopathic effects of a recombinant MYXV that expresses a red fluorescent protein were determined via fluorescent microscopy to detect protein production by the virus within the cells and visualize cell death. Additionally, assays that quantitatively measure cell viability were performed with wild type MYXV. Detection of a late MYXV protein within infected cells was confirmed by a Western immunoblot. The study results suggest that the cells grown in primary culture are of OSA origin, indicate that these cells are susceptible to MYXV infection, and document the cytopathic effects of MYXV on canine OSA cells. These data illustrate the potential of MYXV as an efficacious treatment option for canine cancer patients.

DVM Student/ Microbiology, Immunology and Pathology

101 | FADS2 Overexpression Promotes Metabolic Syndrome in Mice: Influence of Maternal Dietary Polyunsaturated Fatty Acid Composition

Connor M. Whitaker, Christopher M. Mulligan, Amanda J. Evans, Lance C. Li Puma, and Adam J. Chicco

Accumulating evidence indicates risk of developing metabolic syndrome (MSx) is influenced by maternal genotype and the nutritional environment encountered during fetal and early postnatal development. The dietary n6:n3 polyunsaturated fatty acid (PUFA) ratio has been postulated to influence development of MSx, but results from dietary PUFA intervention studies on metabolic health have been inconsistent. Common FADS2 haplotypes and elevated serum indices of PUFA metabolism by its gene product, delta-6 desaturase (D6D), are associated with obesity and MSx in children and adult populations. In support of this, we recently found that transgenic overexpression of FADS2 results in progressive MSx in mice. Based on epidemiological evidence for beneficial effects of perinatal n3-PUFA supplementation on infant metabolic status, we tested the hypothesis that reducing the n6:n3 PUFA ratio to ~3:1 in the maternal diet of FADS2-tg mice would attenuate development of MSx in her offspring. FADS2-tg females were fed standard chow supplemented with 2% w/w flaxseed oil (providing ~1% n3 α-linolenic acid; ALA), or 1% n-3 docosahexaenoic acid (DHA) beginning 2 weeks prior to pairing with a WT male until litters were weaned. All offspring were maintained on a standard chow diet after weaning, and compared to litters from the same mother without n3-PUFA supplementation during pregnancy/nursing, with >3 weeks of washout between dietary interventions. Interestingly, compared to offspring from non-supplemented mothers, maternal ALA and DHA supplementation significantly reduced weight gain and glucose intolerance to near WT levels in offspring for at least 2 months post weaning, despite similar food intake across groups. These studies suggest a potential interaction between maternal FADS2 expression and prenatal dietary PUFA composition on the development of MSx in their offspring. The cellular mechanisms responsible for MSx induced by FADS2 overexpression, and its suppression by maternal ALA and DHA supplementation are currently being investigated in our laboratory.

Graduate Student/ Biomedical Sciences

102 | Elucidating mother to offspring transmission of chronic wasting disease using a transgenic mouse model

Kassandra Willingham and Candace Mathiason

Chronic wasting disease (CWD) is the transmissible spongiform encephalopathy (TSE), or prion disease of free-ranging and captive cervids (deer, elk and moose). CWD was first reported in a captive mule deer herd in 1967. Clinical signs include weight loss, polydipsia, polyphagia and gait impairment. CWD is recognized as the most readily transmitted TSE and since its discovery has been detected in cervid populations in 23 states, 2 Canadian provinces, and the Republic of Korea. The presence of infectious prions in the tissues, bodily secretions/excreta and environments of CWD-infected animals is thought to account for its high transmission efficiency. Recently it has been recognized that transmission from mother to offspring may contribute to this facile transmission. Although the mechanism of maternal transmission has yet to be elucidated, the extended asymptomatic TSE carrier phase, lasting years to decades, suggests that maternal transmission may have implications in the spread of prions. Our work aims to identify whether prions are transmitted from a CWD-positive mother to her offspring, and identify whether they are sufficient to transmit disease. We employed a transgenic mouse that expresses cervid prion protein to elucidate the role of mother to offspring CWD transmission. Females were inoculated with CWD-positive material and subsequently bred with CWD-naïve males at various timepoints post inoculation. Resultant offspring were monitored over a period of 500 days for clinical signs, and at this time sacrificed for tissue analysis seeking CWD-prion deposition. We have demonstrated that CWD-infected transgenic females successfully breed and bear offspring irrespective to TSE disease stage. PrP^{CWD} was detected in brain, reproductive tissue and spleens from these females. While offspring born to CWD-infected females did not exhibit signs of TSE disease and lacked detectible PrP^{res} via conventional methodologies, conversion competent prions were identified in the brains of offspring by highly sensitive amyloid seeding assays.

Graduate Student/ Microbiology, Immunology and Pathology

103 | Central corneal pachymetry measurements in dogs using the Pentacam Scheimpflug topography system

Allison E. Wolfel, Sami Pederson, Allison Cleymaet, Ann Hess, and Katie Freeman

Assessing corneal thickness (pachymetry) is a key part of evaluating ocular health and vision. The Pentacam-HR® is an instrument routinely used in human ophthalmology to perform pachymetry, keratometry, and topography. Using a slit lamp and a camera, the Pentacam-HR® employs Scheimpflug technology to generate 50 detailed sectional images yielding 138,000 distinct elevation values of the cornea. To date, we have not had a tool in canine ophthalmology to measure all of these values with one instrument nor have we been able to assess corneal topography accurately. The purpose of this study is to validate Pentacam-HR® pachymetry in canine eyes by comparing Pentacam-HR® measurements of central corneal thickness to those obtained with the current gold standard devices, high-resolution ultrasound biomicroscopy [UBM] and spectral-domain optical coherence tomography [SD-OCT]. Clinically normal corneas were evaluated in 20 sedated canine patients generating data from 30 eyes, as only non-diseased corneas were included. Corneal images were acquired via Pentacam-HR®, SD-OCT, and UBM. Machine-calculated values of central corneal thickness from Pentacam-HR® and SD-OCT were compared to hand-measured values from UBM. The results found mean corneal thickness at the corneal apex ± standard deviation from each device were as follows: Pentacam-HR® 634.07 ± 67.8 µm, SD-OCT 614.59 ± 57.58 µm, and UBM 693.19 ± 58.95 µm. Statistical analysis will further evaluate the data. The preliminary results indicate that the Pentacam-HR® accurately measures corneal thickness in dogs, as compared with the currently accepted gold standards for pachymetry. Thus, the Pentacam-HR®, and the multitude of novel data about anterior segment parameters and health that it generates, can now be used for canine ophthalmology. The results from this study aid in optimal patient care, provide necessary baseline data for future research, and contribute to overall understanding of canine vision.

DVM Student/ Clinical Sciences

104 | Lactobacillus paracasei fermentation of rice bran extract reduces Salmonella Typhimurium growth in vitro

Colette R Worcester, Nora J. Nealon, and Elizabeth P. Ryan

Salmonella Typhimurium causes illness in people and animals worldwide, and probiotic bacteria and prebiotic molecules have been shown to reduce infection and promote a healthy intestinal microflora. Rice bran provides a variety of nutrients and may play protective roles in the intestinal tract. Mice fed rice bran in previous studies had reduced *Salmonella* fecal shedding after being challenged with *S. Typhimurium*. In addition, *Lactobacillus* numbers increased in those experiments, and *Lactobacillus paracasei* has been shown to reduce *Salmonella* infections. This study aims to determine if rice bran could enhance the ability of *Lactobacillus paracasei* to reduce *Salmonella Typhimurium* growth *in vitro*. *L. paracasei* cultures (ATCC27092) were grown in de Man, Rogosa, and Sharpe media (MRS) alone, or in MRS with the addition of Lijiangxintuanheigu (LTH) rice bran extract (100µg/ml) for 24 hours at 37°C. The cell-free supernatant from these fermentations were collected, pH titrated to 4.5, and added to *Salmonella enterica* supsp. *enterica serovar* Typhimurium 14028s Kan^r (rPSM::GFP) cultures in different concentrations, diluting with sterile, unfermented media (MRS alone or 100µg/ml LTH in MRS) titrated to a pH of 4.5. Growth curves were obtained from optical density readings over 16 hours at 37°C. *S. Typhimurium* growth was reduced significantly ($p<0.05$) with increasing amounts of fermented media in a dose-dependent effect. Even as the lowest dose, 20% fermented LTH supernatant significantly reduced S*. Typhimurium* growth ($p<0.05$) starting 3 hours into the experiment) compared to S*. Typhimurium* grown with sterile LTH in MRS. 100% *Lactobacillus*-fermented LTH also reduced S*. Typhimurium* growth significantly ($p<0.05$) more than 100% fermented MRS alone, starting at 8.67 hours. Future work to profile fermented rice bran metabolites will give insight to which small molecules produced by *L. paracasei* reduce growth of *Salmonella* Typhimurium.

Undergraduate Student/ Environmental and Radiological Health Sciences

105 | A comparison of tricaine methanesulfonate and alfaxalone as an immersion anesthetic in two tropical fish species

Amanda K. Zellar and Terry W. Campbell

Alfaxalone's use as an induction anesthetic has been evaluated in case reports and studies of goldfish and koi. The purpose of this study was to evaluate the effectiveness of alfaxalone as an immersion anesthetic in tropical fish species compared to MS-222. The species studied were black spot barbs, Puntius filamentosis, measuring (mean +/- SD) 11.4 +/- 1.4cm in body length, and peacock cichlids, Auloncara, 8.4 +/- 1.6cm. Fish were placed in water baths containing 100mg/L of MS-222 buffered with 200mg/L of bicarbonate, then observed for a two-week dry-off period before being immersed in 5mg/L of alfaxalone. During anesthetic trials, behavior was monitored during induction for self-righting, spontaneous swimming movements, opercular movement and response to noxious stimuli. Anesthetic depth assessment was based on a modified published scale. All 22 barbs reached surgical anesthesia when treated with either drug. 100mg/L of MS-222 induced surgical anesthesia in 20 of 22 cichlids. 5mg/L of alfaxalone induced surgical anesthesia in 20 of 21 cichlids. Induction and recovery times for barbs treated with 100mg/L of MS-222 was 6 +/- 2min and 3 +/- 1min respectively. Induction and recovery times for cichlids treated with 100mg/L MS-222 were 15 +/- 5min and 3 +/- 1min respectively. Induction and recovery times for barbs treated with 5mg/L alfaxalone were 3 +/- 2min and 9 +/- 3min respectively. Induction and recovery times for cichlids treated with 5mg/L alfaxalone were 11 +/- 10min and 23 +/- 11min respectively. In both species there was no statistically significant difference observed in induction time between treatment with MS-222 and alfaxalone, however, recovery times in both species were significantly longer from anesthesia induced with alfaxalone than with MS-222, $p < 0.0001$ by T-test.

DVM Student/ Clinical Sciences

106 | Localization and Stability of a Toxic mRNA in a Cell Culture Model of Type I Myotonic Dystrophy

Annie Zhang, Mary Schneider, and Carol J. Wilusz

Myotonic Dystrophy 1 (DM1) is a multisystem inherited disease caused by expanded CTG repeats within the 3'UTR of the DMPK gene. The encoded CUG repeat-containing RNAs are toxic to the cell and accumulate in nuclear foci where they sequester cellular RNA-binding proteins such as the splicing factor Muscleblind (MBNL1) leading to profound changes in gene expression. Here we describe a new regulatable cell culture model to study metabolism of CUG-repeat containing RNA, and its application in analyzing decay of the repeat-containing mRNAs. This model recapitulates the accumulation of mutant DMPK mRNA in nuclear foci as assessed by in situ hybridization and nuclear/cytoplasmic fractionation. We have determined that both wild type and mutant DMPK transcripts decay rapidly with half-lives of ~ 1 hour in our myoblast cells. This is significantly less stable than reported previously, likely due to the fact that earlier studies were forced to employ Actinomycin D to inhibit all cellular transcription prior to measuring decay rates. We have also discovered that cycloheximide treatment, which inhibits cytoplasmic translation-dependent decay pathways, results in increased accumulation of the mutant transcript suggesting that a significant portion of the mutant mRNA actually exits the nucleus and is degraded in the cytoplasm. We are currently investigating the pathways and factors influencing export and turnover of the mutant transcripts. A better understanding of the decay mechanisms of wild type and mutant DMPK mRNA may lead us to additional therapeutics for DM1, as well as providing valuable information to explain how current pre-clinical therapies affect metabolism of DMPK mRNA.

Graduate Student/ Microbiology, Immunology and Pathology

CONGRATULATIONS AGAIN TO 2015 RESEARCH DAY WINNERS!

ORAL PRESENTATIONS

FIRST BASIC — **Tanya Gustafson**, veterinary resident, CS, "Inhibition of hedgehog signaling inhibits proliferation in canine transitional cell carcinoma." Mentor: Barb Biller

SECOND BASIC — **Kristin Davenport**, DVM/PhD student, MIP, "Novel in vitro assessments of prion disease species barriers." Mentor: Ed Hoover

FIRST CLINICAL — **Stacie Summers**, post-doctoral fellow, CS, "Effect of parenteral administration of modified live or inactivated FVRCP vaccine on clinical signs in a feline herpesvirus challenge model." Mentor: Mike Lappin

SECOND CLINICAL — **Aimee Colbath**, veterinary resident, CS, "Single paralumbar fossa laparoscopy for elective ovariectomy in standing mares." Mentor: Eileen Hackett

POSTER PRESENTATIONS

FIRST — **Lauren Radakovich**, veterinary resident, MIP, "Reticulocyte hemoglobin content (CHr) does not differentiate true from functional iron deficiency in dogs." Mentor: Christine Olver

SECOND — **Sophia Moore**, DVM student, "Potential target for cancer therapy: Expression of cellular prion protein found in canine cancer cell lines with unique glycosylation patterns." Mentor: David Argyle, University of Edinburgh

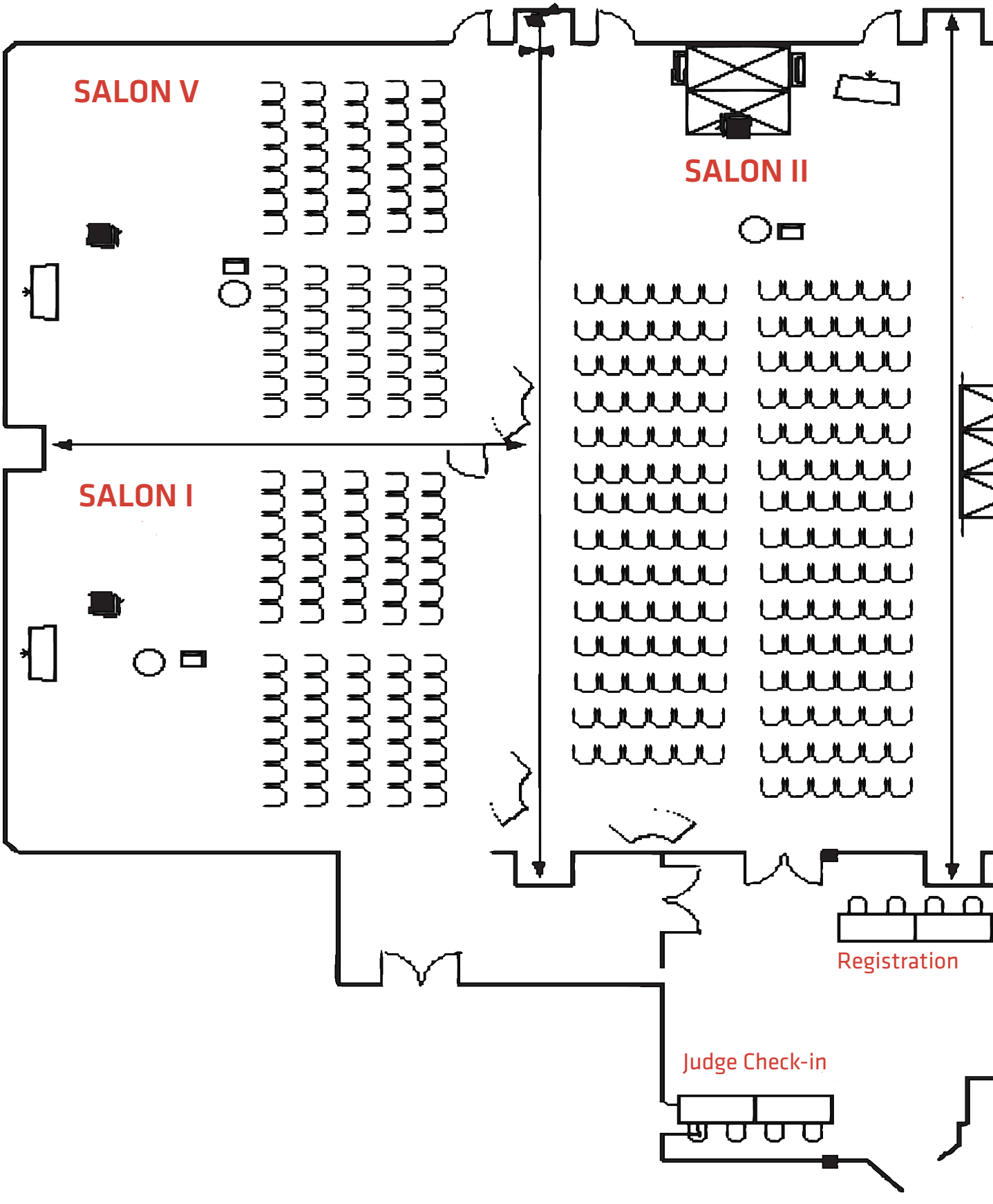
THIRD — **Gustavo Diaz**, graduate student, MIP, "Proteomic characterization of exosomes released from human macrophages infected with Mycobacterium tuberculosis." Mentor: Karen Dobos

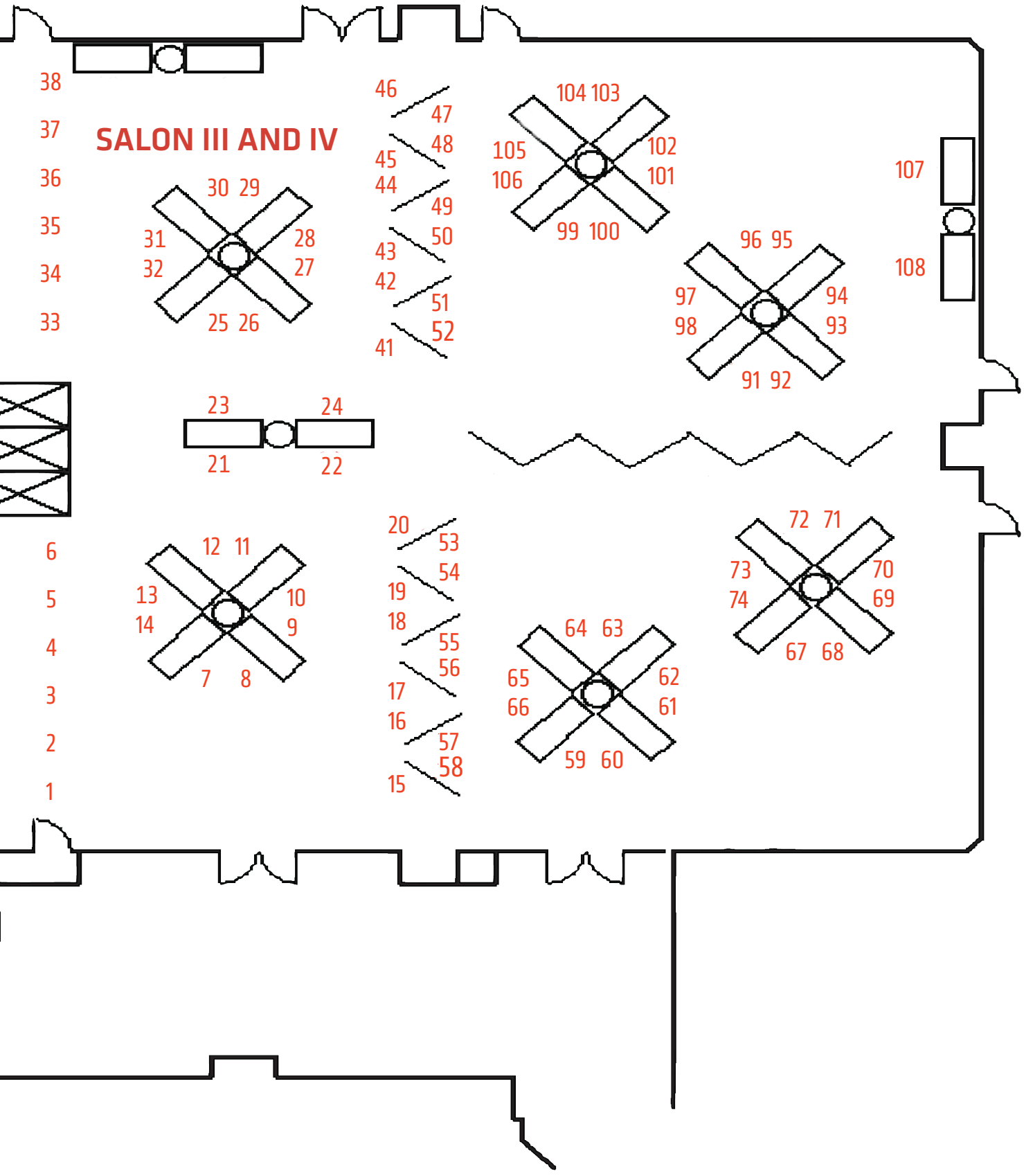
GOLDEN PIPETTE AWARD – Department of Clinical Sciences

2016 CVMBS RESEARCH DAY ORGANIZING COMMITTEE

Claudia Wiese – Faculty Chair – Environmental and Radiological Health Sciences
Brad Borlee – Faculty Co-Chair – Microbiology, Immunology, and Pathology
Ashley Turnidge – Biomedical Sciences
Jimmy Singh – Biomedical Sciences
Val Johnson – Clinical Sciences
Sarah Shropshire – Clinical Sciences
Nadia Sampaio – Environmental and Radiological Health Sciences
Hailey Conover – Environmental and Radiological Health Sciences
Mike Mangalea – Microbiology, Immunology, and Pathology
Danielle Adney – Microbiology, Immunology, and Pathology
Aimee Oke – Committee Coordinator – CVMBS Dean's Office

Floor Plan Colorado State Ballroom | Hilton Fort Collins




SALON III AND IV
38
37
36
35
34
33
30 29
31
32
28
27
25 26
46
47
45
48
44
49
43
50
42
51
41
52
104 103
105
102
106
101
99 100
96 95
97
94
98
93
91 92
107
108
23
24
21
22
6
5
4
3
2
1
12 11
13
10
14
9
7 8
20
53
19
54
18
55
56
17
16
57
15
58
64 63
65
62
66
61
59 60
72 71
73
70
74
69
67 68

NOTE: POSTER NUMBERS CORRESPOND TO THOSE SHOWN IN RED ON THE FLOOR MAP, ALLOWING ATTENDEES TO EASILY LOCATE POSTERS OF INTEREST.