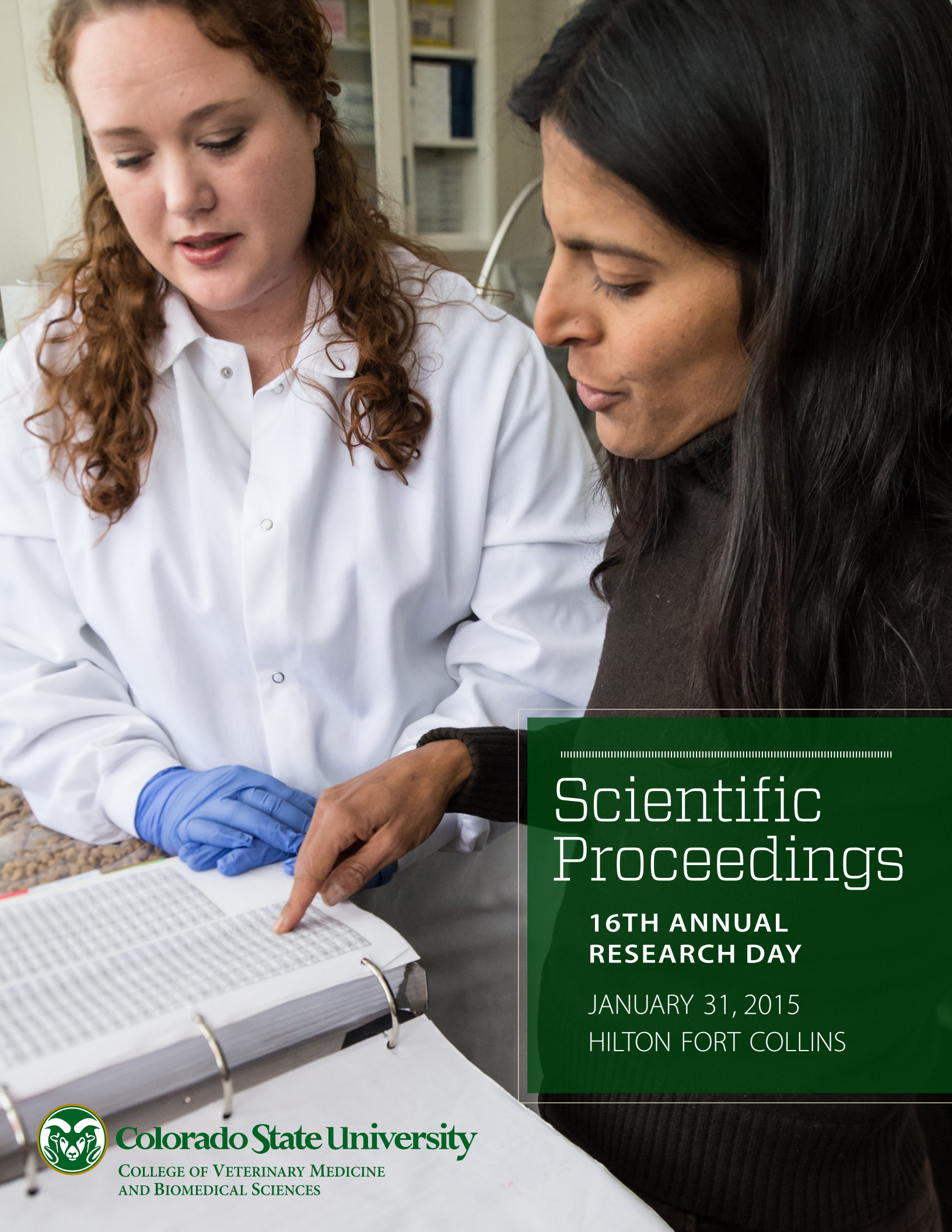

Scientific Proceedings

16TH ANNUAL RESEARCH DAY

JANUARY 31, 2015

HILTON FORT COLLINS

Colorado State University

College of Veterinary Medicine and Biomedical Sciences

ON THE COVER:

Colorado State University graduate student Genevieve Forster, working alongside Dr. Elizabeth Ryan, is among more than 140 undergraduate students, graduate students, veterinary residents, and post-doctoral fellows participating in 2015 Research Day. The day gives trainees in the College of Veterinary Medicine and Biomedical Sciences a showcase for their research efforts and findings.

2015 CVMBS-ZOETIS EARLY CAREER RESEARCH AWARD

Two researchers in the College of Veterinary Medicine and Biomedical Sciences have been honored with the 2015 CVMBS-Zoetis Early Career Research Award. The honorees, selected by colleagues and peers, are Dr. Candace Mathiason and Dr. Elizabeth Ryan. Each will receive a plaque and $1,000 honorarium. The two awardees also will kick off 2015 Research Day by presenting talks about their work. In keeping with a proud tradition, global animal health company Zoetis sponsors Research Day and the Early Career Research Awards.

Mathiason investigates prion disease to shed light on human neurologic diseases

DR. CANDACE MATHIASON is an assistant professor and member of the Prion Research Center in the Department of Microbiology, Immunology, and Pathology. She established her research program in 2012. Her talk at 2015 Research Day is titled, "Stealthy Prions."

Mathiason received a Ph.D. in prion pathobiology from Colorado State University under the mentorship of Dr. Edward Hoover, University Distinguished Professor and member of the National Academy of Sciences. She had earlier worked as manager of the Hoover Laboratory, training in retrovirology and prion biology.

The Mathiason Laboratory investigates the biological mechanisms associated with covert prion infection and transmission. Studies examine chronic wasting disease (CWD) prions in native cervid hosts using novel *in vitro* detection methodologies.

This work is designed to provide insight for all transmissible spongiform encephalopathies (TSEs), along with human neurologic diseases similar to prion diseases, including Alzheimer's, Huntington's, and Parkinson's diseases, as well as type II diabetes.

Animal prion diseases, including CWD, are remarkably similar to human prion diseases, recapitulating trafficking, dissemination, and even transmission patterns. In exposed mammals, including humans, prions establish and maintain a silent carrier, or asymptomatic phase of disease, that lasts many months or years prior to the development of clinical TSE disease.

Mathiason collaborates with researchers from Colorado, Nebraska, Kansas, Montana, Illinois, Wisconsin, New York, Georgia, Tennessee, Canada, Scotland, France, England, and Germany. She is a board member for the Rocky Mountain Virology Association and has played key roles in several regional, national and international conferences addressing prion and TSE research. Mathiason also mentors young scientists seeking to develop careers and is an alternate member on the CSU Institutional Animal Care and Use Committee.

Mathiason's research is funded by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service; and National Institutes of Health, National Institute of Allergy and Infectious Diseases.

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Ryan examines rice bran as a route to reducing cancer and diarrheal disease

DR. ELIZABETH RYAN is an assistant professor of toxicology in the Colorado State University Department of Environmental and Radiological Health Sciences. She established her CSU research laboratory in 2010. Her talk at 2015 Research Day will be, "Rice bran: cross-kingdom and One Health perspectives."

Ryan received her doctorate in molecular toxicology from the University of Rochester School of Medicine, Department of Environmental Medicine. She received postdoctoral training with a National Cancer Institute fellowship in clinical cancer control and prevention and focused her research in the field of immunotoxicology at the James P. Wilmot Cancer Center, University of Rochester Medical Center in New York.

The Ryan Laboratory employs molecular biological, immunological and biochemical techniques and implements clinical dietary studies to explore enteric diseases, namely diarrhea and colon cancer, affecting people in both developing and developed countries. Studying mechanisms of pathogen infection and metabolic disturbances in animals and people provides essential information that is leading to novel dietary strategies and molecular targets.

Ryan's research program focuses on the interaction of dietary compounds with the immune system and lymphoid tissues in the gut. Her studies in mice, dogs, pigs and humans have led to numerous publications detailing translational knowledge about the promising role for the agricultural byproduct rice bran to promote health. Her laboratory collaborates with researchers from Fort Collins, University of North Carolina, University of Illinois, Virginia Polytechnic Institute and State University, Michigan State University and University of Colorado. International research collaborations are established in Mali, Kenya, Nicaragua, Nepal, and India.

Ryan has also distinguished herself in global public health and food security. Her work is funded through National Institutes of Health, National Cancer Institute; the Bill and Melinda Gates Foundation, University of Colorado Cancer Center, and the Dry Bean Health Research Group.

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SCHEDULE of Events

11:30–NOON	POSTER SET UP	SALON III, IV
NOON	OPENING REMARKS Dr. Melinda Frye, Associate Dean for Veterinary Academic and Student Affairs	SALON II
12:05 P.M.	CVMBS-Zoetis Early Career Research Award Winner Dr. Candace Mathiason "Stealthy Prions"	SALON II
12:25 P.M.	CVMBS-Zoetis Early Career Research Award Winner Dr. Elizabeth Ryan "Rice Bran: Cross-Kingdom and One Health perspectives"	SALON II
1-5 P.M.	ORAL PRESENTATION I: Clinical Sciences	SALON I
1-5 P.M.	ORAL PRESENTATION II: Basic Sciences	SALON II
1-5 P.M.	ORAL PRESENTATION III : Clinical/Basic Sciences	SALON V
1-3 P.M.	POSTER SESSION I JUDGING: Odd-numbered Posters	SALON III, IV
2:45 P.M.	BREAK	
3:15-5 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

OUR 16TH ANNUAL RESEARCH DAY

showcases the academic work of more than 140 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 researches 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!

SESSION 1: Clinical Science

1:00–5:00 PM | SALON I

1:00	Bartner	Bartonella spp. PCR assay results using cerebrospinal fluid of naturally exposed dogs with central nervous system disease | CS
1:15	Bromberek	Canine B-cell chronic lymphocytic leukemia shows strong breed-specific risk | MIP
1:30	Colbath	Single Paralumbar Fossa Laparoscopy for Elective Ovariectomy in Standing Mares | CS
1:45	Coleman	Investigation of gastrointestinal motility in dogs undergoing prophylactic laparoscopic gastropexy | CS
2:00	Fagre	Improved characterization of Salmonella enterica shedding among reptile patients at the James L. Voss Veterinary Teaching Hospital | CS
2:15	Felumlee	Intra- and interobserver variability of GFR rate determination in cats with chronic kidney disease via gamma camera uptake of Tc-99m-DTPA | ERHS
2:30	Fisher	Carbon dioxide induced pulmonary hemorrhage | MIP
2:45	BREAK	
3:00	Forster	Navy bean powder diets modulate serum cholesterol and fecal lipid metabolites in healthy companion dogs | ERHS
3:15	Freund	Assessment of novel digital and smartphone goniometers for joint angle measurement of the canine stifle | CS
3:30	Hart	Comparison of owner satisfaction with stifle orthoses or tibial plateau leveling osteotomy for the treatment of cranial cruciate ligament disease | BMS
3:45	Hoaglund	Effect of intravenous fluid volume administration on development of post-operative ileus in horses with colic | CS
4:00	Lakin	The estrus cycle influences the bacterial microbiome in the normal mare reproductive tract | CS
4:15	Lantz	Assessing pain and trauma in early lactation dairy cattle | CS
4:30	Lee	Cationic liposomes-oligonucleotide complexes as an alternative adjuvant for polyclonal antibody production in rabbits | MIP
4:45	Martin	Canine Gait Analysis Using Inertial Measurement Units During Outdoor and Treadmill Activity | CS

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SESSION 2: Basic Science

1:00–5:00 PM | SALON II

1:00	Adney	Efficient replication and shedding of MERS CoV from the upper respiratory tract of experimentally infected dromedary camels | MIP
1:15	Ali	Neuron-specific (pro)renin receptor deletion regulates renin-angiotensin components and contributes to the attenuation of DOCA-salt hypertension | BMS
1:30	Brown	Evaluation of pathogenesis and immune response of Francisella tularensis in cottontail rabbits | MIP
1:45	Cerda	Wolf (Canis lupus) population dynamics and taeniid cestode prevalence in Isle Royale National Park, Michigan, USA | MIP
2:00	Chotiwan	Dengue Virus Infection Induces Lipid Alterations in the Aedes aegypti Mosquito Vector | MIP
2:15	Dang	Reactive Oxygen Species Modulate Local L-type Calcium Channel Signaling in Gonadotropes | BMS
2:30	Davenport	Novel in vitro assessments of prion disease species barriers | MIP
2:45	BREAK	
3:00	Faulhaber	In vivo expression of programmed death ligand 1 by canine tumors | CS
3:15	Fowles	Interspecies gene expression models for predicting doxorubicin response in canine osteosarcoma | CS
3:30	Gullberg	Dengue virus requires the unsaturated fatty acid biosynthesis pathway for its infection in the mammalian host | MIP
3:45	Gustafson	Inhibition of Hedgehog signaling inhibits proliferation in canine transitional cell carcinoma | CS
4:00	Kane	Complement, complement receptors, and complement regulatory proteins bind prions and assist in pathogenesis | MIP
4:15	Mapesa	Rice-bran phytochemical extracts inhibit invasion and intracellular replication of Salmonella in mouse intestinal and porcine jejunal epithelial cells | ERHS
4:30	McNeil	Developing the dairy disease adjusted lactation yield model (DALY) | CS
4:45	Moore	Interactions between Diet and Exposure to Secondhand Smoke Exposure on Childhood Obesity: National Health and Nutrition Examination Survey (NHANES) 2007-2010 | ERHS

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SESSION 3: Clinical / Basic Science

1:00–5:00 PM | SALON V

1:00	Ouyang	Use of patient body temperatures in surveillance for healthcare-associated infections in a veterinary hospital | CS
1:15	Regan	Role of monocyte recruitment in hemangiosarcoma metastasis in dogs | CS
1:30	Smith	Precision of a Horse-Mounted Inertial Sensor Device Following Re-Application | CS
1:45	Summers	Effect of parenteral administration of modified live or inactivated FVRCP vaccine on clinical signs in a feline herpesvirus challenge model | CS
2:00	Thigeel	Validation of a polymerase chain reaction assay for the subtyping of Cryptosporidium spp. isolates of human origin | CS
2:15	Tuttle	Guinea pigs, Tuberculosis, and negative results- A research conundrum | MIP
2:30	Warrit	Gastrointestinal Motility Changes in Dogs During Hospitalization | CS
2:45	BREAK	
3:00	Wendland	Evaluation of Gait Patterns in Dogs: The Pace | CS
3:15	Ortega	Prions in plants: assaying grasses from Rocky Mountain National Park for PrP^{CWD} | MIP
3:30	Rosenberg	Rapid Isolation of Neptunium from Solution and Soil | ERHS
3:45	Rout	Characterization of immunoglobulin gene use and mutation status in canine B cell chronic lymphocytic leukemia | MIP
4:00	Sims	Evaluation the elution of polyhexamethylene biguanide from two wound dressing materials | CS
4:15	Sullivan	Neuronal overexpression of the human (pro)renin receptor increases sympathetic tone that is masked by upregulation of endothelial nitric oxide synthase | BMS
4:30	Verma	Reversal of Mycobacterium abscessus antimicrobial resistance using biofilm inhibitors | MIP
4:45	Willingham	Assessing mother to offspring transmission of chronic wasting disease using a transgenic mouse model | MIP

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POSTER PRESENTATIONS

SESSION 1 | ODD NUMBERED POSTERS | 1:00–2:45 PM | SALON III AND IV

SESSION 2 | EVEN NUMBERED POSTERS | 3:00–4:45 PM | SALON III AND IV

NOTE: POSTER NUMBERS PROCEEDE NAMES AND CORRESPOND TO NUMBERS LISTED ON THE FLOOR PLAN AT THE BACK OF THIS PROCEEDINGS BOOK.

1	Aanstoos-Ewen	Influence of mesenchymal stromal cells on pulmonary metastases and local recurrence following primary tumor removal in a murine osteosarcoma model | CS
2	Aldrich	Radiographic Localization of the Origins and Insertions Associated with the Tendons and Ligaments of the Equine Stifle Joint | CS
3	Anna	Comparison of 2 commercially available PCR assays for the amplification of Ehrlichia spp. DNA from blood of naturally exposed dogs in Oklahoma | CS
4	Ball	Genetic modification of mesenchymal stem cells with scAAV-equine-BMP-2 to induce osteogenesis | CS
5	Barnes	Serum D-lactate concentrations in dogs with parvoviral enteritis | CS
6	Barron	Comparison of proliferation and immunomodulatory potential of adipose-derived mesenchymal stem cells from young and geriatric feline patients | CS
7	Bell	Effects of venom from the Prairie Rattlesnake (Crotalus viridis) on canine coagulation and fibrinolysis: In vitro evaluation using thromboelastography | BMS
8	Benally	Mesenchymal Stem Cell Responses to Activation With TLR Ligands | CS
9	Bender	Encapsulated siRNA as a therapeutic for prion diseases | MIP
10	Benson	Pharmacodynamics of transdermal mirtazapine in healthy client-owned cats | CS
11	Borresen	Increasing dietary rice bran and navy bean intake for colorectal cancer control and prevention: A randomized-controlled pilot investigation | ERHS
12	Bradley	Early Assessment of Clinical Effects of Mesenchymal Stem Cell Therapy in Dogs with Chronic Hepatitis | CS
13	Brandes	Individual animals versus the dung pile: which sampling strategy is best for herd-based fecal egg count surveillance programs? | MIP
14	Brown	Pharmacokinetics of Atopica capsules stored at -20C° | CS
15	Brown	Metabolomics investigation of human colorectal cancer tissue, adjacent mucosa, and stool collected from CRC patients | ERHS
16	Bryan	Prevalence of Dirofilaira Immitis in non-endemic Northern Colorado animal shelters | CS
17	Caress	Effect of Enterococcus faecium strain SF68 on gastrointestinal clinical signs of healthy cats administered amoxicillin-clavulanate | CS

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18	Cerda	Urine protein:creatinine ratio variability when comparing single, serial, or pooled urine samples from dogs with varying magnitudes of proteinuria currently receiving medical therapy | CS
19	Charley	RNA viruses target exoribonuclease XRN1 to promote pathogenesis | MIP
20	Chiu	Bovine herpesvirus 4 not detected in free-ranging domestic cats from California, Colorado, and Florida | MIP
21	Chow	Derivation of Mesenchymal Stem Cells From Canine Induced Pluripotent Stem Cells | CS
22	Christofferson	Identification of Particulate Matter 2.5 as a Possible Trigger for Idiopathic Pulmonary Fibrosis | MIP
23	Coleman	Expression of the mutant DMPK mRNA in immortalized myotonic dystrophy patient cells alters cellular mRNA stability | MIP
24	Conover	Investigation of genetic predispositions to copy number variation in the parents of autistic children | ERHS
25	Contreras	Effect of the probiotic, Enterococcus faecium (SF-68), on resolution of acute diarrhea in young shelter cats | CS
26	Cooley	Consumption of Viyo Recuperation – Renal in cats with stable chronic kidney disease and its effects on biochemical parameters | CS
27	Curran	Acute leukemia in dogs: diagnostic criteria, treatment and outcome | MIP
28	DeHaan	Incidence rate and effects of persistent mating-induced endometritis (PMIE) in Quarter Horse mares | CS
29	Diaz	Proteomic characterization of exosomes released from human macrophages infected with Mycobacterium tuberculosis | MIP
30	Doster	Detection of Salmonella enterica in the dairy environment using a commercially available lateral flow immunoassay | CS
31	Eddy	Isolation and Characterization of Primordial Follicles from Canine Ovaries | BMS
32	Edmondson	Identification of susceptibility loci for sarcomatoid change and metastasis in pulmonary adenocarcinoma | ERHS
33	Fauver	West Nile virus surveillance in Fort Collins, 2006-2013 | MIP
34	Fletcher	Changes in leukocyte population subsets in goats experimentally infected with Mycobacterium avium subsp. paratuberculosis | MIP
35	Genis	Cytokine enzyme-linked immunosorbent assays for the detection of Johne's disease during pre-clinical stages in experimentally infected goats | MIP
36	Gibas	Development of an immunocytochemistry mast cell tumor profile | MIP
37	Goldsmith	Serosurveillance for Brucellosis in Pacific Walrus (Odobenus rosmarus) in Alaska | CS

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38	Hafferman	Vitamin D deficiency in sled dogs | BMS
39	Harbison	Relationships between inflammatory cytokines and leukocyte telomere length in healthy adults with and without a history of colorectal cancer | ERHS
40	Harcy	Investigation of mechanisms of mitotic recombination in yeast: A mutational avalanche for some | ERHS
41	Hartley	Immune regulation of PD-L1 expression on canine tumors | CS
42	Hernandez	Survey of Whole Exome Sequence Data of Canine Bladder Transitional Cell Carcinomas | CS
43	Hoover	Early prion trafficking in deer exposed to chronic wasting disease | MIP
44	Hornig	Validation of the Heska Element POC Blood Gas and Electrolyte Analyzer for use in alpacas and goats | CS
45	Immesberger	Pre-ride serum amyloid A in endurance horses is not predictive for completion of a 160-km ride | CS
46	Jalkanen	Post-transcriptional mechanisms coordinate expression of zinc finger protein mRNAs | MIP
47	Jeon	Reversal of phenotypic and intrinsic antimicrobial drug resistance in Mycobacterium tuberculosis with 2-aminoimadazole based small molecule adjuvants | MIP
48	Johnson	Harnessing the antimicrobial properties of activated mesenchymal stem cells: A clinical trial in dogs with multi-drug resistant infections | CS
49	Kopanke	Marked trematodiasis in a population of wild-caught Trinidadian guppies | MIP
50	Kurth	Relationship between Androgen Receptor (AR) and LIN28A in the mouse placenta | BMS
51	Lake	Building a virtual cat: towards a physiologic-based pharmacokinetic (PBPK) model for investigating drug dosing in cats | CS
52	Lakey	Activated immune cells demonstrate decreased glucose receptor expression as potential antimicrobial mechanism in early stage Mycobacterium tuberculosis infection | MIP
53	Ledesma-Feliciano	Does feline foamy virus cause disease in domestic cats? | MIP
54	Lee	L-type Ca2+ channel knock-out model in αT3-1 cells using the novel CRISPR-Cas9 system with gRNA | BMS
55	Loncar	Evaluation of biofilms produced by Gram-negative bacteria isolated from the equine uterus | CS
56	Loughridge	Computed tomography reveals higher bone density at the distal articular surface of racehorses with third metacarpal fractures | CS
57	Maeda	Investigation of radiosensitivity gene signatures in canine tumor cells | ERHS

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58	Malmlov	Serological evidence that Tacaribe virus is circulating among bats in Trinidad and Tobago | MIP
59	McClure	Brain regions involved in seasonally dependent A1AR induced torpor in Arctic ground squirrels | BMS
60	McGuire	Experimental Infection of Deer Mice with Maporal Hantavirus | MIP
61	McNamara	Do mesenchymal stromal cells abrogate the host immune response in massive cortical allograft recipients? | CS
62	Moore	Potential target for cancer therapy: Expression of cellular prion protein found in canine cancer cell lines with unique glycosylation patterns | RDSVS
63	Moreno-Garcia	Impact of insecticide resistance on the immune response of Aedes aegypti mosquito | MIP
64	Nealon	Dry bean (Phaseolus vulgaris) consumption supports long-term weight loss in companion dogs | CS
65	Ngai	Fecal cortisol and other measures of stress before and after clicker training cats in two Colorado animal shelters | BMS
66	Noyes	Using Metagenomics to Unlock the Ecology of Antimicrobial Resistance in Cattle Production Systems | CS
67	Nuckols	One Health approach to review of questionnaires for clinical trials of bean-based dog food | ERHS
68	Plumley	The regulatory role of cyclic diguanylate in Burkholderia pseudomallei motility and biofilm formation | MIP
69	Pyuen	In Vitro Effects of PI3K/mTOR Inhibition in Canine Hemangiosarcoma | CS
70	Radakovich	Reticulocyte hemoglobin content (CHr) does not differentiate true from functional iron deficiency in dogs | MIP
71	Rico	Encephalitic alphavirus E1 glycoprotein-liposome-nucleic acid complexes protect mice from lethal challenge with multiple alphaviruses | MIP
72	Rotcheewaphan	The Mycobacterium leprae specific protein ML1419c functions as a diguanylate cyclase to produce cyclic-di-GMP | MIP
73	Ruggeri	Cytoskeletal alterations of equine oocytes that failed to cleave after ICSI: Evaluation of maternal and cell aging | BMS
74	Salmon	The effect of docosahexaenoic acid (DHA) on cardiac myocyte adiponectin protein expression | BMS
75	Schmitz	Community-academic partnership to reduce cardiovascular disease risk in Northern Colorado children with elevated cholesterol | ERHS
76	Schwartz	Pilot investigation of peripheral blood natural killer cell populations in colorectal cancer survivors | ERHS

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77	Sharif	Environmentally induced Copy Number Variation in diploid strains of the budding yeast Saccharomyces cerevisiae | ERHS
78	Shatila	Occupational Radiation Doses during Fluoroscopically Guided Procedures in a Hybrid Operating Room | ERHS
79	Shoup	Long-term behavioral consequences of fetal glucocorticoid excess | BMS
80	M. Smith	Dynamic changes in high affinity yet polyreactive B cells during development of human type 1 diabetes suggests pathophysiologic function | MIP
81	K. Smith	Hematologic immune markers and the relationship between disease, environment, and immunocompetence in French mountain ungulates | BMS
82	Steel	Dengue virus alters central carbon metabolism and induces a Warburg-like effect for successful replication | MIP
83	Stenkamp-Strahm	Predictive modeling indicates climate and pharmaceutical variables have an influence on shedding prevalence of dairy E.coli O157:H7 | CS
84	Stutzman-Rodriguez	Detection of antigenic proteins of Felis catus gammaherpesvirus 1 | MIP
85	Tangtrongsup	Effect of Toll-like receptor-induced inflammation on chondrogenesis of bone marrow-derived mesenchymal stem cell | CS
86	Tenne	Palatability and clinical effects of an oral recuperation fluid during the recovery of dogs with parvoviral enteritis | CS
87	Therio	Iron dysregulation is evident in canine hemangiosarcoma: preliminary studies | CS
88	Timmons	In Vitro Evaluation of the Effects of Pre-conditioning and Gender on Feline Adipose-derived Mesenchymal Stem Cell Cytokine Production | CS
89	Trundell	Intrauterine application of ciprofloxacin in the mare | CS
90	Trusiano	The effects of docosahexaenoic acid (DHA) on cardiac microvascular endothelial cell adiponectin production | BMS
91	Wanty	Effect of Azaperone on Blood Pressure in Immobilized Bull Elephants in Kruger National Park, South Africa | CS
92	Wennogle	Measurement of plasma fibrinogen in dogs with hepatobiliary disease | CS
93	West	Regulation of Human and Sheep Trophoblast Cell Differentiation by Lin28B | BMS
94	Woodard	Comparing transfection efficacy of superfect transfection reagent and electroporation in mesenchymal stem cells | CS
95	Yashari	Evaluation of a novel canine activity monitor for at-home physical activity analysis | CS
96	Yurkon	Ironed out: a new way to differentiate anemia etiologies | MIP
97	Zellar	H7N9 Influenza A virus Transmission in a Model Wet Market | BMS

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CONGRATULATIONS AGAIN TO

2014 RESEARCH DAY WINNERS!

ORAL PRESENTATIONS

FIRST BASIC	Alan M. Elder, Ph.D. student, MIP, "Determining the presence of blood-borne prions at various time points throughout infection." Mentor: Candace Mathiason
SECOND BASIC	Nathan Grubaugh, Ph.D. student, MIP, "West Nile virus population dynamics in wild-caught birds." Mentor: Gregory D. Ebel
FIRST CLINICAL	Kaitlin Curran, veterinary resident, CS, "CHOP versus LAP for treatment of CHOP-relapsed canine lymphoma." Mentor: Douglas Thamm
SECOND CLINICAL	Audrey Ruple-Czerniak, Ph.D. student, CMB, "Risk factors for the development of canine lymphoma in North American dogs: 18,826 cases (1990-2009)." Mentor: Paul Morley

POSTER PRESENTATIONS

FIRST	Kara Mosovsky, Ph.D. student, MIP, "Interferon-gamma enhancement of antibiotic activity against Burkholderia is mediated by induction of reactive oxygen species." Mentor: Steven Dow
SECOND	Jared Fowles, Ph.D. student, CMB, "Canine COXEN: cross-species genomic applications for predicting chemosensitivity in dogs." Mentor: Daniel Gustafson
THIRD	Aimee Ortega, Ph.D. student, MIP, "Prions in plants: potential assay for detection of PrPres in grasses from Rocky Mountain National Park." Mentor: Mark Zabel

GOLDEN PIPETTE AWARD – Department of Clinical Sciences

2015 CVMBS RESEARCH DAY ORGANIZING COMMITTEE

Brad Borlee, faculty chair, Microbiology, Immunology, and Pathology
Ashley Turnidge, Biomedical Sciences
An Dang, Biomedical Sciences
Dan Regan, Clinical Sciences
Shannon McLeland, Clinical Sciences
Rabab Sharif, Environmental and Radiological Health Sciences
Cory Sicard, Environmental and Radiological Health Sciences
Mike Mangalea, Microbiology, Immunology, and Pathology
Danielle Adney, Microbiology, Immunology, and Pathology
Sue VandeWoude, CVMBS Associate Dean of Research
Aimee Oke, committee coordinator, CVMBS College Office

VETERINARY SUMMER SCHOLARS PROGRAM

DVM STUDENTS DIVE INTO RESEARCH WITH PROJECTS AND FIELD TRIPS
APPLY BY 1 P.M. FEB. 6, 2015! / FOR MORE INFORMATION: HTTP://COL.ST/K7POL

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

VETERINARY STUDENTS IMMERSE THEMSELVES in research – and gain invaluable insights into career opportunities in veterinary medical research – through the Colorado State University Veterinary Summer Scholars Program. The application deadline is Feb. 6 for the summer 2015 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 successful program in summer 2015.

Last summer, 29 students from Colorado State's DVM Program and other veterinary schools participated in the CSU Veterinary Summer Scholars Program. They spent the summer working in laboratories on their own research with guidance from CSU faculty mentors. They also attended weekly research seminars and field trips to university, federal and state research facilities, getting an inside look at career opportunities in veterinary medical research.

Many research projects conducted by CSU students last summer are presented today at our 16th Annual Research Day.

Merial, a multinational animal health company, supports the program, along with several other organizations, the college, and faculty mentors who help to provide stipends for program participants.

We encourage students to apply for experiential learning in veterinary medical research!

BY THE NUMBERS

- 29 SCHOLARS in the 2014 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.
- 222 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 2014 PROGRAM:

Merial Limited

National Institutes of Health

Morris Animal Foundation

American Humane Association

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

American Veterinary Medical Association

YOUNG INVESTIGATOR GRANT PROGRAM:
funding research and boosting vet students

CENTER FOR COMPANION ANIMAL STUDIES, DEPARTMENT OF CLINICAL SCIENCES

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 2014, corporate and non-corporate sponsors donated more than $77,000 to the program. This funding was distributed to 26 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 eight years, the program has grown to support five times the number of research projects that it supported in its first year – a credit to sponsors who understand the importance of bolstering young scientists, and a credit to our DVM students for the impressive quality of their research efforts.

The College of Veterinary Medicine and Biomedical Sciences thanks all program sponsors. These supporters are helping to advance veterinary science while also involving more DVM students in important clinical research.

To view the grants funded in 2014 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

Colorado State veterinary student Robert Cerda, shown examining a puppy at the James L. Voss Veterinary Teaching Hospital, is part of a research team whose study of urine protein in dogs was funded by the Young Investigator Grant Program. He is participating in 2015 Research Day.

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Novartis Animal Health

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Canine Rehabilitation Institute
International Veterinary Seminars
Royal Canin

ORAL PRESENTATIONS Clinical Science

1:00–5:00 PM | SESSION I - SALON I

BARTONELLA SPP. PCR ASSAY RESULTS USING CEREBROSPINAL FLUID OF NATURALLY EXPOSED DOGS WITH CENTRAL NERVOUS SYSTEM DISEASE

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

Among the multitude of infectious agents known to cause focal or multifocal central nervous system dysfunction in dogs, the role of *Bartonella* spp., including *B. henselae*, *B. vinsonii* subsp. *berkhoffii,* and *B. clarridgeaie*, in clinical diseases has not been widely explored. 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 in an endemic area. CSF samples from 56 pure or mixed breed dogs were submitted by the Washington State University Neurology Department to Colorado State University between February 2012 and August 2014 for evaluation in a *T. gondii* PCR assay. The CSF samples were stored at -80C until evaluated in this study. Dogs with focal and multifocal neurologic dysfunction and CSF pleocytosis (total nucleated cell count >5 nucleated cells/µl and RBC count <4,000 cells/µl) were included. The CSF was 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. Seventeen dogs met the inclusion criteria, none of which was positive for *Bartonella* spp. DNA in CSF. Of the other 39 CSF samples, one was positive for *B. henselae* DNA. The CSF from this dog contained red blood cells (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 of dogs must be interpreted in light of RBCs within the sample as well as the presence of inflammation or systemic infection.

CANINE B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA SHOWS STRONG BREED-SPECIFIC RISK

Julia L. Bromberek, Michael R. Agnew, Janna Yoshimoto, Paul S. Morley, and Anne C. Avery

Background: B cell chronic lymphocytic leukemia (CLL) is the most common human hematopoietic malignancy in the West. The primary risk factor for developing this disease is genetic, with first-degree relatives of B cell CLL patients at a substantially higher risk. Dogs also develop B cell CLL, but there is no systematic description of the canine disease. Understanding of the epidemiology of canine B cell CLL will help practitioners recognize this disease, and position the dog as a model for future genetic studies. Purpose: To describe canine B cell CLL patient presentation, clinical signs, and breed predisposition. Methods: Retrospective cross-sectional study of dogs for which samples were submitted to the Colorado State University Clinical Immunology laboratory for immunophenotyping between September 2010 and June 2014. Four hundred ninety-one dogs with B cell CLL were identified. Breed distribution of dogs with B cell CLL was compared to those with other lymphoproliferative disorders using logistic regression. Results: The median age at diagnosis of B cell CLL was 11 years old with no sex predilection. Half of the dogs presented with peripheral lymphadenopathy or splenomegaly, and 26% with anemia. Eleven small breed dogs had significantly increased odds of B cell CLL. In addition, English Bulldogs had an increased risk and a unique presentation: these dogs were diagnosed at a median of 6 years of age and expressed significantly lower levels of class II MHC and CD25. Conclusions: Canine B cell CLL appears to be overrepresented in small breed dogs. Future genetic studies focusing on these breeds may be useful in localizing genetic risk factors. In addition, the unique presentation of English Bulldogs provides evidence that there may be more than one form of this disease. Further studies are necessary to determine whether presenting signs are associated with survival.

SINGLE PARALUMBAR FOSSA LAPAROSCOPY FOR ELECTIVE OVARIECTOMY IN STANDING MARES

Aimee C. Colbath, Dean A. Hendrickson, Eileen S. Hackett

Purpose: The purpose of this study was to describe a novel technique of bilateral ovariectomy performed through a unilateral left paralumbar fossa approach. Materials/methods: Healthy, adult, mares were presented for bilateral ovariectomy due to undesirable estrus behavior. Mares were placed in stocks and sedated with intravenous detomidine hydrochloride and butorphanol tartrate. Four portal sites were utilized in the left paralumbar fossa. The first portal was made midway between the tuber coxae and the last rib at the level of the distal tuber coxae. The second and third portals were made 5-10cm craniodorsal and caudoventral to the first portal. In horses 1-4, the final portal was made just distal to the first portal. In horses 5-8, the final portal was made within the 17th intercostal space at the level of the middle of the tuber coxae. Both ovaries were amputated and removed from the left paralumbar fossa. Following surgery, horses were given four weeks convalescence. Follow-up conversations with the owners were performed between 2 weeks and 6 months post-surgery. Results: Eight mares were enrolled into the surgical study. Mares ranged from 5-17yo (median: 14.5yo). The median height of mares was 15hands, and median weight was 431kg. All ovaries were removed successfully with no complications experienced during surgery. Short-term complications were experienced in 2 horses and included anorexia, azotemia and hypertriglyceridemia in a single mare, and fever and neutropenia in a single mare. All owners were able to be contacted between 2 weeks and 6 months post-surgery. No complications occurred following discharge from the veterinary hospital, and all clients were satisfied with the outcome of the procedure. Conclusion: A unilateral, single left paralumbar approach is a novel technique for bilateral ovariectomy in the mare with few post-operative complications.

INVESTIGATION OF GASTROINTESTINAL MOTILITY IN DOGS UNDERGOING PROPHYLACTIC LAPAROSCOPIC GASTROPEXY

Kristin A. Coleman, Eric Monnet, Pedro Boscan, Leah E. Ferguson, David C. Twedt, Kristy Dowers

Previous studies regarding therapeutic and prophylactic gastropexy for gastric dilatation-volvulus (GDV) syndrome have raised concerns about the effect of gastropexy on gastric motility. Recently, the trend for prophylactic gastropexies has been towards minimally invasive techniques, such as the total laparoscopic gastropexy. The objective of the study presented here was to assess possible changes in gastrointestinal motility in normal dogs undergoing prophylactic laparoscopic gastropexy. Prophylactic gastropexy was performed via laparoscopy with the aide of barbed unidirectional suture and an Endostitch™ device in nine otherwise healthy client-owned dogs. Three weeks after initiating a standardized GI-health kibble diet, each dog was administered a canine-validated wireless motility capsule (WMC), a SmartPill™ device, to evaluate pre-operative GI motility. The SmartPill™ is capable of measuring GI motility based on the changes in pH, pressure, and temperature throughout the entire GI tract, which was transmitted to a receiver worn by the patient. The data recorded by this receiver included pressure measurement, frequency of contractions, motility index, temperature, and total transit time (including gastric emptying time), as well as minimum, median, and maximum pH. A second SmartPill™ device was administered to evaluate post-operative GI motility approximately three weeks after surgery while continuing the standardized diet. Following data and statistical analyses, no significant differences in mean or median (+/- SD) pH, pressure measurements, contraction frequency, motility index, and total time of passage within the stomach, pylorus, and small intestines were found between pre- and post-operative measurements. Shapiro-Wilk test was used to assess normality, and each parameter was analyzed using Wilcoxon Signed Rank test for matched pairs comparison before and after surgery. Therefore, no statistically significant differences were found between the two measurements when using each dog as its own control. In conclusion, gastrointestinal motility does not change in healthy dogs undergoing prophylactic laparoscopic gastropexy.

IMPROVED CHARACTERIZATION OF SALMONELLA ENTERICA SHEDDING AMONG REPTILE PATIENTS AT THE JAMES L. VOSS VETERINARY TEACHING HOSPITAL

Anna C. Fagre, Brandy A. Burgess, Matthew Johnston, Kristy L. Pabilonia, and Paul S. Morley

In the U.S., an estimated 6% of approximately 1.2 million sporadic human *Salmonella enterica* infections annually are attributed to reptile or amphibian exposure. Reptiles continue to be relatively popular pets posing a significant public health risk. Sample collection and isolation poses several challenges, including difficulty in patient handling, intermittent shedding, and insensitive culture methods. The goal of this study was to improve the characterization of *Salmonella* shedding among reptilian patients, determining shedding prevalence, assess sampling methods, and compare sensitivity of detection methods. A cross-sectional study of reptile and chelonian patients was undertaken at the CSU-VTH. Briefly, patient samples included, 1) cloaca swab cultured in TET for 18hrs at 43°C and plated on XLT4 for 18hrs at 43°C; 2) a body Swiffer sample (sampling approximately 80% of the body surface including dorsum, ventrum and feet) pre-enriched in BPW for 18hrs at 43°C, then passed into TET for 18hrs at 43°C, then plated on XLT4 as above. In addition, environmental Swiffer samples were also collected before and after exam table cleaning and disinfection, pre-enriched in BPW for 18hrs at 43°C, then passed into TET for 18hrs at 43°C, then plated on XLT4 as above. All samples were also tested via PCR and, for each body Swiffer sample, tested using a lateral flow immunoassay. Approximately 45 reptiles and chelonians that were at least 30 grams by weight were enrolled in this study. Estimated shedding prevalence was much lower than expected at 13.6% (3/22). In general, there was poor agreement among testing methodologies. Reptiles and chelonians present a potential source for environmental contamination and infections among animals and people. The results of this study provide evidence that will allow for prevention efforts to be tailored to the hospital as well as home environment.

INTRA- AND INTEROBSERVER VARIABILITY OF GFR RATE DETERMINATION IN CATS WITH CHRONIC KIDNEY DISEASE VIA GAMMA CAMERA UPTAKE OF TC-99M-DTPA

Amy E. Felumlee, Angela J. Marolf, Elissa K. Randall, Annette M. Bachand, Jessica M. Quimby

The measurement of glomerular filtration rate (GFR) via gamma camera uptake of 99mTc-diethylenetriamine pentaacetic acid (DTPA) is a useful way to evaluate renal function in cats. Drawing of kidney and background region of interest (ROI) is a major source of variability. The aim of this study was to determine the intra- and interobserver variation in the calculation of GFR and to determine whether the renal insufficiency classification of each cat changed between the same or different observers. A total of 29 GFR studies performed on 16 cats at CSUVTH between 2010-2013 were examined. Each study was read twice, 6 months apart with two measurements per reading. The global GFR value was calculated for each reading and the cat was classified as having normal function, subclinical renal insufficiency, or clinical renal insufficiency. Intra- and interobserver reliability was evaluated using the % limit of agreement. Changes in clinical classification were determined between readings and observers. Independent of observer or reading, there is a 95% chance that the second GFR measurement falls within 10-20% of the first. Based on average measurements, experienced radiologists have a 95% chance of reporting a second reading within 13% of the first; for non-experienced observers a learning effect may take place between readings resulting in a higher % limit of agreement (40%). For each reading, the average GFR values reported by different experienced radiologists have a 95% chance of falling within 15-20% of each other. Comparing the average GFR value reported by an experienced radiologist to that of a non-experienced observer, the % limit of agreement is higher (35%). Results show high intra- and interobserver reliability among experienced radiologists; for non-experienced observers reliability may be affected by a learning effect. Variations in GFR measurements will rarely lead to differences in patient clinical classification.

CARBON DIOXIDE INDUCED PULMONARY HEMORRHAGE

Suhrim Fisher, Winona L. Burgess, Kenneth D. Hines, Gary L. Mason and James R. Owiny

Carbon dioxide (CO2) is the most commonly used method of euthanasia for rodents. AVMA Guidelines recommend CO2 displacement rate of 10-30% per minute and recommend against placing conscious animals in prefilled chambers. An investigator reported pulmonary hemorrhagic lesions in Balb/c mice euthanized with slow-fill method that were not previously observed with prefilled method. This study aims to determine whether or not slow-fill CO2 euthanasia method induces pulmonary lesions in Balb/c and C57Bl/6 mice compared to prefilled method. In a pilot study, 6 week old Balb/c mice (n=6) and C57BL/6 (n=4) were euthanized using either slow-fill method or prefilled method followed by cervical dislocation. All procedures were approved by the IACUC. Tissues (lung, heart, brain, liver, spleen, kidneys, nasal turbinate, muscle and sexual organs) were collected for gross and histological evaluation. Results showed that three Balb/c mice euthanized with slow-fill method had extensive pulmonary hemorrhage while no hemorrhage was noted in three Balb/c mice euthanized with prefilled method. One of two C57Bl/6 mice euthanized with slow-filled method had mild pulmonary hemorrhage while no hemorrhage was noted in two C57Bl/6 mice euthanized with prefilled method. Three Balb/c mice euthanized with slow-fill method had mild to moderate nasal hemorrhage while one of three Balb/c mice euthanized with prefilled method had mild nasal hemorrhage. Two C57Bl/6 euthanized with slow-fill method had mild to moderate nasal hemorrhage while one of two C57Bl/6 mice euthanized with prefilled method had mild nasal hemorrhage. No gross or histologic lesions were noted in other organs. Based on these findings, we have concluded that slow-fill CO2 euthanasia method induces pulmonary and nasal hemorrhage in Balb/c mice compared to prefilled method. In C57Bl/6 mice, there were insignificant and inconsistent hemorrhagic responses. Further studies are planned to validate whether the lesions are attributable to chamber filling rate or due to strain differences.

NAVY BEAN POWDER DIETS MODULATE SERUM CHOLESTEROL AND FECAL LIPID METABOLITES IN HEALTHY COMPANION DOGS

Genevieve M. Forster, Adam L. Heuberger, Corey D. Broeckling, Kelly S. Swanson, John E. Bauer, and Elizabeth P. Ryan

Common beans (*Phaseolus vulgaris*, L.) have been shown to beneficially alter serum lipids in humans and lab animals. We have shown that common beans are safe and digestible for dogs and modulate serum lipids in overweight dogs undergoing calorically restricted weight loss. The objective of this study was to determine the effects a navy bean diet on the fecal metabolome of healthy dogs and the phytochemical diversity of the diets. Twenty-one healthy adult, male and female, companion dogs completed a 28 day, randomized, double-blinded, controlled, dietary intervention and consumed either a 25% weight/weight cooked navy bean powder (n=10) or control (n=11) diet. Physical exams, urinalysis, and serum biochemistry panels were used to assess the health of each dog throughout the study. Aqueous-methanolic extracted diet and fecal samples were analyzed using non-targeted GC-MS at the Proteomics and Metabolomics Core Facility. Nutrient profiles of each diet were also assessed. Serum cholesterol was significantly lower in navy bean group compared to control group ($p < 0.05$). Bile acid excretion decreased by 6% in the control group and 43-48% in the navy bean group. Fecal excretion of three steroid-like compounds increased in the navy bean group by 1.3-9 fold. Targeted diet analysis revealed that soluble fiber content was higher in the navy bean diet compared to control diet (4.69% and 3.68%, respectively). In the navy bean diet fructose was 15% higher and sucrose was 60% higher, compared to control. These results support that common bean intake may have similar effects on lipid and carbohydrate metabolism in healthy dogs as has been demonstrated in people and that bean modulation of serum lipids may be associated with fecal elimination of steroid like compounds. Further evaluation of fecal steroids and bile acids are needed to determine the mechanisms by which beans modulate serum lipids in dogs.

ASSESSMENT OF NOVEL DIGITAL AND SMARTPHONE GONIOMETERS FOR JOINT ANGLE MEASUREMENT OF THE CANINE STIFLE

Kristin A. Freund, Nina R. Kieves, Juliette L. Hart, Sasha A. Foster, Unity Jeffery, Felix M. Duerr

Objective: To evaluate accuracy and precision of three novel goniometers in comparison to the universal goniometer (UG) in the canine stifle. Design: Cadaveric study Animals: 8 canine cadaver hind limbs Procedures: Reliability with concurrent validity of stifle goniometry measured with two smartphone-based applications, iHandy Carpenter (iHandy) and DrGoniometer (DrG), and a novel digital goniometer (HALO). Stifle joint angles were compared to angles obtained with the UG and radiographic stifle joint angle measurements. Cadaveric limbs were secured to a wooden platform with bolts applied through the tibia and femur in three random stifle angles. Three evaluators performed all measurements in triplicate resulting a total of 864 measurements. Coefficient of variation (CV), correlation coefficient (R), bias, and total error were calculated Results: CV was lowest for UG (4.88), followed by DrG (7.37), iHandy (7.57) and HALO (12.71). R was highest for UG (0.97) followed by DrG (0.93), iHandy (0.90) and HALO (0.78). Constant bias was present for all devices except DrG. The UG and iHandy exhibited positive constant bias; HALO exhibited negative constant bias. Total error observed at 50 and 100 degrees was greater than 15% for all devices. Conclusions and Clinical Relevance: The UG was the most accurate and precise of the devices tested. None of the devices accurately represent radiographically measured stifle joint angles. Additional veterinary studies are indicated prior to the use of novel goniometers. Further investigation into a superior goniometer for the veterinary market is warranted.

COMPARISON OF OWNER SATISFACTION WITH STIFLE ORTHOSES OR TIBIAL PLATEAU LEVELING OSTEOTOMY FOR THE TREATMENT OF CRANIAL CRUCIATE LIGAMENT DISEASE

Juliette L. Hart, Kimberly D. May, Nina R. Kieves, Patsy Mich, Clara S..Goh, Ross H. Palmer, Felix M. Duerr

PURPOSE: Owner-reported online survey to compare pet-owner satisfaction following surgical and non-surgical (orthosis) treatment of canine cranial cruciate ligament disease (CCLD). MATERIALS & METHODS: Dogs receiving either a custom-made, hinged stifle orthosis (ORTHOSIS) or tibial plateau leveling osteotomy (TPLO) for the management of naturally occurring CCLD. RESULTS: The response rate for the ORTHOSIS group was 25% (n = 203/819) and 40% for the TPLO group (n = 76/203). Greater than 85% of owners in both groups replied they would repeat the chosen treatment again if they were given the choice (P = 0.32). A greater number of dogs in the TPLO group showed no/mild lameness following intervention (98%, n = 62/63, P = 0.04) compared to the ORTHOSIS group (88%, n = 142/161). A larger number of owners in the TPLO group rated the treatment as excellent (TPLO: 68%, n = 43/63; ORTHOSIS: 41%, n = 68/167, P = 0.003). Forty-four percent of owners in the ORTHOSIS group (n = 73/166) reported skin issues. Thirteen percent of dogs in the ORTHOSIS group (n = 21/167) subsequently underwent a surgical procedure on the treated leg and eight percent (n = 14/167) reported their dog never adjusted to the orthosis. CONCLUSION: In this study population, a high percentage of owners reported positive satisfaction with surgical and non-surgical treatment of CCLD. However, pet owners selecting functional stifle orthoses should be advised of the potential for complications including persistent lameness, skin issues, non-acceptance of the orthosis, and the possible need for subsequent surgical intervention.

EFFECT OF INTRAVENOUS FLUID VOLUME ADMINISTRATION ON DEVELOPMENT OF POST-OPERATIVE ILEUS IN HORSES WITH COLIC

Elizabeth L Hoaglund, Diana M Hassel, Ann M Hess

Aggressive intravenous fluid therapy is a routine practice in horses with disease of the gastrointestinal tract, particularly those that develop ileus of the small intestine with generation of large volumes of gastric reflux. Recent studies in critically ill humans have demonstrated that excessive fluid administration is associated with increases in patient morbidity and mortality. We propose that current fluid therapy protocols in horses may result in prolongation of ileus and increased duration and cost of hospital stay. This retrospective study aims to evaluate cases of ileus in horses undergoing surgery for gastrointestinal disease with development of ileus post-operatively with matched controls between the years of 2004 and 2012. We examined signalment, electrolyte concentrations, electrolyte supplementation type and quantity, the duration and severity of ileus, the cost of hospitalization, survival outcome and volume of fluid therapy taking into account maintenance requirements, hydration status, and ongoing losses. Our results indicate that horses that developed ileus in the post-operative period were significantly more likely to have received less than 10 liters of net fluids for 1 or more days of hospitalization than matched controls without ileus (P<0.01). However, they received a significantly larger volume of intravenous fluids during anesthesia and net fluids on days 3 and 4 post-operatively (P<0.03). No association was found between electrolyte supplementation and development of ileus. Horses that developed ileus were approximately 4 times more likely to develop complications and to receive lidocaine as a component of therapy (P<0.01). The odds of developing ileus was 7-fold higher in horses undergoing repeat celiotomy and 8-fold higher in horses undergoing resection and anastomosis (P<0.01). PCV at presentation was significantly higher in horses that developed ileus (P<0.02). Results of this study fail to support our hypothesis that excess fluid administration and decreased electrolyte concentrations lead to an increased risk of ileus.

THE ESTRUS CYCLE INFLUENCES THE BACTERIAL MICROBIOME IN THE NORMAL MARE REPRODUCTIVE TRACT

Steven M Lakin, Bradley R Borlee, Patrick M McCue, Ryan A Ferris

Understanding normal fluctuations in a patient's bacterial flora is essential to understanding the pathogenesis of bacterial disease. This study aims to describe the microbiome of the healthy mare reproductive tract and the changes in bacterial flora between estrus and diestrus. Mares characterized as estrus or diestrus (n = 6) were sampled from the clitoral fossa, vaginal vestibule, vaginal vault, and uterus using guarded swabs and uterine low volume lavage. DNA from the resulting samples was extracted and prepared for paired read sequencing on the Illumina MiSeq using the 16S gene v4 region protocol established by Patrick Schloss (Kozich et al. 2013). Analysis was performed using mothur and the DESeq and phyloseq packages for R. Bacterial diversity and abundance were highest in the clitoral samples and decreased cranially for both estrus and diestrus mares. Bacterial richness and evenness were higher in the vaginal vault of the estrus mares than the diestrus mares. The samples clustered into either cranial or caudal locations based on Dirichlet multinomial models established by Holmes et al. 2012. The samples were more often significantly differentiated by location than by estrus status based on concordance from alpha and beta diversity measurements and a variety of non-parametric statistical tests (e.g. UniFrac, AMOVA/HOMOVA, principle component analysis, non-metric multidimensional scaling). Bacterial classifications most closely resembled fecal microflora in the caudal reproductive tract. These results suggest that while mares in estrus and diestrus have similar bacterial profiles throughout the reproductive tract, bacterial abundance and diversity is higher in the cranial reproductive tract of the estrus mares than the diestrus mares. This could be clinically significant, as most of the bacteria isolated from uterine infections are also found in the intestinal microbiome, and this study shows that these bacteria are found more cranially in the reproductive tract of estrus mares.

ASSESSING PAIN AND TRAUMA IN EARLY LACTATION DAIRY CATTLE

Emma L Lantz, Craig S McConnel, Jessica A McArt, Franklyn B Garry

Pain can be a difficult vital sign to measure. Objective measurements are often expensive and/or impractical, particularly when working with populations. The goal of this study was to investigate the use of subjective variables to assess pain and trauma in dairy cattle during early lactation – their most stressful life stage. We developed a scoring system based on 11 behavioral and appearance indicators for cows one to sixteen days in milk (DIM). We validated subjective pain scores using serum creatine kinase (CK) and haptoglobin (Hpt) quantified as biochemical markers of muscle damage and inflammation. Blood samples were drawn from each subject up to four times during this period (1-3 DIM=A, 4-6 DIM=B, 7-10 DIM=C, 11-16 DIM=D). Records of daily milk yield, illness, and removal from the herd were also examined. An average of eight subjective assessments were completed per individual for a total of 752 records and 313 blood samples on 94 cows. For each range (A-D), 44-49% of the animals scored zero when subjective indicators were added. However, at least one variable was abnormal at some point during the first 16 DIM for all but eight subjects. In addition, CK and Hpt concentrations exceeded standard reference ranges. In spite of this, quantification of these biomarkers may still provide insight. Using a cut off based on risk factors for metritis (80mg/dl), milk weights for cows with high Hpt in our study were consistently lower than those with low Hpt. In animals with a subjective pain score greater than zero, CK was higher for range A (p-value = 0.07), while Hpt was elevated in ranges A (p-value = 0.11), C (p-value = 0.06), and D (p-value = 0.01). We will ultimately develop a matrix of subjective factors that correlate with objective measurements and serve to predict adverse production outcomes.

CATIONIC LIPOSOMES-OLIGONUCLEOTIDE COMPLEXES AS AN ALTERNATIVE ADJUVANT FOR POLYCLONAL ANTIBODY PRODUCTION IN RABBITS

Erin S.Lee, Jake E Moskowitz, Valerie A Johnson, Elisa French, Lon V Kendall

Rabbits are routinely used for polyclonal antibody production and the most common adjuvants used are Freund's and TiterMax. While Freund's adjuvants induce robust antibody responses there are some animal welfare concerns related to the granulomas complete Freund's adjuvant causes. TiterMax is an alternative adjuvant to induce antibody response without animal welfare concerns, but the antibody response may not be as robust as Freund's adjuvant. Cationic liposome-oligonucleotide complexes (CLDC) are potent activators of the immune response without reported animal welfare concerns. This study assessed the antibody response to CLDC compared to Freund's adjuvant and TiterMax. Eight rabbits were immunized subcutaneously with ovalbulin in either complete Freund's adjuvant (CFA, n=2), TiterMax (n=3) or CLDC (n=3). Two weeks after immunization, blood was collected for antibody responses and rabbits were given a booster immunization with incomplete Freund's adjuvant, TiterMax or CLDC. Additional blood samples were collected two weeks after the booster immunization. An enzyme linked immunosorbent assay was performed to assess antibody responses based on optical density values. Although there was no statistical differences, CFA resulted in a higher antibody response than TiterMax and CLDC at two week post immunization, while CLDC resulted in a higher antibody response following the booster immunization. Injection site swellings were noted in the CFA groups, and mild bruising in the TiterMax and CLDC groups. One drawback to the use of CLDC is the ability to reconstitute the antigen in an appropriate volume for injection which resulted in a 5 ml immunization for the rabbits due to the charge of the antigen. These results suggest that CLDC may be used as an alternative adjuvant to produce polyclonal antibodies with mild clinical side effects similar to TiterMax; however, antigens with negative charges are more easily formulated with CLDC.

CANINE GAIT ANALYSIS USING INERTIAL MEASUREMENT UNITS DURING OUTDOOR AND TREADMILL ACTIVITY

Kyle W Martin, Theresa M Wendland, Molly A Vitt, Wayne J Board, Colleen G Duncan, and Felix M Duerr

Introduction – Conventional gait analysis systems require a dedicated lab space, expensive equipment, and adequate time for data acquisition. Since data acquisition with these systems is limited to the gait lab, it does not allow for evaluation of dogs in a natural environment. Inertial measurement units (IMUs) have the potential to address these issues. We assessed stride length using wireless IMUs in normal dogs outdoors and on a treadmill, as stride length has been shown to be a reliable indicator of orthopedic pathology in dogs. We hypothesized that there would be no difference between stride outdoors and on a treadmill. Materials & Methods – Data was collected outdoors while dogs were trotted on a lead and on a treadmill at a consistent trotting velocity. The mean stride length for each dog was compared using correlation coefficients and a Paired T-test stratified by thoracic vs. pelvic limbs. Results – The stride length obtained outdoors and on the treadmill was highly correlated for both the thoracic and pelvic limbs, however, it was significantly longer on the treadmill than outdoors. Discussion/Conclusions – IMUs can be used to acquire stride length for dogs in a natural outdoor setting. This may provide a rapid method of objective outcome measurement that is highly correlated with stride length under controlled velocity. Contrary to our hypothesis, stride length on the treadmill was significantly longer than the stride length obtained outdoors. This finding may be a result of a true change in canine gait associated with the treadmill or due to a difference in velocity between the two settings. We cannot rule out the latter since real-time GPS data only estimates the velocity, which may have resulted in an overall slower speed outdoors. Investigation and validation of IMUs for the acquisition of further kinematic data is currently ongoing.

ORAL PRESENTATIONS Basic Science

1:00–5:00 PM | SESSION II - SALON II

EFFICIENT REPLICATION AND SHEDDING OF MERS COV FROM THE UPPER RESPIRATORY TRACT OF EXPERIMENTALLY INFECTED DROMEDARY CAMELS

Danielle R Adney, Neeltje van Doremalen, Vienna R Brown, Trenton Bushmaker, Dana Scott, Emmie de Wit, Vincent J Munster and Richard Bowen

The Middle East respiratory syndrome coronavirus (MERS CoV) is a novel coronavirus first recognized in 2012 and is associated with severe respiratory disease in humans. Virus has been isolated from dromedary camels in endemic areas, and many camels also have neutralizing antibodies against the virus, suggesting that they are likely a reservoir host. In order to better understand the role of camels in virus transmission we experimentally infected 3 adult, male dromedary camels with a human isolate of MERS CoV. All animals developed a transient, upper respiratory tract infection associated with very minor clinical disease. Large quantities of infectious virus were isolated from nasal secretions from each animal through 7 days post-inoculation, and viral RNA was detected much longer. Although our study design was limited to 3 animals, these data indicate that MERS CoV readily infects camels, which shed large amounts of virus and likely can efficiently transmit virus to other camels and humans.

NEURON-SPECIFIC (PRO)RENIN RECEPTOR DELETION REGULATES RENIN-ANGIOTENSIN COMPONENTS AND CONTRIBUTES TO THE ATTENUATION OF DOCA-SALT HYPERTENSION

Asghar Ali, Wencheng Li, Michelle N Sulllivan, and Yumei Feng

We previously reported that neuronal deletion of the (pro)renin receptor (PRR) attenuates DOCA-salt hypertension, but the underlying mechanisms are unclear. To test our hypothesis that changes in renin-angiotensin system (RAS) components contribute to beneficial effects of PRR deletion during DOCA-salt hypertension. Wildtype (WT) and neuron-specific PRR knockout (PRRKO) mice underwent SHAM or DOCA (50 mg) pellet implantation and were given 0.9% NaCl water. After 3 weeks, real time PCR was performed on the paraventricular nucleus of the hypothalamus to compare mRNA levels of angiotensin II type 1 or type 2 receptors (AT1R, AT2R), Mas receptors (MasR), and angiotensin-converting enzyme 2 (ACE2). In SHAM mice, PRR deletion increased AT2R (1.46 ± 0.03 vs. 1.0 ± 0.04), MasR (2.14 ± 0.07 vs. 1.0 ± 0.05), and ACE2 (1.25 ± 0.01 vs. 1.0 ± 0.05) mRNA levels compared with WT mice, but no difference in AT1R expression was observed (1.26 ± 0.24 vs. 1.0 ± 0.07). DOCA-salt treatment increased AT1R (2.33 ± 0.19) but lowered ACE2 mRNA levels (0.69 ± 0.03) compared with SHAM treated WT mice. There was a slight, but statistically insignificant, decrease in AT2R (0.82 ± 0.12) and MasR (0.83 ± 0.06) mRNA following DOCA-salt treatment compared to SHAM in WT mice. Importantly, neuronal PRR deletion attenuated increases in AT1R (1.59 ± 0.20) and normalized the reduction of ACE2 mRNA levels (0.90 ± 0.04) compared with WT following DOCA-salt treatment. However, AT2R and MasR mRNA levels remained lower in DOCA-salt treated PRRKO relative to WT mice. In summary, neuron-specific PRR deletion improves the balance of vasoconstrictory and vasodilatory arms of the RAS that may contribute to the attenuation of DOCA-salt hypertension.

EVALUATION OF PATHOGENESIS AND IMMUNE RESPONSE OF FRANCISELLA TULARENSIS IN COTTONTAIL RABBITS

Vienna R Brown, Danielle R Adney, Helle Bielefeldt-Ohmann, Richard A Bowen

Francisella tularensis is a highly virulent, zoonotic bacterium that causes significant natural disease and is also of concern as an organism for bioterrorism. Serologic testing of wildlife is frequently used to monitor spatial patterns of infection and quantify exposure. Cottontail rabbits are a natural reservoir for this bacterium in the U.S., although very little work has been done experimentally to determine how these animals respond to infection; thus, information gathered from field samples can be difficult to interpret. The objective of this study was to provide an initial characterization of clinical disease, bacteremia, pathology, and antibody kinetics of Desert cottontail rabbits (*Sylvilagus audubonii*) experimentally infected with five strains of *F. tularensis*. We infected rabbits with four field strains, including MA00-2987 (A1b strain), WY96-3418 (type A2), KY99-3387 and OR96-0246 (type B strains), and with SchuS4 (type A1a strain), a widely used, virulent laboratory strain. The results clearly indicate that infection with different strains of the bacterium resulted in significantly varied patterns of disease as well as gross and histopathology. We also characterized long term humoral immune responses and the ability of cottontail rabbits to clear infection of type B strains of *F. tularensis*. Furthermore, we found that previous infection with a type B strain afforded partial protection against challenge with a virulent type A strain. Understanding *F. tularensis* infection in a natural reservoir species can guide sero-surveillance projects as well as generate new insights into environmental maintenance of this pathogen.

WOLF (CANIS LUPUS) POPULATION DYNAMICS AND TAENIID CESTODE PREVALENCE IN ISLE ROYALE NATIONAL PARK, MICHIGAN, USA

Jacey R Cerda, Danielle Buttke, Leah Vucetich, John Vucetich, Rolf Peterson, and Lora R Ballweber

Taeniid cestodes (i.e., *Taenia, Echinococcus*) are present in wild canid populations throughout North America; however, very little is known about the influence of host population dynamics and ecology on the biology of taeniid cestodes in wolves, particularly given the pressures of a changing landscape. This is especially true of the zoonotic tapeworm genus *Echinococcus*. To address these data gaps, this study examines the prevalence of taeniid cestodes, with a particular focus on *Echinococcus* spp., in the wolves of Isle Royale National Park (ISRO). From 1999 to 2014, complete demographic, pack, and genetic data were collected from the wolves in ISRO, along with >300 fecal samples. This long-term data provides a unique opportunity to evaluate the influence of host population dynamics on parasite transmission over time. Analysis of 141 fecal samples collected from 70 wolves from 2004 to 2014 found taeniid eggs in 28 (40%) individual animals. Five of these individuals were infected for more than one year, but no more than two years. Four individual wolves were infected with *Taenia* spp. *Echinococcus canadensis* was confirmed in 7 individuals (6 with G8 genotype, 1 with G10 genotype) and *Taenia hydatigena* was confirmed in 1 individual. Chi-square analysis of results to date showed a significant difference in prevalence between the year 2006 and the years 2004 and 2008, but no other year pairs were significant. This research serves as a platform for further investigations to determine the role that ecological factors, as well as host population genetics and dynamics have on the distribution, prevalence, and public health consequences of *Echinococcus* spp. and other taeniid cestodes in North America.

DENGUE VIRUS INFECTION INDUCES LIPID ALTERATIONS IN THE AEDES AEGYPTI MOSQUITO VECTOR

Nunya Chotiwan, Irma Sanchez-Vargus, Barbara Andre, Jeffrey M Grabowski, Amber Hopf-jannasch, Victoria Hedrick, Erik Gough, Ernesto Nakayasu, Devika Sirohi, Catherine A Hill, Richard J Kuhn and Rushika Perera

Aedes mosquitoes are the essential vector for transmitting dengue virus (DENV) among human populations. Unlike in humans, DENV infects and replicates in the mosquito without causing detrimental effects indicating that the virus-host interactions benefit both vector survival and virus replication. Our study has explored global metabolic changes during early and late infection in the *Aedes aegypti* midgut and salivary glands upon DENV2. The expression of the majority of membrane building blocks are suppressed early during infection but enhanced during the active replication phase in both the midgut and salivary glands. Interestingly, molecules involved in cellular signaling, cell survival and the stress response are either highly abundant or highly suppressed during active virus replication in both tissues indicating an active response of the mosquito vector to infection and possibly virus manipulation of the host. We also observed that expression patterns of energy storage lipids and intermediates in lipid metabolic pathways are different between midgut and salivary glands. These data suggest that the virus might alter the metabolic environment of the two tissues differently.

REACTIVE OXYGEN SPECIES MODULATE LOCAL L-TYPE CALCIUM CHANNEL SIGNALING IN GONADOTROPES

An K Dang, Dilyara Murtazina, Christianne Magee, Amy M Navratil, Colin M Clay, Gregory C Amberg

The binding of gonadotropin-releasing hormone (GnRH) to its receptor on gonadotrope cells in the pituitary initiates signaling cascades that result in ERK activation and enhanced luteinizing hormone biosynthesis. Previously, we have directly visualized GnRH-induced Ca^{2+} influx ("Ca^{2+} sparklets") mediated by L-type Ca^{2+} channels necessary for ERK phosphorylation. We now want to examine molecular events that reside between the activation of the GnRHR and these biologically relevant localized subplasmalemmal Ca^{2+} signals. Reactive oxygen species (ROS) are recognized as cognate signaling molecules that regulate cell function, but has not been examined in gonadotropes. We hypothesize that ROS play a role in GnRH signaling and local L-type Ca^{2+} channel function. To test our hypothesis, we first determined if GnRH receptor stimulation increased ROS production. Using TIRF microscopy and a cell-permeant ROS indicator (DCF; 1μM) to monitor subplasmalemmal fluorescence, acute stimulation of the GnRH receptor (with GnRH; 3nM) produced localized ROS "puncta" in gonadotropes. If ROS visualized near the plasma membrane modulate the activity of nearby L-type Ca^{2+} channels, then application of exogenous ROS should result in an increase in Ca^{2+} sparklets. Consistent with a stimulatory role, exposing gonadotropes to hydrogen peroxide (H2O2;100 μM) increased Ca^{2+} sparklet activity within 5min (control n*P*s=0.013±0.005, H2O2 n*P*s=0.30 ± 0.07; $P<0.05$, n=7). We next examined if endogenous ROS generators (e.g. NADPH oxidase) are involved with GnRH-dependent activation of L-type Ca^{2+} channels. To test the involvement of NADPH-derived ROS, we pharmacologically inhibited NADPH oxidase activity with apocynin (25μM pretreatment for 5min) followed by GnRH (3nM). Apocynin pretreatment abolished stimulation of Ca^{2+} sparklets by GnRH ($P>0.05$, n=11). Cells treated with catalase (500U/mL), an enzyme to decompose intracellular H2O2, also decreased GnRH-induced Ca^{2+} sparklets compared to GnRH control ($P>0.05$, n=6). Taken together, these data provide strong evidence that ROS signaling plays an important role in GnRH-dependent Ca^{2+} channel activity in gonadotropes.

NOVEL IN VITRO ASSESSMENTS OF PRION DISEASE SPECIES BARRIERS

Kristen A Davenport, Davin M Henderson, Candace K Mathiason, and Edward A Hoover

Prion diseases are unique among protein misfolding disorders in their transmissibility within and between species. Yet, the mechanisms that dictate cross-species transmissibility are poorly understood. Neuropathology results when a misfolded prion protein, PrP^{RES}, coerces the natively folded prion protein, PrP^{C}, to misfold and aggregate. Here, we use an in vitro assay in which infectious brain material seeds the conversion of recombinant Pr^{PC} to PrP^{RES}, the aggregation of which is measured in real time, making it possible to analyze the kinetics of conversion. We have used several PrP^{C} substrates, including bovine, feline, human and white-tailed deer, and a number of infectious brain seeds (bovine spongiform encephalopathy, BSE; feline spongiform encephalopathy, FSE; chronic wasting disease, CWD; feline-adapted chronic wasting disease, fCWD) to assess the propensity of each prion to convert PrP^{C} of other species. We show that prions from the brains of cats or cows infected with BSE have similar patterns of trans-species conversion; specifically, both more efficiently convert bovine PrP^{C} than feline PrP^{C}. There is little evidence that BSE adapts to the new (feline) host. By contrast, when cervid CWD infects felines (fCWD), the resultant prions adapt to the new host and become a more efficient converter of feline than white-tailed deer PrP^{C}. Because the assay is conducted in the absence of other cellular factors, these results support the critical role for prion structure (tertiary and quaternary) in determining the propensity for cross-species prion transmission.

IN VIVO EXPRESSION OF PROGRAMMED DEATH LIGAND 1 BY CANINE TUMORS

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

Introduction. Programmed cell death ligand 1 (PD-L1) is a cell surface molecule expressed by tumor cells and by tumor infiltrating macrophages. The interaction of PD-L1 with its receptor PD-1 on T-cells leads to inhibition of T-cell function and suppression of tumor immunity. Rodent models and human trials have shown that blocking this interaction augments anti-tumor immunity and leads to inhibition of tumor growth. Therefore, a better understanding of tumor PD-L1 expression by canine tumors is an important first step in targeting this molecule for tumor immunotherapy. The primary study objective was to determine the expression patterns of PD-L1 by a representative panel of canine tumor biopsies. Approach. Tumor biopsies from 48 dogs were immunostained for PD-L1 expression, using immunofluorescence and a canine PD-L1 specific mAb. Specificity of PD-L1 expression was assessed using an isotype control antibody. Images were obtained using an Olympus IX83 confocal microscope. Results. High levels of PD-L1 expression was observed on canine melanoma, soft tissue sarcoma, and mammary gland adenocarcinoma biopsies. However, there was however a high degree of variability in PD-L1 expression, even within tumors of the same histotype. The majority of PD-L1 expression was membranous, though intense cytoplasmic expression was also observed in a number of tumors. The highest PD-L1 expression was observed on a histiocytic sarcoma biopsy. In addition, PD-L1 expression was also noted on infiltrating tumor associated macrophages in several different tumors. Conclusions. We conclude that most canine tumors express PD-L1, though there is a considerable degree of variability in expression. Studies are underway to determine whether tumor PD-L1 expression levels are correlated with T cell or myeloid cell infiltration or tumor grade. These findings suggest that PD-1 or PD-L1 blockade is an attractive target for tumor immunotherapy in dogs.

INTERSPECIES GENE EXPRESSION MODELS FOR PREDICTING DOXORUBICIN RESPONSE IN CANINE OSTEOSARCOMA

Jared S Fowles, Kristen C Brown, Ann M Hess, Dawn L Duval, and Daniel L Gustafson

Purpose: Gene expression models can improve outcome to cancer therapy in human patients. Osteosarcoma is the most common primary bone cancer in dogs. Our purpose is to explore the co-expression extrapolation (COXEN) method for the intra- or interspecies extrapolation of cancer cell line data for prediction of chemosensitivity in canine osteosarcoma patients. Methods: Gene expression and chemosensitivity data to doxorubicin (DOX) for human and canine cell line(NCI60 and ACC29) and tumor panels (COS16 and COS33)were obtained publicly or generated via Alamar Blue assay and microarray analysis. Differentially expressed genes (DEGs) were identified by SAM or t-test analysis. Probe matching between species was done by sequence homology or selecting one probe per gene based on maximum variance between samples. Hierarchical clustering was performed using CIMminer. DEGs sharing strong co-expression between reference and co-expression sets were used for prediction model building using linear discriminant analysis. Results: ACC29 and COS16 panels clustered according to DOX sensitivity 67% and 100% correctly when DEGs from NCI60 were used. NCI60-trained models using DEGs co-expressed with the ACC29 were 83% accurate in predicting sensitivity in ACC29 (p=0.019, binomial). NCI60-trained models using DEGs co-expressed with COS49 were 67% accurately predicted response in the combined COS49 (p=0.049, binomial, p=0.045, Log Rank). Models with NCI60 DEGs but co-expressed and built on COS16 were 73% accurate in COS33 (p=0.026, binomial, p=0.001, Log Rank). ACC29-trained models using DEGs co-expressed with COS49 were 73% accurate in COS49 (p=0.008, binomial). Models with ACC29 DEGs but co-expressed and built on COS16 were 70% accurate in COS33 (p=0.047, binomial, p=0.071, Log Rank). Conclusions: Human and canine COXEN models accurately predicted response of canine osteosarcoma patients to DOX. The best model involved human DEGs that were built on dog tumors, suggesting that genomic strategies in canine oncology could benefit from the wealth of available human genomic data.

DENGUE VIRUS REQUIRES THE UNSATURATED FATTY ACID BIOSYNTHESIS PATHWAY FOR ITS INFECTION IN THE MAMMALIAN HOST

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

Dengue virus (DENV) is a significant public health concern with approximately 400 million infections each year leading to 20,000 deaths with no currently available therapeutics or vaccines. As a flavivirus it is enveloped with a positive sense single-stranded RNA genome, and utilizes the endoplasmic reticulum (ER) to accomplish its replication. During the course of infection the ER membrane of the host cell is expanded and forms vesicles which house replicating RNA. These vesicles serve to condense viral and host substrates for replication, provide a scaffold for the complex, and protect the dsRNA from the host immune response. It is now understood that fatty acid synthesis is upregulated during infection to meet the substrate demands, moreover specific lipid species are required to drive the curvature and fluidity of these specialized membranes. We interrogated a library of siRNAs directed at the unsaturated fatty acid biosynthesis pathway to identify bottlenecks in viral replication. We found that stearoyl co-A desaturase (SCD), the rate-limiting enzyme responsible for the conversion of steric to oleic acid, was critical for viral replication. Furthermore, virus that was grown in the presence of an SCD inhibitor was less infectious than control, indicating a change to the virus particle resulting in a possible fusion defect. SCD is a high-profile target for treating cancer, obesity and metabolic syndrome, and has led to the development of therapeutics that have potential to be repurposed as antivirals.

INHIBITION OF HEDGEHOG SIGNALING INHIBITS PROLIFERATION IN CANINE TRANSITIONAL CELL CARCINOMA

Tanya Gustafson, Barbara E Kitchell, and Barbara Biller

Background: Transitional cell carcinoma (TCC) is the most commonly diagnosed tumor of the canine urinary system. Overall survival with traditional chemotherapy in this disease has not improved for many years, and so additional research into new therapeutic targets is needed. Hedgehog signaling, which regulates normal embryonic development, represents one such target. When activated in adult cells, Hedgehog signaling promotes oncogenesis and has been found to play a central role in human bladder cancer. Methods: In this study, TCC cell lines highly expressing the Hedgehog pathway were treated with the inhibitors cyclopamine and GANT61. Down-regulation of the pathway was confirmed by real time RT-PCR for Patched 1 (PTCH1), GLI1 and GLI2. Cell growth was assessed at 48 and 96 hours after inhibitor treatment. Cell viability was assessed through an alamar blue assay. Apoptosis was assessed through Annexin V staining. Results: In these cell lines, Hedgehog pathway mediators are expressed at high levels. Inhibition of Hedgehog signaling with cyclopamine and GANT61 did lead to decreased expression of the targets PTCH1, GLI1 and GLI2. Further, Hedgehog inhibition led to decreased cell proliferation, but had a lesser effect on apoptosis. Conclusions: This study suggests that Hedgehog signaling does have a role in the pathogenesis of canine TCC. Further studies using silencing RNA techniques to inhibit signaling alone and in combination with chemotherapy may provide additional evidence of the importance of this pathway in canine TCC and provide the basis for future clinical trials.

COMPLEMENT, COMPLEMENT RECEPTORS, AND COMPLEMENT REGULATORY PROTEINS BIND PRIONS AND ASSIST IN PATHOGENESIS

Sarah J Kane, Taylor K Farley, Brady A Michel, Aimee E Ortega, and Mark D Zabel

Former research implicates Complement, a component of the innate immune system, as crucial for establishing prion disease, and the work in this study aimed to biochemically characterize the interactions between Complement and prions, as well as determine the role of a regulatory protein in establishing disease. We employed surface plasmon resonance (SPR) to observe interactions between a panel of Complement proteins and prions enriched from the brain of an elk with Chronic Wasting Disease (CWD). Upon identification of a putative prion receptor by SPR, we injected fluorescent prions into the footpad and performed intravital microscopy of the popliteal lymph to test co-localization with this receptor *in vivo*. Lastly, to determine the role of complement regulatory protein factor H (CFH) in manifesting disease, we inoculated CFH-/- mice with mouse-adapted Scrapie and compared disease onset and prion accumulation with hemizygous (CFH+/) and wild type (CFH+/+) littermates. Our findings suggest Complement Receptors 1/2 (CD21/35) are prion receptors because not only did we observe an interaction between CD21/35 bind prions via SPR, fluorescent prions co-localized with CD21/35 *in vivo* on B and follicular dendritic cells in the popliteal lymph node within an hour of inoculation. We also determined other Complement proteins, including C3 cleavage products and C1q, bound infectious prions using SPR. We observed a gene-dose effect of CFH in establishing terminal disease. CFH deficient mice survived longer and accumulated less prions than their hemizygous or wild type littermates. In conclusion, we propose our findings provide viable targets for therapeutic intervention. Previous work from our lab revealed mice deficient in CD21/35 entirely resisted disease in a CWD model. In conjunction with the findings reported here, we believed we have identified CD21/35 as prion receptors. Prion diseases are invariably fatal, but perhaps preventing the interactions outlined in this study could delay or prevent disease.

RICE-BRAN PHYTOCHEMICAL EXTRACTS INHIBIT INVASION AND INTRACELLULAR REPLICATION OF SALMONELLA IN MOUSE INTESTINAL AND PORCINE JEJUNAL EPITHELIAL CELLS

Job Mapesa, Irfan A Ghazi, Jessie Wilburn, Jan Leach, Sangeeta Rao, Corey Broeckling, Anna McClung and Elizabeth P Ryan

Environmental enteric dysfunction is a persistent problem in children living in poor and non-sanitary conditions in developing countries due to microbial contaminants. Despite increased efforts to provide clean water and nutritious foods, enteric infections, which present as chronic diarrheal disease, remain a major risk to malnutrition. To combat this problem, there is a need for development of new approaches for enteric infections treatment, as well as assessment of dietary compounds to control and prevent severity of infections. The aim of this study was to determine if rice bran extracts from two distinct varieties differentially regulate invading microorganisms such as *Salmonella Typhimurium* enterica in vitro, using mouse intestinal (MSIE) and porcine jejunal (IPEC-J2) cell lines. Rice-bran included in the diet has previously been validated in experimental work *in vivo* using piglets and mice. Rice bran from two rice cultivars (SHZ and LTH) with differential gene expression patterns were extracted in methanol. LTH, the most pigmented variety blocked *Salmonella* invasion and replication in a dose-dependent manner as opposed to SHZ the less active. *Salmonella* growth was reduced 100 fold by 1 mg in MSIE and 5 mg in IPEC-J2. We applied metabolomics for identification of bioactive compounds distinguishable by rice variety. From the metabolite profiles by UPLC-MS, oryzalic acid, catechin, procyanidin-B, 6-methoxychromanone, and lysoPC(16:0) showed significantly ($p<0.05$) higher relative abundance in LTH. The relative abundance of coumaric acid, hydroxylinolenic acid and andrographolide was significant ($p<0.05$) SHZ compared to LTH. Two promising bioactive candidates include lysophosphatidylcholine, which induces arachidonic acid release and catechin, a strong antioxidant, merit further bioactivity assessment. We conclude that rice-bran differentially regulates Salmonella growth *in vitro*. Understanding the mechanisms of action and the bioactive compounds involved will provide information for developing not only rice genotypes with tolerant disease traits but genes regulating functional food and bioactive compounds.

DEVELOPING THE DAIRY DISEASE ADJUSTED LACTATION YIELD MODEL (DALY)

Ashleigh A McNeil and Craig S McConnel

Within human epidemiology, the economic burden of years lost due to ill-health or early death is measured through the World Health Organization's use of disability adjusted life years (DALY). Traditionally, dairy farm management has focused solely on singular pathologies without quantifying economic costs tied to disease. Our research focuses on mimicking the DALY to combine direct and indirect costs with milk production lost due to disease and early removal on dairy farms. The Dairy DALY is estimated by combining days of milk lost due to disease (DLD) and days of milk lost due to early death or removal (DLL). The DLD reflects the number of cases during a certain period, multiplied by the average duration of disease, multiplied by a factor reflecting the severity of disease. Severity scores are derived from an expert opinion survey of dairy producers, managers and veterinarians, and combined with average duration and prevalence of diseases on a given farm. For comparison, on-farm data is being collected from 7,500 cows over an 18-month period. Severity scores were gathered for 13 common dairy cow diseases ranging from 0 (no adverse effects) to 10 (death). Ranked most severe was right displaced abomasum (6.45) and least severe was diarrhea (3.27). Likelihood of culling scores were gathered for the same diseases and qualified by days in milk and pregnancy status. To date, 5,553 cows have been enrolled in the on-farm component of the study with mastitis the most prevalent disease (35.4%) and ketosis the least prevalent (0.7%). The DLD and DLL components will be combined with farm-supplied direct costs associated with each disease to quantify the economic effects of morbidity and mortality. The on-farm data will be used as a comparison of variation between farm severity of diseases and the expert weights of the Dairy DALY model.

INTERACTIONS BETWEEN DIET AND EXPOSURE TO SECONDHAND SMOKE EXPOSURE ON CHILDHOOD OBESITY: NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY (NHANES) 2007-2010

Brianna F Moore, Maggie L Clark, Annette Bachand, Stephen J Reynolds, Tracy L Nelson, Jennifer L Peel

Background: Obesity is a global crisis that affects all age groups, particularly children. High calorie diets and low physical activity levels are universally accepted as risk factors; however the extent of obesity prevalence cannot be entirely explained by these risk factors. Exposure to secondhand smoke (SHS) may play a role in the onset of childhood obesity, but few studies have evaluated these relationships. Objectives: We evaluated the effects of exposure to SHS on childhood obesity using a novel biomarker (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol [NNAL]), an established biomarker (cotinine) and self-report to characterize exposure to SHS. Additionally, we examined the interaction between exposure to SHS and diet on childhood obesity. Methods: Weighted multinomial logistic regression models were used to examine the associations of SHS exposure and obesity among children (ages 6-19) who participated in the 2007-2010 National Health and Nutrition Examination Survey. We assessed interactions between exposure to SHS and several nutrients (dietary fiber, eicosapentaenoic acid [EPA], docosahexaenoic acid [DHA], vitamin C, and vitamin E) and nutrient patterns on both the additive and multiplicative scales. Results: Approximately half of the children had levels of NNAL and cotinine above the limit of detection, indicating exposure to SHS. Interaction results suggest that increases in obesity risk among children with both high exposure to SHS and low levels of certain nutrients (dietary fiber, DHA, or EPA) are greater than would be expected due to the effects of the individual exposures alone. For example, children with high NNAL levels and low intake of dietary fiber were more than twice as likely to be obese as compared to children with low NNAL exposure and high fiber intake (odds ratio=2.6 [95% confidence interval: 1.6, 4.0]). Conclusions: Childhood obesity prevention strategies aimed at reducing SHS exposures and improving diets may exceed the expected benefits based on targeting either risk factor alone.

ORAL PRESENTATIONS Clinical/Basic Science

1:00–5:00 PM | SESSION III - SALON V

USE OF PATIENT BODY TEMPERATURES IN SURVEILLANCE FOR HEALTHCARE-ASSOCIATED INFECTIONS IN A VETERINARY HOSPITAL

Z.B. Ouyang, B.A. Burgess, P.S. Morley

There is an ethical and legal responsibility to optimize infection control programs in hospitals. Surveillance is a key component to these efforts. Syndromic surveillance has been proposed as an efficient tool to screen for infection control problems. These efforts can be facilitated by increasing utilization of electronic medical records (EMR) and other information management systems. Fever is a sequella of infection and rectal temperatures are typically assessed with every physical examination. This study evaluates rectal temperature data obtained from hospitalized patients as an efficient and effective tool to assist with surveillance for issues related to healthcare associated infections (HCAI) in veterinary patients. Data for rectal temperatures and patient information recorded in EMR for a veterinary teaching hospital were abstracted from 1/1/2012 to 7/15/2014. This included a total of 43,059 visits to the hospital, during which 6.1% (2,621) of patients were identified as febrile. For small animal (SA; canine and feline) visits, 3.5% (1232/35,562) of patients were febrile, compared to 19.2% (678/3,523) of patients during equine visits and 21.9% (1157/5293) of visits for livestock animals (LA; camelid, bovine, caprine, ovine and porcine). A total of 109,692 rectal temperatures were recorded, of which 5.9% (6,517) were febrile. Among these, 3.0% (2,441/80,466), 10.2% (2,475/24,162), and 23.3% (1,025/4,407) of individual temperature measurements were febrile for SA, equine, and LA patients, respectively. Febrile events were analyzed to identify associations with hospital and patient factors. This study provides an initial examination of trends in febrile temperatures recorded among hospitalized veterinary patients. Deviations from these baseline trends may indicate an increase in nosocomial infections, allowing for prompt implementation of infection control measures. Further, once infection control measures have been implemented, a return to the baseline would indicate an effective protocol. This information would be vital for the establishment of protocols based on successful outcomes.

ROLE OF MONOCYTE RECRUITMENT IN HEMANGIOSARCOMA METASTASIS IN DOGS

Daniel Regan, Andrea Escaffi, Jonathan Coy, Jade Kurihara, and Steven Dow

Canine hemangiosarcoma is a highly malignant tumor, which is associated with poor long-term survival due to the development of early and widespread metastatic disease. Currently, little is known regarding the biology of canine hemangiosarcoma, and the mechanisms accounting for the highly metastatic nature of the tumor are poorly understood. In humans and rodents, monocytes have been shown to play key roles in metastasis through promotion of tumor cell extravasation, seeding, growth, and angiogenesis, as well as suppression of anti-tumor immunity. However, there has been little investigation into the role of monocytes in canine tumor metastasis. Therefore, we investigated the potential role of monocyte infiltration in the regulation of tumor metastasis in dogs. To address this question, we initially performed immunohistochemistry for CD18 to determine the degree of monocyte infiltration in necropsy samples obtained from several common metastatic tumors of dogs, including hemangiosarcoma, osteosarcoma, and various carcinomas. We found that compared to other tumor types, hemangiosarcoma metastases had significantly greater infiltration of CD18+ monocytes. Next, migration assays were used to compare the ability of tumor cell lines to stimulate monocyte migration in vitro. Hemangiosarcoma cell lines were among the strongest at stimulating monocyte migration, and were also found to be the highest producers of the monocyte chemoattractant CCL2. In addition, hemangiosarcoma metastases in vivo were found to produce large amounts of CCL2, compared to other tumor metastases. These results are consistent therefore with the hypothesis that overexpression of CCL2 and recruitment of large numbers of monocytes may explain in part the aggressive metastatic nature of canine hemangiosarcoma. Moreover, these findings suggest that immunotherapeutic interventions designed to block monocyte recruitment or mobilization may be an effective adjuvant strategy for suppressing tumor metastasis in dogs with hemangiosarcoma.

PRECISION OF A HORSE-MOUNTED INERTIAL SENSOR DEVICE FOLLOWING RE-APPLICATION

Bernadette L. Smith, Valerie J. Moorman, Melissa E. King

Orthopedic disease in the equine athlete is all too common, and lameness is one of the most common reasons a horse is seen by a veterinarian. Lameness is typically detected using the subjective lameness evaluation, but subtle lameness can be difficult to detect and observers do not always agree on lameness grade and limb affected. Great leaps have been made in the avenues of lameness diagnostics, including the development of horse mounted objective lameness detection systems like the Equinosis Lameness Locator®. While short term repeatability of the system has been evaluated, there has been no evaluation on the precision of the system when it is applied by multiple evaluators. The objective of this project was to test the inter-user repeatability of the system. Six horses from another investigation that had mild, naturally occurring lameness where used in this study. Horses were assigned a subjective lameness score using the AAEP Lameness Scale. The horses each underwent 6 trials (2 trials per individual; 3 individuals). The same handler trotted all horses. The order of individuals applying the system was determined randomly (random number generator). Statistical Analysis was performed using a Mixed model ANOVA model with horse as a random variable, and individual as a fixed variable. Significance was set at $P < 0.05$. Sensors where placed based on anatomical location as described by Equinosis® From this study of 6 horses, only one variable showed a significant difference between observers: forelimb Max Diff Mean (p-value= 0.0369). Potential sources of variation include placement of sensors or changes in lameness. Limitations in this study were few horses, few applicators, and narrow range of lameness. We concluded that we cannot rely on one variable to suggest the horse is lame and potentially less emphasis should be placed on Max Diff Mean variable.

EFFECT OF PARENTERAL ADMINISTRATION OF MODIFIED LIVE OR INACTIVATED FVRCP VACCINE ON CLINICAL SIGNS IN A FELINE HERPESVIRUS CHALLENGE MODEL.

Stacie C Summers, Rebecca Ruch-Gallie, Jennifer R Hawley, Michael R Lappin

In a previous study, cats administered one SQ dose of an inactivated FHV-1, feline calicivirus (FCV), and panleukopenia virus (FPV) vaccine (FVRCP) developed protective antibody titers against FHV-1 significantly faster than cats administered a modified live FVRCP vaccine (MLV). However, a FHV-1 challenge was not performed. The objective of this study was to determine the effect of one dose of an inactivated FVRCP vaccine or one dose of a MLV FVRCP vaccine on the clinical signs of FHV-1 in SPF kittens. A total of 24 5–month-old purpose bred kittens from a FHV-1 naïve barrier facility were purchased and randomly divided into 3 groups of 8 kittens. Group 1 kittens were maintained as unvaccinated controls, Group 2 kittens were administered 1 dose of the inactivated FVRCP vaccine SQ on Day 0, and Group 3 kittens were administered 1 dose of the MLV FVRCP vaccine SQ on Day 0. A standardized clinical score system was applied to each Group of kittens by 2 masked observers for each day of the study. On Day 7, all 24 kittens were administered a USDA challenge strain of FHV-1. Group clinical scores were compared among study periods using Kruskal Wallis with pair-wise comparison and significance defined as $p < 0.05$. In the 21 days (Days 8-28) after FHV-1 challenge, both groups of vaccinated cats were less likely to be clinically ill (indicated by lower cumulative clinical scores) than control cats ($p < 0.001$). There was no statistical difference in total clinical score between the two vaccinated groups ($p = 0.97$). Parenteral administration of either of these inactivated or modified live FVRCP vaccines decreased clinical signs of illness due to FHV-1 on a Day 7 challenge when compared to controls. Use of either vaccine product is indicated in cats at risk of acute exposure to FHV-1.

VALIDATION OF A POLYMERASE CHAIN REACTION ASSAY FOR THE SUBTYPING OF CRYPTOSPORIDIUM SPP. ISOLATES OF HUMAN ORIGIN

Hanaa A Thigeel, Andrea V Scorza, Francisco J Olea-Popelka, and Michael R Lappin

Cryptosporidium species have been isolated as a cause of diarrhea in a wide range of hosts including humans. The objective of this ongoing study is to present the optimization and validation of a PCR assay to subtype *C. parvum* and *C. hominis* positive human isolates for future application in a study of veterinary students. A published 60 kDa glycoprotein (gp60) gene-based polymerase chain reaction (PCR) is currently being optimized for subtyping of *C. parvum* and *C. hominis*. The assay was validated in fecal samples experimentally inoculated with *C. parvum* oocysts and *C. hominis* DNA. Optimal primer concentration (0.2µM), DNA volume (2µl) with (55 C) annealing temperature were selected after the optimization to achieve the highest diagnostic sensitivity. The gp60 assay detected DNA from *C. parvum* and *C. hominis* as expected. To date, we have received 25 human fecal samples. For detection of *Cryptosporidium* spp. oocysts, a commercially available immunofluorescence assay (IFA) (MERIFLUOR® *Cryptosporidium/Giardia*, Meridian Biosciences) was used. One *Cryptosporidium* oocyst was detected in one sample (4.0%) using the IFA method, but none of the human samples tested positive using this PCR assay. The one positive sample detected by IFA that was negative by the PCR was interpreted as a false negative PCR assay result that was likely related to decreased sensitivity of the PCR assay, especially when the number of oocysts is very low. Overall, the low prevalence for *Cryptosporidium* spp. to date is attributed to the small sample size. Additional samples will be collected to further identify the presence of *Cryptosporidium* spp. in human samples.

GUINEA PIGS, TUBERCULOSIS, AND NEGATIVE RESULTS - A RESEARCH CONUNDRUM

Wendy R Tuttle, Elizabeth Creissen, JoLynn Troudt, Lonnie Kendall, and Angelo Izzo

Guinea pigs are commonly used animal models to study infectious disease. Specifically, guinea pigs are used in our lab to test vaccine candidates for Tuberculosis. There are many reasons guinea pigs are used as animal models to study human disease. In particular, they are a docile species that are easy to work with. More importantly, guinea pigs share many similarities with the human immune system, which results in nearly identical lung pathology upon infection with *Mycobacterium tuberculosis*. Unfortunately, guinea pigs are also a prey species, which results in excellent masking of any illness. Clinically, it is very difficult to identify disease in guinea pigs, which is particularly cumbersome when evaluating the efficacy of potential vaccines. Therefore, it became our focus to identify biomarkers in the serum and urine of guinea pigs infected with *Mycobacterium tuberculosi*s that would correlate to disease severity; with a hope of mitigating disease at earlier time points, resulting in overall better welfare of the animal. Our findings show that not only do guinea pigs mask their disease outwardly; they also have an ability to hide disease inwardly. Monthly sampling of guinea pig blood and urine after low dose aerosol inoculation of *Mycobacterium tuberculosis* H37rV resulted in very few clinically relevant changes over the life of the guinea pig. None of the parameters evaluated were significant predictors of disease severity. Our overwhelmingly negative results have prompted more questions not only in our sampling methods, but also about basic guinea pig physiology and immunology. Additionally, our work is a good example of how negative results can benefit the scientific community and prevent unnecessary duplication of science.

GASTROINTESTINAL MOTILITY CHANGES IN DOGS DURING HOSPITALIZATION

Kanawee Warrit, Pedro Boscan, Leah Ferguson, Allison Bradley, David Twedt

Objective: To determine if hospitalization affects gastrointestinal motility in dogs. Animal: Prospective study in 9 adult healthy dogs. Methods: A wireless motility capsule (WMC) given orally measures gastrointestinal tract pressures, transit time and pH. The WMC was administered to each dog during their normal home environment and then repeated during hospitalization. All dogs were fasted overnight and the WMC was administered following a standard meal between 7:30-8 am. During in home testing all dogs followed their normal daily routine. During hospitalization, the dogs were kenneled, abdominal X-rays were performed daily and walked 4-6 times a day. All dogs ate Purina EN™ twice a day (8 am and 7 pm). Once the WMC exited the gastrointestinal tract data was analyzed and the results compared using either paired t-test or Wilcoxon Signed Rank test for analysis. Results: Differences in mean gastric emptying time was observed between the home environment and hospitalization. At home, the mean gastric emptying was 1716 minutes (95%CI 495-2937) and during hospitalized the mean gastric emptying increased to 3695 minutes (95%CI 1909-5478; p= 0.0375). All other parameters measured; gastric motility index, gastric pH, small bowel transit time, small bowel motility index, large bowel transit time and large motility index were comparable between at home and during hospitalization. However, the 95%CI observed for each parameter was large. Conclusion and clinical relevance: The study suggests that hospitalization could induce a delay of gastric emptying time in dogs.

EVALUATION OF GAIT PATTERNS IN DOGS: THE PACE

Theresa M Wendland, Kyle W Martin, Molly A Vitt, Colleen G Duncan, Angela J Marolf, Felix M Duerr

The pace is a two beat "lateral-couplet" symmetrical gait in which ipsilateral limb pairs move in cadence. Not all dogs pace and there is conflicting evidence whether the pace is normal or abnormal in orthopedically healthy dogs. Furthermore, little documentation exists regarding pacing speeds or the effect that controlled speed has on gait patterns between walk and trot. This study primarily investigates whether pacing in dogs is a normal gait pattern or associated with orthopedic disease. Secondarily it establishes the impact controlled speed has on occurrence of pacing and the speeds at which pacing occurs. Healthy, medium to large breed dogs were included in the study. Each dog was evaluated for orthopedic abnormalities and lameness by a board certified veterinary surgeon. Abnormalities found on physical exam were further defined with radiography of the affected area(s). Dogs were then separated into non-lame and mildly-lame groups based on findings. Gait was assessed using digital video imaging under three conditions: off-lead unrestricted movement, lead-controlled, and on a land treadmill after a period of treadmill habituation. Pacing was observed in 17/20 non-lame and 10/19 mildly-lame dogs. Non-lame dogs were significantly more likely to pace than mildly-lame dogs under lead-controlled conditions. Comparison of pacing frequency between groups showed no significant difference under treadmill and unrestricted-motion conditions. All pacing occurred at speeds between walk and trot with speeds ranging from 3.52 km/h-10.24 km/h (2.20-6.36 mph, mean 6.51 km/h [4.04 mph]). Pacing was demonstrated in orthopedically normal and abnormal dogs under all study conditions and may be a normal gait observed in clinically normal dogs. Therefore, pacing alone should not be considered a clinical indicator of orthopedic pathology. Relative speed was also determined to be a key factor in pacing and controlled speed conditions appear to affect frequency of pacing.

PRIONS IN PLANTS: ASSAYING GRASSES FROM ROCKY MOUNTAIN NATIONAL PARK FOR PRP^{CWD}

Aimee Ortega, Jeffrey Seligman, Jan Leach, 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. It has spread to 19 states within the United States, 2 Canadian provinces, and South Korea. 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. We want to further explore environmental aspect of CWD transmission and determine whether prions can be detected in grasses and other plants in ROMO by use of the protein misfolding cyclic amplification (PMCA) assay. This past summer three sites within ROMO were surveyed and a total of 32 plants collected. 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 PMCA for detection of PrP^{CWD}. Discovery of PrP^{CWD} in plants would be a novel finding but it could also help and provide park management with a new area to target in order to curtail the spread of CWD.

RAPID ISOLATION OF NEPTUNIUM FROM SOLUTION AND SOIL

Brett L Rosenberg, Georg S Steinhauser, and Katsumi Shozugawa

Actinides are great sentinels for ascertaining the condition of nuclear fuel elements following the meltdown of a nuclear reactor. Isotopic and activity ratios of environmental plutonium significantly different from the background ratios established by nuclear explosions indicate that there was a breach in the fuel elements of a reactor. Measuring these ratios employ mass spectrometry and alpha spectrometry. We propose using ^{239}Np as a sentinel; the gamma rays emitted from short-lived ^{239}Np have intensities and energies that make detection of this radionuclide rapid by gamma spectroscopy. Potential problems with spectroscopic analysis are interferences by volatile radionuclides, such as radiotellurium and radioiodine. Therefore, the goal was to isolate ^{239}Np by ion specific extraction chromatography using Eichrom® resin columns. UTEVA, RE, TRU, and Actinide resins were evaluated for their ability to isolate volatile radionuclides from neptunium. UTEVA and RE resins loaded and eluted with nitric acid of varying concentrations can isolate neptunium from more than 99% of the iodine in a sample and effectively exclude tellurium and cesium. This exclusion was observed in both aqueous and soil matrices. Therefore, following an accident, rapid evaluation of reactor core conditions can be assessed by isolating ^{239}Np using extraction chromatography on soil or rainwater samples and detecting this radionuclide with gamma spectroscopy.

CHARACTERIZATION OF IMMUNOGLOBULIN GENE USE AND MUTATION STATUS IN CANINE B CELL CHRONIC LYMPHOCYTIC LEUKEMIA

Emily D Rout, Robert C Burnett, Courtney Abbott, Stacey George, and Anne C Avery

Canine B cell chronic lymphocytic leukemia (CLL) is common in dogs and shares many features with human CLL. In human CLL, immunoglobulin (Ig) gene usage and mutation status are important markers for disease behavior, but these factors have not been assessed in canine B-CLL. We sequenced the immunoglobulin heavy chain variable region (VH) genes from neoplastic peripheral blood B cells in 48 dogs with B-CLL. Thirteen VH genes were used. Excluding the Boxer breed, the most commonly used genes were VH1-44, VH1-62, and VH1-35. In non-neoplastic B cells, VH1-44 and VH1-62 are the most commonly used genes. 56% of all genes used in the B-CLL patients had greater than 2% mutations compared to germline sequence and were classified as mutated, while 44% were unmutated. Of the cases using VH1-44, one third used an unmutated gene. All the cases using VH1-62 and VH1-35 used mutated genes. VH-41 is overrepresented in Boxers with B-CLL, but is not preferentially used in normal Boxer dogs or in Boxers with other B-cell neoplasms. Of 11 Boxer dogs with B-CLL that were sequenced, 9 used an unmutated VH1-41 gene. These findings suggest that antigen selection may play a role in the pathogenesis of B-CLL in Boxers. These data lay the foundation to correlate VH gene usage and mutation status with outcome in dogs, further establish canine patients as good models of human CLL, and identify groups, like the Boxers, that may be useful in studying the pathogenesis of CLL.

EVALUATION THE ELUTION OF POLYHEXAMETHYLENE BIGUANIDE FROM TWO WOUND DRESSING MATERIALS

Helen F Sims, Dean Hendrickson, Luke Wittenburg, Doreene R. Hyatt.

Polyhexamethylene biguanide (PHMB), a polymer with broad spectrum efficacy, is a promising local treatment for wound infections. Clinical observation suggests that PHMB may elute from Kerlix™ AMD gauze (G+) and Kendall™ AMD foam (F+) dressings, allowing for inhibition of bacterial growth distant from the dressing. In this study, G+ and F+ dressings were incubated in equine serum, as were plain Kerlix™ gauze (G-) and plain Kendall™ foam (F-) dressings, both without PHMB. Daily aliquots from the F+, G+, F-, G- and plain serum conditions were transferred onto filter disks and dried for 8 consecutive days. The inhibitory properties of PHMB was evaluated against three clinically obtained equine isolates of each *Pseudomonas aeruginosa*, *Streptococcus equi* ssp. *zooepidemicus*, *Escherichia coli,* and *Staphylococcus aureus*. Commercially available ATCC cultures of *Pseudomonas aeruginosa*, *Escherichia coli, Enterococcus faecalis* and *Staphylococcus aureus* were used for comparison. Three replicates of each disk type were placed onto Muller-Hinton plates inoculated with the different bacterial isolates. After 24 hours of incubation the absence of bacterial growth beneath the disks was recorded. Inhibition of bacterial growth occurred directly beneath G+ and F+ disks on plates inoculated with the four *Staphylococcus aureus* isolates. No inhibition occurred on the plates inoculated with the other bacteria, nor under the control disks (F-, G-, plain serum or blank). The average number of replicate disks causing inhibition increased over time for both F+ and G+. Enough PHMB eluted off both the Kerlix™ AMD gauze and Kendall™ AMD foam dressing into equine serum to produce inhibition against both equine and ATCC *Staphylococcus aureus* isolates showing that these dressings may be effective against bacterial contamination within wounds. In order to generate clinical bandage change recommendations further research using a more sensitive analytical technique is needed to quantify the elution behavior.

NEURONAL OVEREXPRESSION OF THE HUMAN (PRO)RENIN RECEPTOR INCREASES SYMPATHETIC TONE THAT IS MASKED BY UPREGULATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE

Michelle N Sullivan, Wencheng Li, Curt D Sigmund, Scott Earley, and Yumei Feng

Binding of (pro)renin to the (pro)renin receptor (PRR) contributes to generation of angiotensin II (Ang II) but can also induce Ang II-independent signaling. It is unclear whether the latter is important for blood pressure (BP) regulation. To address this question we created transgenic mice that overexpress the human PRR (hPRR) selectively in neurons (syn-PRR mice). Activated human (pro)renin (hPRO) cannot cleave mouse angiotensinogen to generate Ang II. Therefore, administration of hPRO to syn-PRR mice can be used to examine Ang II-independent PRR signaling. Intracerebroventricular infusion of hPRO has no effect on wildtype (WT) mice (ΔMAP: 2 ± 0.8) but increases BP in syn-PRR (ΔMAP: 23 ± 4.6) (n = 8/group). This increase is unaffected by treatment with the Ang II type 1 receptor blocker losartan (ΔMAP: 19 ± 5.2), suggesting that hPRR activation increases BP independent of Ang II generation. Interestingly, although basal MAP (101 ± 1.5 vs. 101 ± 1.3) and HR (552 ± 3.3 vs. 544 ± 7.3) are similar between syn-PRR and WT mice, syn-PRR have increased basal cardiac (ΔHR: -68 ± 1.3 vs. -45 ± 1.4) and vasomotor (ΔMAP: -41 ± 2.1 vs. -29 ± 0.4) sympathetic tone. We hypothesized that endothelial nitric oxide synthase (eNOS) activity was augmented in the periphery to compensate for increased sympathetic activity. qPCR revealed that eNOS mRNA is increased in mesenteric arteries from syn-PRR vs. WT mice (expression-ratio 1.54 vs. 1.0). To examine eNOS activity we inhibited NO production by daily intraperitoneal injection of L-NAME (400 mg/kg). 24 hrs post-L-NAME injection, WT mice displayed normal BP (MAP: 106 ± 6.7). In syn-PRR mice L-NAME treatment resulted in a sustained elevation of BP (MAP: 123 ± 2.6) 24 hrs post-injection. Together, these data provide evidence that neuronal overexpression of hPRR increases sympathetic activity that is masked by subsequent upregulation of eNOS.

REVERSAL OF MYCOBACTERIUM ABSCESSUS ANTIMICROBIAL RESISTANCE USING BIOFILM INHIBITORS

Deepshikha Verma, Ajay Grover, Kim Arnett, Megan Blackledge, Anne Lenaerts, Christian Melander, and Diane Ordway

Rationale: Over the last 10 years, *Mycobacterium abscessus* group strains have emerged as important human pathogens and are associated with a higher fatality rate than any other rapidly growing mycobacteria (RGM). Although the pathogenesis of these organisms remains unknown—contamination of water sources and medical equipment have been implicated. *Mycobacterium abscessus* outcomes are much worse than other RGMs, in fact this organism seems to be essentially untreatable, with only ~50% sputum conversion seen in multiple chemotherapy studies. Two major limitations to developing new therapies for NTM are the lack of understanding of bacterial pathogenesis and the contribution of biofilms on phenotypic drug resistance in the host Methods: We orally infected mice with mCHERRY-expressing *M. massiliense* and used whole body xenogeny imaging to track bacterial pathogenesis. *Mycobacterium abscessus* was infected *in vitro* with standard anti-antimicrobial compounds and biofilm inhibitors to assay minimum inhibitory concentration and minimum bactericidal concentration. Results: Our studies have made some progress in dissecting pathogenesis of these organisms using mCHERRY-expressing *M. massiliense* and xenogeny imaging which support the notion that infection can occur by the gastrointestinal and pulmonary route. Our preliminary studies provide strong evidence that through inhibition of biofilm formation with small molecule inhibitors during *in vitro* infection with *M. abscessus* increased bactericidal activity is present when combined with the standard anti-NTM treatment. Conclusion: Our results are the first report of reversal of nontuberculosis mycobacterial phenotypic antimicrobial resistance using biofilm inhibitors.

ASSESSING MOTHER TO OFFSPRING TRANSMISSION OF CHRONIC WASTING DISEASE USING A TRANSGENIC MOUSE MODEL

Kassandra Willingham, Erin McNulty, Kelly Anderson, Jeanette Hayes-Klug, Amy Nalls, 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). The presence of sufficient infectious prions in the tissues, bodily fluids (urine, saliva, and blood) and environments of clinical and preclinical 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 the facile transmission of some TSEs. 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. Placental trafficking and/or secretion in milk are two means by which maternal prion transmission may occur. In these studies we explore CWD maternal transmission during early and late CWD infection using a transgenic mouse model (TgCerPRP) expressing cervid prion protein. Naïve and CWD-infected dams were bred during early (45 dpi) and late (120 dpi) infection and were allowed to bear and raise their offspring. Milk was collected from the dams for prion analysis, and the offspring were observed for TSE disease progression. Terminal tissues harvested from these dams and offspring were analyzed for prions. We have demonstrated: 1) that CWD-infected TgCerPRP females successfully breed and bear offspring, 2) the presence of PrP^{CWD} in reproductive and mammary tissue harvested from CWD-infected dams, and 3) clinical disease progression in offspring born to CWD-infected dams. We are currently analyzing terminal tissue harvested from offspring born to CWD-infected dams for the detection of PrP^{CWD} and amplification competent prions. These studies will provide insight into the potential mechanisms and biological significance associated with mother to offspring transmission of TSEs.

POSTER PRESENTATIONS

SALON III AND IV

1/ INFLUENCE OF MESENCHYMAL STROMAL CELLS ON PULMONARY METASTASES AND LOCAL RECURRENCE FOLLOWING PRIMARY TUMOR REMOVAL IN A MURINE OSTEOSARCOMA MODEL

Megan Aanstoos-Ewen, Dan Regan, Ruth Rose, Laura Chubb, Nicole Ehrhart

Mesenchymal stromal cells (MSCs) improve bone healing; conversely, MSCs also promote primary and metastatic tumor growth in the presence of gross tumor. However, MSCs are unlikely to be utilized in a clinical setting when gross tumor is present. Previously, we reported on pilot data regarding the safety of MSC usage in a model of osteosarcoma. In this follow-on project, we hypothesized that MSCs would have no influence on pulmonary metastases or local disease recurrence in a minimal disease setting. A syngeneic primary tumor was established in the tibia of mice and confirmed using bioluminescence imaging (BLI). At ten days, either a coxofemoral amputation or a femorotibial amputation was performed. Mice were randomized to receive no MSCs, $5x10^5$ MSCs locally into the surgical site, or $5x10^5$ MSCs intravenously. Development of pulmonary metastases or local recurrence was monitored by BLI and caliper measurements at the amputation site. Mice were sacrificed at 31 days (*metastasis study group*) or 38 or 41 days (*local recurrence study group*). Lungs and amputation sites were examined histologically and using calipers. A Kruskal-Wallis test and a Fisher's Exact test were used with significance set at $p < 0.05$. In the metastasis study group, mice receiving IV MSCs had faster BLI expression of pulmonary disease, higher tumor area percent relative to total area, and the median number of metastatic nodules was 5-fold higher relative to local delivery of MSCs or no MSCs. No statistical difference was noted between groups or between pairwise comparisons. In the local recurrence study group, no significant difference was seen between groups with respect to time to tumor recurrence or size of recurrent tumor in any group. Local MSC administration appears to be safe; however, further study is warranted to evaluate the safety and influence of IV MSCs on minimal residual pulmonary metastatic disease.

2/ RADIOGRAPHIC LOCALIZATION OF THE ORIGINS AND INSERTIONS ASSOCIATED WITH THE TENDONS AND LIGAMENTS OF THE EQUINE STIFLE JOINT

Ellison Aldrich, Laurie R Goodrich, Meaghan Monohan, James D Conway, Alejandro Valdés-Martínez

Radiography is the most commonly used modality for the evaluation of the equine stifle joint, following localization of lameness. While typically a way to assess bone, valuable information may be gleaned regarding soft tissue structures through careful assessment of the entheses of the stifle. Few published reports exist that detail these origins and insertions radiographically. In this study, the origins and/or insertions of all twelve tendons and ligaments of the equine stifle were identified individually with radiopaque marking in four radiographic views (caudocranial, lateromedial, caudolateral-craniomedial oblique, and caudomedial-craniolateral oblique views). Placement of barium paste was based on dissection of multiple specimens, plus the use of plastinated specimens and published descriptions of origins and insertions. The entheses were visible in all radiographic projections and key osseous landmarks were noted. The images created in this study serve as a radiographic guide for evaluation of the entheses of the stifle joint to detect and interpret radiographic changes associated with soft tissue injury. This study also highlights the fact that certain structures are best evaluated in a particular projection, for instance, the non-traditional caudomedial-craniolateral oblique projection for the cranial cruciate ligament. This work should provide important guidelines for clinicians in imaging techniques that will highlight entheses associated with the soft tissue structures commonly injured in stifle disease.

3/ COMPARISON OF 2 COMMERCIALLY AVAILABLE PCR ASSAYS FOR THE AMPLIFICATION OF EHRLICHIA SPP. DNA FROM BLOOD OF NATURALLY EXPOSED DOGS IN OKLAHOMA

Madeline M Anna, Melissa M Brewer, Jennifer R Hawley, and Michael R Lappin

Ehrlichia canis, *E. chaffeensis*, and *E. ewingii* are vector borne pathogens of dogs and people. PCR assays to amplify DNA of these agents are available commercially, and positive PCR assay results often occur prior to detection of specific antibodies against *Ehrlichia* spp. The purpose of the study was to compare the results of 2 different commercially available PCR assays for the amplification of *Ehrlichia* spp. DNA using samples from naturally exposed dogs in an endemic area. Dogs (n = 72) being sterilized at a low cost clinic in Checotah, OK during July 2014 were selected as those likely to be exposed to Amblyomma americanum, the tick vector for 2 *Ehrlichia* spp.. Blood (1 ml) in EDTA collection tubes was stored in a refrigerator overnight, and the next day were sent on cold packs to the first testing laboratory via overnight shipping. On arrival, the samples were split into 2 aliquots with 1 aliquot being frozen at -80 °C and 1 aliquot being prepared for evaluation in a conventional *Ehrlichia* spp. PCR assay (www.dlab.colostate.edu). The frozen EDTA blood was shipped to the second PCR laboratory on dry ice for evaluation in a real time multiplex *Ehrlichia* spp. PCR assay (www.antech.com). Results from samples with sequence confirmation were compared using the kappa statistic. In the first laboratory, DNA of *E. canis* (1), *E. ewingii* (6), and *E. chaffeensis* (1) were amplified from some dogs (11.1%). In the second laboratory, DNA of *E. ewingii* was amplified from 9 dogs (12.5%). Agreement between the assays at the genus level was good (kappa = 0.667 [SE 0.139]; 95% CI = 0.395 to 0.938) with an overall percentage agreement of 93.1%. The dog with DNA most homologous with *E. chaffeensis* in the first laboratory showed homology with *E. ewingii* in the second laboratory.

4/ GENETIC MODIFICATION OF MESENCHYMAL STEM CELLS WITH SCAAV-EQUINE-BMP-2 TO INDUCE OSTEOGENESIS

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

Fracture treatment in horses is fraught with difficulties. During the repair of long-bone fractures, equine surgeons work at the mechanical limits of implants available for bone and regions where implants are applied often have a paucity of soft tissue coverage. Further, the plight of recovery is characterized by high incidences of secondary complications such as support limb laminitis, infection, and mal or non-union callous formation. Bone marrow derived mesenchymal stem cells (MSCs) have shown efficacy in their ability to accelerate healing of connective tissue injuries, including bone. Further, literature supports an osteo-induction of MSCs in response to bone morphogenic protein-2 (BMP-2). Using a novel gene therapy vector, self-complementary Adenoassociated Viral Vector (scAAV), we are able to transduce equine MSCs with equine-BMP-2 and achieve a high level of prolonged equine-BMP-2 expression. Our hypothesis postulated that genetic modification of MSCs overexpressing BMP-2 will induce osteogenesis in cell culture monolayer and protein expression will not be reduced if cells are cryopreserved. Our specific aims are to 1) determine the most appropriate dose of scAAVequine-BMP-2 to induce osteogenesis of MSCs in cell culture monolayer, and 2) to compare BMP-2 expression in MSCs before and after cryopreservation. Preliminary data suggests MSCs should be transduced with 48,000 viral particles per cell (vpc) as they appeared more osteogenic and stained more positively for calcium than cells transduced with doses ranging from 4,000-96,000 vpc. Our data also suggests that cells should be maintained for 7 days post-transduction before cryopreservation. Cells maintained for only 4 days scored and stained less osteogenic, although their BMP-2 protein levels were still elevated. Data is pending following cryopreservation. In the future, cells will be placed in an alginate or fibrin glue to determine if changes in protein expression occur. If not, a delivery method for fracture repair in the horse can be perfected.

5/ SERUM D-LACTATE CONCENTRATIONS IN DOGS WITH PARVOVIRAL ENTERITIS

Alexander J Barnes, Emilee C Venn, Ryan J Hansen, Pedro L Boscan, David C Twedt, Lauren A Sullivan

Dogs infected with canine parvovirus (CPV) are at risk for bacterial translocation due to destruction of small intestinal crypt cells and sloughing of the protective mucosa layer. D-lactate is a byproduct of bacterial metabolism and could serve as a surrogate marker for bacteremia in dogs with CPV. This study hypothesized that serum D-lactate concentrations would be elevated in CPV dogs when compared to healthy age-matched controls, and that serum D-lactate concentrations in CPV dogs would correlate with other markers of disease severity and acid-base derangements. Naturally infected CPV dogs were hospitalized and treated supportively for their disease. Blood samples were obtained at admission and then daily for the first four days of hospitalization. A complete blood count, venous blood gas electrolyte panel, and serum D-lactate concentration were performed on each sample. Serum D-lactate concentrations were determined using a commercially available colorimetric assay in both CPV (n=40) and control dogs (n=9). CPV dogs exhibited higher baseline serum D-lactate concentrations (469 ± 173 μM) compared to control dogs (306 ± 45 μM, $p<0.001$). Regression analysis showed no significant changes in serum D-lactate concentration over time in CPV dogs, despite standardized treatment interventions ($p=0.46$). Additionally, serum D-lactate concentrations obtained at hospital admission did not differ between CPV survivors [36/40 (90%), 474±28 μM and non-survivors 4/40 (10%), 424±116 μM, $p=0.70$. Serum D-lactate concentrations appeared to intermittently correlate with other clinicopathologic variables including base excess, pH, presence of toxic neutrophils and blood L-lactate concentration. In conclusion, dogs with CPV have elevated serum D-lactate concentration compared to healthy controls, but the significance of these elevations is unknown. Larger studies are warranted to determine the clinical significance of elevated D-lactate in CPV dogs and if associations with other clinicopathologic values or survival definitively exist.

6/ COMPARISON OF PROLIFERATION AND IMMUNOMODULATORY POTENTIAL OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS FROM YOUNG AND GERIATRIC FELINE PATIENTS

Lara Barron Zajic, Jessica M Quimby, Tracy L Webb, Andrea K Herndon, Polly Webb, and Steve W Dow

Adipose-derived mesenchymal stem cells (aMSC) have recently been explored as an immunomodulatory therapy in cats, however, the influence of age on the proliferative and immunomodulatory potential of feline aMSC has not been explored. The purpose of this study was to compare telomere length, ability to proliferate in culture and immunomodulatory potential of aMSC collected from young and geriatric feline patients. Adipose tissues from six young and six geriatric cats were harvested and cryopreserved for subsequent aMSC isolation and culture. aMSC proliferation in culture was compared via determination of time until passage two (P2) and a standard MTT proliferation assay. Immunomodulatory ability was compared in a lymphocyte proliferation assay. Telomere length was measured using fluorescent in situ hybridization. All assays were performed on aMSC cell populations between passage two and three (P3). Adipose-derived MSC from geriatric cats exhibited significantly longer time ($p=0.03$) to P2 (average 13.5 days, median 11 days, range 6-14 days) when compared to aMSC from young cats (average 8.2 days, median 7 days, range 9-22 days) but no difference in MTT proliferation assay performed at P3 was detected. No significant difference was found between the two populations in telomere length or ability to suppress lymphocyte proliferation at P2. Geriatric aMSC demonstrated a significantly decreased ability to proliferate in culture when compared to young aMSC, however, telomere length and and immunomodulatory potential were similar between the two groups. Further assays are required to elucidate the mechanism of this disparity in proliferation.

7/ EFFECTS OF VENOM FROM THE PRAIRIE RATTLESNAKE (CROTALUS VIRIDIS) ON CANINE COAGULATION AND FIBRINOLYSIS: IN VITRO EVALUATION USING THROMBOELASTOGRAPHY

Amy Bell, Christine Olver, Raegan Wells, Tyler Johnson, Stephen MacKessy

Rattlesnake envenomation affects thousands of human and veterinary patients each year. Prairie rattlesnake (*crotalus viridis*) envenomation causes bleeding in a subset of canine patients. Bleeding may be the result of either hypocoagulation or hyperfibrinolysis due to the phospholipases and proteases contained in venom. This pilot study's purpose was to identify whether *C. viridis* venom causes hypocoagulation or hyperfibrinolysis, or both, using thromboelastography (TEG) of canine whole blood and plasma. TEG is a point-of-care coagulation analysis that assesses the viscoelastic properties of both clot formation and lysis. This method of analysis is considered a more global assessment of coagulation, as it is influenced by the components of both primary and secondary hemostasis and of the fibrinolytic pathway. There is no existing data describing TEG changes in veterinary patients envenomated by the Prairie rattlesnake. Whole blood and plasma from ten healthy canine subjects was collected in citrate tubes and incubated with varying concentrations of *C. viridis* venom (0.12 mcg/mL, 0.6 mcg/mL and 6 mcg/mL) prior to running native (whole blood) or tissue factor activated (plasma) thromboelastography. TEG tracings were allowed to run until maximum amplitude (MA) was achieved or a minimum of 15 minutes. Measurements included time to clot formation (R), speed of clot formation (K), clot strength (maximum amplitude, MA) and time to clot lysis. When whole blood or plasma is incubated with *C. viridis* venom, there is a dose-dependent decrease in R, K, and MA, but no inherent fibrinolysis. At high doses of venom, the whole blood TEG was relatively hypocoagulable compared to native whole blood. However, even at high doses of venom, fibrinolysis was not reliably induced in our healthy canine patients. Further analysis of naturally envenomated dogs may provide valuable information related to the pathophysiology, treatment monitoring, clinical outcome and prognosis of envenomated patients.

8/ MESENCHYMAL STEM CELL RESPONSES TO ACTIVATION WITH TLR LIGANDS

Roxanne Benally, Andrea Escaffi, Lyndah Chow, Jonathan Coy, Val Johnson, and Steven Dow

Introduction/Significance. Mesenchymal stem cells (MSCs) are shown to stimulate wound healing and to enhance the clearance of chronic bacterial infections. From previous studies conduct in our lab, we have discovered that activation of MSCs with Toll-like receptor (TLR) ligands can enhanced their beneficial properties, particularly with respect to clearance of chronic bacterial infections. However, the mechanism(s) of TLR enhancement of MSC properties has not been previously elucidated. Therefore, the purpose of these studies was to determine how TLR activation altered MSCs properties in relation to the control of wound infection. Approach. Primary cultures of mouse MSC were established from adipose tissue biopsies. MSC were activated in vitro using LPS, plasmid DNA, or polyI:C and the effects on cell migration in response to SDF-1 were assessed using Boyden chambers. Effects of TLR activation on upregulation of the CXCR4 receptor (SDF-1 receptor) were assessed using flow cytometry and the release of chemokines were determined with TLR ligands, using specific ELISA assays. Results. Activation of MSC with the TLR3 ligand (polyI;C) resulted in significant stimulation of MSC migration, which was further enhanced by co-incubation with SDF-1. Interestingly, activation with polyI;C did not produce significant upregulation of CXCR4 expression on MSC. Incubation of MSC with LPS, polyI;C, and pDNA all produced significant release of the chemokines MCP-1, KC, and CXCL10. Studies are currently underway to compare the potency of TLR ligands for induction of chemokine release and for migration stimulation. Conclusions. We conclude that MSC activation with TLR ligands can lead to cell activation. For instance, the activation of MSC with TLR ligands can resulted in the present of an inflammatory site to recruit innate immune cells in order to enhance wound healing. In understanding the mechanism behind MSCs migration can provide valuable insight to therapeutic targets for MSCs.

9/ ENCAPSULATED SIRNA AS A THERAPEUTIC FOR PRION DISEASES

Heather Bender, Mark Zabel

Prion diseases, or Transmissible Spongiform Encephalopathies (TSEs), primarily affect sheep, cattle, cervids, and humans. The prion hypothesis suggests that these diseases are caused by the conversion of a native protein, PrP^{C}, into a misfolded protease resistant isomer called PrP^{Res}. The emergence of prion diseases in wildlife populations and the increasing impact of prion diseases on human health has led to an increase in the study of antiprion compounds. Recent studies have found antiprion compounds that can inhibit the infectious prion isomer (PrP^{Res}) or down regulate the normal cellular prion protein (PrP^{C}). These compounds are often found through the screening of drug or chemical compound libraries. However, most of these chemicals cannot cross the blood brain barrier to effectively inhibit PrP^{Res} formation in brain tissue or to specifically target neuronal PrP^{C}. Also, these compounds tend to have multiple off target effects, and are often too toxic to use in animal or human subjects. Therefore, we have proposed using intravascular siRNA that is targeted towards PrP^{C} as a safer and more effective antiprion compound. To protect the siRNA from serum degradation and RES elimination, we have encapsulated it within anionic PEGylated liposomes using protamine sulfate. Encapsulation of the siRNA with protamine sulfate results in ~90% encapsulation efficiency as compared to 40-50% efficiency without protamine. We are also using the small peptide RVG-9r to target the siRNA to nicotinic acetylcholine receptors within the CNS. In order to reduce the elimination of the peptide, we have covalently bonded it to the PEG groups of the liposomes using carbodiimide reactions. In future experiments, we will determine the effectiveness of this drug delivery system using flow cytometry.

10/ PHARMACODYNAMICS OF TRANSDERMAL MIRTAZAPINE IN HEALTHY CLIENT-OWNED CATS

Kellyi K Benson, Lara Zajic, Paul J Lunghofer, Ryan J Hansen, Luke A Wittenburg, Daniel L Gustafson, and Jessica M Quimby

Transdermal mirtazapine (TM) would be beneficial for ill cats intolerant of pill administration. The purpose of this study was to evaluate the pharmacodynamics of TM in client-owned cats. Ten client-owned cats (1-7 years) with unremarkable CBC, chemistry and urinalyses were enrolled in a randomized double-blind placebo-controlled three-way crossover study. Treatments consisted of six days of aural application of 7.5mg/dose TM or placebo gel by owner, and 1.87mg mirtazapine once orally in clinic (washout period: 7 days). Owners documented appetite, rate of food ingestion, begging, activity and vocalization daily in the home environment. On day 6 cats were observed for 8 hours in hospital; food consumed, activity and vocalization were documented hourly and trough and peak serum levels were obtained. Serum alanine aminotransferase (ALT) levels were measured after TM administration. Serum mirtazapine and compounded gel concentrations were measured using liquid chromatography/tandem mass spectrometry. Cats receiving TM for 6 days had a significant increase in appetite ($p=0.0078$), rate of food ingestion ($p=0.0078$), activity level ($p=0.0078$), begging ($p=0.0078$) and vocalization ($p=0.0039$) when compared to placebo. On day 6, cats receiving TM had a significant increase in food consumption in hospital ($p=0.0435$) when compared to placebo. Vocalization and activity were not statistically different between treatments. Gel concentrations were 105% +/- 11% of target dose. Average +/- SD trough and peak serum mirtazapine concentrations after 6 days of TM administration were 21 +/- 14.9 ng/mL and 35 +/- 18.2 ng/mL, respectively. Serum ALT remained normal after 6 days of TM administration. Average +/- SD peak serum mirtazapine concentrations after one oral dose of 1.87 mg mirtazapine were 65.1 +/- 33.5 ng/mL. Daily 7.5 mg TM administration in healthy cats resulted in a significant increase in appetite in comparison to placebo with serum concentration within the same range of single oral 1.87 mg mirtazapine administration.

11/ INCREASING DIETARY RICE BRAN AND NAVY BEAN INTAKE FOR COLORECTAL CANCER CONTROL AND PREVENTION: A RANDOMIZED-CONTROLLED PILOT INVESTIGATION

Erica C Borresen, Dustin Brown, Melissa Wdowik, Tiffany Weir, Sangeeta Rao, Corey Broeckling, Jessica Prenni, J oanne O'Malia, Marlon Bazan, Regina Brown, Rebecca Davidson, and Elizabeth P Ryan

People who consume whole grains and legumes are repeatedly highlighted for having increased longevity and lower burden of major chronic diseases. Emerging evidence supports that dietary rice bran (RB) and navy beans (NB) inhibit colon carcinogenesis. BENEFIT (Beans/Bran Enriching Nutritional Eating For Intestinal health Trial) is a community-academic partnership to advance dietary RB and NB chemoprevention research in northern Colorado (NCT01929122). Our main objectives were to pilot the feasibility of increased consumption in colorectal cancer (CRC) survivors and to examine changes in dietary intake, stool microflora, and plasma metabolome. A total of 29 CRC survivors completed the three-arm, single-blinded, randomized-controlled, 28-day dietary intervention trial with dietary RB (30g/day), NB (35g/day), or neither. Blood, urine, saliva, and stool samples were collected at baseline, 2-week, and 4-week time points and participants recorded weekly 3-day dietary food logs. We achieved 80-100% intervention compliance and participants consumed an average range of 4-9% daily caloric intake from RB or NB in prepared meals and snacks. Total dietary fiber intake was significantly increased in RB and NB diet groups by week 4 ($p<0.05$). Pilot stool microflora of 15 survivors' stool microbiome (5 participants per group) analyzed using 16S Illumina showed significant changes at the phylum level in Firmicutes, Bacteroidetes, Actinobacteria, and Verrucomicrobia. Blood plasma metabolite analysis detected more than 1000 compounds and a majority of the detected compounds responded to sex and age and roughly 20% responded to diet. These results suggest unique dietary modulation of metabolism through digestion, microbiota, and biotransformation with RB or NB consumption. Our pilot findings warrant further evaluation of biomarkers for these specific foods in a larger cohort for CRC control and prevention.

12/ EARLY ASSESSMENT OF CLINICAL EFFECTS OF MESENCHYMAL STEM CELL THERAPY IN DOGS WITH CHRONIC HEPATITIS

Allison Bradley, David Twedt, Steven Dow

Purpose & Hypothesis: Chronic hepatitis is a common condition in dogs, characterized by inflammation leading to hepatocellular loss and fibrosis. Without treatment, it often progresses to cirrhosis, liver failure, and death. Mesenchymal stem cells (MSC) are a promising new treatment for chronic hepatitis due to their anti-inflammatory, pro-regenerative, and anti-fibrotic properties. MSC therapy has shown efficacy in experimental models of liver disease, but its use in naturally occurring chronic hepatitis in dogs has not been reported. We hypothesized that MSC treatment of dogs with chronic hepatitis would suppress ongoing liver damage. Methods: Four dogs diagnosed with chronic hepatitis according to histologic and clinical criteria were treated with a series of three intrasplenic MSC injections given at two-week intervals. Parameters of liver health and function were monitored, with serum concentration of ALT (alanine aminotransferase), the most specific indicator of active liver damage, being the most informative. Changes in serum ALT concentration were compared before and after the MSC therapy using a two-tailed Wilcoxon matched pairs test (GraphPad Prism). Results: The median increase in ALT over the two measurements immediately prior to MSC therapy was 12%. The median decrease in ALT following completion of MSC therapy was 49% ($p = 0.0975$). Two of the four patients demonstrated normalization of their serum ALT concentrations. Conclusions: These data suggest that intrasplenic MSC therapy may limit ongoing hepatocellular damage due to chronic hepatitis, however, additional study in more patients is needed.

13/ INDIVIDUAL ANIMALS VERSUS THE DUNG PILE: WHICH SAMPLING STRATEGY IS BEST FOR HERD-BASED FECAL EGG COUNT SURVEILLANCE PROGRAMS?

Katy Brandes, Stacey Byers, Robert L Callan, Jessica A McArt, Lora R Ballweber

Fecal egg counts (FEC) are used widely to determine parasite loads. Although well understood in horses and sheep, more data is needed on FEC to assess surveillance programs for alpaca owners. Therefore, the purpose of this study is to: (1) Confirm that spring fecal nematode egg count patterns in intensively managed alpacas conform to the expected negative binomial distribution (overdispersed); (2) Estimate repeatability of fecal eggs per gram (EPG) values within an individual animal; (3) Estimate number of animals required to evaluate herd/pen mean; and (4) Determine whether dung pile samples accurately reflect herd/pen mean EPG. Fecal samples from individual animals from four alpaca herds (Colorado n = 3; Oregon n = 1) were collected as were composite samples from communal dung piles. FECs were conducted on all samples in duplicate and parasite elements identified to species, genus or group. One herd was also sampled over a 3-day period to determine variability of FECs within individual alpacas. Overall, FECs were low in Colorado herds, with many 0 EPGs, and moderate in the Oregon herd; overdispersion of the major parasite genera/groups was confirmed. Analyses of strongyle FECs indicated a wide range present in the coefficient of variation for individual FECs (0.13-1.10) indicating daily variation across the 3-day sampling period for some animals. Initial results, using a stochastic Monte Carlo model to estimate the number of animals necessary to accurately represent the mean herd FEC, indicate at least 5 animals/management group is necessary. No significant difference was detected in dung pile samples vs mean pen samples indicating in herds with low strongyle EPGs, dung pile samples, collected properly, may substitute for individual animal samplings in order to estimate overall herd egg shedding.

14/ PHARMACOKINETICS OF ATOPICA CAPSULES STORED AT -20C°

Anna Brown, Jeremy Bachtel, Jennifer Pendergraft, R.A.W. Rosychuk, D. Gustafson, R. Hansen, P. Lunghofer

Canine environmental skin allergy (atopic dermatitis) negatively impacts the quality of life of 10% of companion dogs. Cyclosporine (Atopica ®) is among the most successful therapies for the management of canine atopic dermatitis with reported response rates as high as 87%. Atopica® is used also to treat many immune-mediated diseases. Vomiting is the most common side effect of Atopica® and occurs in up to 30% of dogs. Vomiting often results in discontinuation of the drug. Anecdotal reports state that placing Atopica® capsules in a household freezer (-20ºC) for 30-60 minutes prior to administration decreases vomiting. This practice has gained international popularity although no published, controlled studies validate this claim. Atopica® is labeled for storage at 15-25ºC. It was prudent, therefore, to determine the pharmacokinetics of encapsulated cyclosporine at -20 ºC to ensure that appropriate cyclosporine levels are obtained prior to making the recommendation to store this product in a household freezer. A blinded, randomized crossover study was performed to compare blood concentrations of cyclosporine dosed in eight beagle dogs (4.9-5.3 mg/kg per os) after Atopica storage for 28 days at -20^{0}C versus storage at 15-25^{0}C with a 7-day washout period. Blood samples were obtained at 0, 0.5, 1, 1.5, 2, 4, 6, 8, 10 and 24h. Both capsule and blood cyclosporine concentrations were assessed via HPLC-MS/MS. There was no significant difference between cyclosporine concentrations of Atopica capsules stored at -20 C and those stored at the recommended temperature range ($p=0.80$). Similarly, in the crossover study, there were no significant differences in pharmacokinetic parameters assessed: AUC ($p=0.9273$), half-life ($p=0.71$), Cmax ($p=0.66$), Tmax ($p=0.41$). Thus, Atopica capsules can be administered to dogs after storage at -20^{0}C for approximately one month with no significant impact on drug stability or pharmacokinetics.

15/ METABOLOMICS INVESTIGATION OF HUMAN COLORECTAL CANCER TISSUE, ADJACENT MUCOSA, AND STOOL COLLECTED FROM CRC PATIENTS

Dustin G. Brown, Sangeeta Rao, Erica Borresen, Joanne O'Malia, Marlon Bazan, and Elizabeth P. Ryan

Metabolomics is an emerging analytical tool that has been applied to cancer biomarker and pathway discovery across several cancer types. Cancer metabolomic studies have revealed variable levels of amino acids, nucleosides, and carbohydrates in tissue that imply perturbations of the pentose-phosphate pathway, glycolysis, and TCA cycle catabolic pathways. Metabolite profile distinctions have been reported between colorectal cancer (CRC) patients and healthy controls. Limited evidence exits for differential expression of metabolites in CRC tissue when compared to adjacent healthy mucosa, and we hypothesize that metabolite profile differences occur across tumor locations in the colon that are involved in CRC pathogenesis. The goal of this study was to identify metabolite profile distinctions between tumor and non-tumor tissue collected from 17 recently diagnosed CRC patients during colonic resection surgery. Three different tumor locations were determined from pathology reports, and were used to correlate the metabolite differences in tissue with their expression in matched stool samples. Stool samples were self-collected by 13 patients just prior to surgery. These samples were analyzed by metabolomic analysis using GC-MS, LC-MS, and UPLC-MS platforms (Metabolon Inc., Durham, North Carolina). Nineteen Metabolites showed significant ($p\leq.05$) differences between tumor and non-tumor tissue, involving lipids, carbohydrates, and amino acids when analyzed by matched t-test. Eighty-six metabolites were significantly ($p\leq.05$) different across 3 locations within the colon after linear regression analysis. Interestingly, one-hundred and sixty-six stool metabolites showed distinctions across tumor location. Metabolic aberrations between tumor and non-tumor tissue in the same individual provides an opportunity for future studies with larger sample sizes to identify biomarkers of CRC pathogenesis by location and potentially inform prognosis and treatment.

16/ PREVALENCE OF DIROFILAIRA IMMITIS IN NON-ENDEMIC NORTHERN COLORADO ANIMAL SHELTERS

Emily Bryan, Rebecca Ruch-Gallie, Lily Ngai, Sarena Olsen, Michael R Lappin

Dirofilaria immitis is endemic to certain areas of the United States and causes a detrimental disease (heartworm disease) that is relatively costly to treat both financially and with regards to animal welfare. Though the Colorado climate is not conducive to year-round replication of mosquito species that carry the organism, microclimates exist potentially allowing year-round transmission of *D. immitis*. Animal shelters on the northern Colorado Front Range frequently transport dogs from other states including those with a high risk of *D. immitis* infection. The hypothesis for this study is that shelter dogs have a higher prevalence than the general pet dog population. The purpose of this study was to determine the prevalence of *D. immitis* antigen in the serum of dogs entering animal shelters in Northern Colorado. Samples were collected from dogs entering three shelters in northern Colorado from May to October 2014. Blood or sera from each dog were evaluated for D. immitis antigen by one of three differently commercially available kits (Heska SoloStep®, Idexx SNAP® 4Dx® Plus, Abaxis VetScan®). Positive results for *D. immitis* antigen were confirmed with a second test differing from the original test used. A total of 5 samples from the 340 shelter dogs were positive (1.5%) for *D. immitis* antigen. Three of the positive dogs came from areas considered low-risk (Northern Colorado and South Dakota). The other two positive dogs came from Arkansas, a known high-risk state. The *D. immitis* prevalence rate of 1.5% in the shelter dogs described here is 3 times greater than the currently reported prevalence rate in Colorado dog populations and 2-7 times greater than the rate reported for the participating counties (capcvet.org). Results of this study indicate *D. immitis* testing should be considered as a receiving procedure in northern Colorado animal shelters for both in-area and out-of area intakes.

17/ EFFECT OF ENTEROCOCCUS FAECIUM STRAIN SF68 ON GASTROINTESTINAL CLINICAL SIGNS OF HEALTHY CATS ADMINISTERED AMOXICILLIN-CLAVULANATE.

Amber Caress, Camille Torres, Stacie C Summers, Michael R Lappin

Amoxicillin-clavulanate is prescribed by veterinarians for a variety of feline conditions including bite wound abscesses and upper respiratory infections. As the drug has a broad spectrum against many bacteria, including anaerobes; diarrhea, vomiting, and loss of appetite can occur when it is prescribed to cats. *Enterococcus faecium* strain SF68 (SF68) is a commercially available probiotic (FortiFlora™; Nestle Purina PetCare) and has been shown to be effective in the management of diarrhea in cats. The purpose of this study was to determine if administration of SF68 in conjunction with amoxicillin-clavulanate could alleviate antibiotic-associated stool abnormalities. In this double blinded, placebo-controlled study a total of 34 young adult, healthy, mixed sex cats were randomized into two rooms. All cats were administered amoxicillin-clavulanate at 62.5 mg/cat PO twice a day for 7 days. Prior to antibiotic administration, the cats were offered canned food mixed with either *E. faecium* SF68 (equivalent to the commercial product) or the palatability enhancer used in the commercial product. The palatability enhancer, SF68, and clinical scoring were continued for an additional 7 days. Cats were monitored daily for appetite, attitude, hydration, vomiting, and consistency of feces (FS) using the following scale; 7=watery puddles to 3=normal. Overall, attitude and hydration was normal for all cats over the course of the study. The major statistical difference between groups during the 14 day SF68 supplementation period was that cats administered amoxicillin-clavulanate and placebo were more likely to have feces with a FS=7 (P=0.013). From this study we concluded that amoxicillin-clavulanate can cause gastrointestinal clinical signs. Severe diarrhea episodes (FS=7), were more likely to be in the placebo group, suggesting the concurrent administration of SF68 may decrease the severity of amoxicillin-clavulanate diarrhea in some cats. Funded by donations to the Center for Companion Animal Studies.

18/ URINE PROTEIN:CREATININE RATIO VARIABILITY WHEN COMPARING SINGLE, SERIAL, OR POOLED URINE SAMPLES FROM DOGS WITH VARYING MAGNITUDES OF PROTEINURIA CURRENTLY RECEIVING MEDICAL THERAPY.

Robert M Cerda, Sarah B Shropshire, and Jessica M Quimby

The purpose of this study was to determine the biologic variability of urine protein:creatinine (UPC) ratios in client-owned dogs on medical therapy and whether single, serial, or pooled urine samples should be used for therapeutic interventions. Seven client-owned dogs receiving medical therapy for the treatment of proteinuria were enrolled. Three urine samples were collected 24 hours apart over three days with the first two samples being collected at home by the owner and the third sample collected in the hospital via cystocentesis. Urinalysis and urine culture was performed using the sample collected in the hospital. A UPC was performed on the samples from day 1, 2, and 3. A pooled and average UPC value from the three days was also calculated. Differences between sample groups were assessed using a non-parametric repeated measures ANOVA test with Dunn's post-hoc analysis. The urinalysis from all of the dogs had a quiet sediment and negative urine culture allowing appropriate UPC interpretation. Day 1 median UPC was 2.31 (range 0.93-3.74), day 2 median UPC was 1.95 (range 0.73-2.87), day 3 median UPC was 1.72 (range 1.2-4.92), pooled median UPC was 2.01 (range 1.0-3.67), and average median UPC was 1.91 (range 0.95-3.59). No statistical differences between a single UPC measurement on day 1, 2, or 3, the pooled UPC value, or the average of the three single day measurements was found. Preliminary results show that a single UPC measurement is valid for the assessment of proteinuria with regards to determining response to therapy and if therapeutic adjustments are warranted. However, all of the dogs had a lower magnitude of proteinuria (UPC < 5.0) thus client-owned dogs with higher magnitudes of proteinuria are needed in order to determine if a single UPC measurement is accurate in this group of dogs as well.

19/ RNA VIRUSES TARGET EXORIBONUCLEASE XRN1 TO PROMOTE PATHOGENESIS

Phillida A Charley, Stephanie Moon, John Anderson, Carol Wilusz, and Jeffrey Wilusz

The 5'-3' exoribonuclease Xrn1 is a key enzyme in a major pathway of mRNA decay in all eukaryotic cells that regulates 20-50% of changes in mRNA levels due to external stimuli. Recent evidence has also indicated that Xrn1 may be a major player in coordinating mRNA transcription and decay so that the steady-state expression of many genes is effectively 'buffered' in cells. Given its key roles in cellular gene expression and its cytoplasmic location, we hypothesized that Xrn1 represents a very attractive target for RNA viruses that wish to disarm the cell by shutting down changes in gene expression that the cell may try to use to combat the viral infection. Consistent with this hypothesis, we have recently demonstrated that members of the *Flaviviridae* (e.g. Dengue, West Nile, Hepatitis C and Bovine Diarrhea viruses) possess structures in either their 3' or 5' untranslated regions (UTRs) that can stall and repress Xrn1 when the cellular enzyme tries to degrade these viral RNAs. Concomitant with the stalling and repression of Xrn1 activity in infected cells, we observe a major dysregulation of cellular mRNA decay, resulting in the stabilization and accumulation of many normally short lived cellular transcripts. This massive dysregulation of cellular gene expression likely has significant implications in viral-induced cytopathology and viral pathogenesis. In order to see if other RNA viruses share a similar strategy and target the cellular Xrn1 enzyme to gain an upper hand during infection, we have recently focused our attention on the segmented *Arenaviridae* (e.g. Junin virus). Interestingly, we have found evidence for Xrn1 decay intermediates produced from the 3'UTRs of Junín virus mRNAs that would be consistent with Xrn1 stalling. Thus Xrn1 may represent a heretofore unrecognized important cellular target that is inactivated by several major RNA viruses during infection with numerous associated pathological implications.

20/ BOVINE HERPESVIRUS 4 NOT DETECTED IN FREE-RANGING DOMESTIC CATS FROM CALIFORNIA, COLORADO, AND FLORIDA

Elliott S Chiu, Ryan M Troyer, Michael R Lappin, and Sue VandeWoude

Previous studies have reported that domestic cats can be naturally infected with bovine herpesvirus 4 (BHV4). Cats experimentally inoculated with BHV4 develop clinical signs involving the urinary tract, which led to the hypothesis that natural infection with BHV4 may be associated with feline urolithiasis. Recent studies have had difficulty reproducing these observations, and the question of whether BHV4 is a pathogen in cats has remained equivocal. We used a sensitive, specific nested PCR protocol specific to the BHV4 thymidine kinase receptor gene to screen free-ranging domestic cat blood DNA samples (n=101) collected from shelters in California, Colorado and Florida. Cats within this cohort were positive for seven other common pathogens of domestic cats, demonstrating the relatively high exposure of this population to endemic feline infections. In contrast, all domestic cat blood samples were negative for BHV4, while BHV4 containing tissue culture extracts were strongly positive. Our results suggest that infection of domestic cats with BHV4 is either rare or nonexistent. We thus conclude that BHV4 is unlikely to be a major common pathogen of domestic cats.

21/ DERIVATION OF MESENCHYMAL STEM CELLS FROM CANINE INDUCED PLURIPOTENT STEM CELLS

Lyndah Chow, Johnson Valerie, Steven W Dow

Mesenchymal Stem Cells (MSCs) are used for their immunosuppressive properties and ability to differentiate into mesenchymal-lineage tissues. Clinical use MSCs are isolated from various tissue sources that require invasive extraction procedures. Methods to grow MSCs from adipose or bone marrow are laborious and time consuming, and the true MSCs grown represent only up to 0.01% of the total cell population. Using ciPSCs to derive MSCs will provide a more easily accessible population that has been uniformly screened and verified, and can be propagated for multiple generations. Previous methods to derive MSCs from iPSCs involve treatment with multiple growth factors, or multiple lineage differentiation steps. Using induced pluripotent stem cells derived from canine skin biopsy fibroblasts, we used a small molecule treatment to generate MSCs for clinical use. This is a simple method that requires the addition of only one small molecule and is less time consuming than all previously published differentiation protocols. To initiate differentiation, a confluent monolayer of epithelial like ciPSC was cultured in serum free media and treated with a small molecule transforming growth factor-β pathway inhibitor SB431542. Differentiation was then completed by switching to conventional MSC media containing 10% fetal bovine serum. Treatment with SB431542 produced a downregulation of pluripotent genes, and after multiple passages in MSC media, retained the typical MSC characteristics and morphology. The resulting population of MSCs derived from ciPSCs maintained MSC like characteristics past 20 generations. Treatment with SB431542 eliminated the need of a feeder culture or an intermediate embryoid body formation, providing a clinically relevant and efficient system for generating MSCs.

22/ IDENTIFICATION OF PARTICULATE MATTER 2.5 AS A POSSIBLE TRIGGER FOR IDIOPATHIC PULMONARY FIBROSIS

Kenny Christofferson, Conner Jackson, and Alan R Schenkel

Particulate matter 2.5 (PM 2.5) is an air-borne pollutant that is commonly found in urban settings and is comprised of acids, organic chemicals, metals, and soil/dust (3). The particles are generally emitted in smoke or haze and are 2.5 micrometers or less in diameter (3). PM 2.5 is capable of being inhaled into the lungs and has been shown to have negative effects on the cardiovascular system (1). PM 2.5 may be a possible trigger for Idiopathic Pulmonary Fibrosis (IPF) because exposure to environmental air-borne particles has been identified as a risk factor for the disease (5). This study proposes that PM 2.5 will degrade endothelial cell monolayers and increase cell permeability in PECAM deficient endothelial cells in vitro. In order to test this hypothesis, both PECAM deficient and PECAM-positive cells need to be analyzed. At the time of experiment, PECAM deficient endothelial cells were not available. We elected to observe the effects of PM 2.5 on PECAM-positive endothelial cells so that further studies can utilize our results. We cultured C166 endothelial cells and then treated the monolayers with PM 2.5 that was derived from Baltimore, MD. We then observed monolayers for cell death and increased cell membrane permeability by measuring electrical resistance of the monolayer. We found that exposure of C166 cells to PM 2.5 did not induce cell monolayer degradation

23/ EXPRESSION OF THE MUTANT DMPK MRNA IN IMMORTALIZED MYOTONIC DYSTROPHY PATIENT CELLS ALTERS CELLULAR MRNA STABILITY

Stephen J Coleman, Alexa Dickson, Annie Zhang, Hend Ibrahim, Wencheng Li, Mainul Hoque, Bin Tian, Jeff Wilusz, and Carol J Wilusz

Type 1 myotonic dystrophy (DM1) is a chronic and progressive neuromuscular disease resulting from the expansion of a trinucleotide CTG repeat in the 3'UTR of the *dystrophia myotonia* protein kinase (DMPK) gene. Pathogenesis is linked to the toxic accumulation of repeat-containing RNA in nuclear foci and sequestration of RNA-binding proteins, such as *Muscleblind* (MBNL1). MBNL1 and CELF1, another protein whose function is affected in DM1, are both implicated as regulators of mRNA decay leading to the hypothesis that the stability of cellular mRNAs may be altered in DM1 which could lead to changes in protein levels and contribute to pathogenesis. To determine the impact of DM1 on cellular mRNA stability, we established a cell culture system using reversibly-immortalized DM1 patient myoblasts. These cells recapitulate important aspects of the DM1 phenotype (presence of nuclear foci, increased CELF1 protein abundance and altered mRNA splicing). Initial results demonstrate that SOX9 mRNA, which encodes an important developmental transcription factor, is stabilized in DM1 patient myoblasts compared to control cells. Knockdown of mutant DMPK mRNA in DM1 patient cells results in restoration of SOX9 mRNA decay to normal rates. To identify other transcripts whose decay is altered in DM1, we applied a global strategy to measure mRNA half-lives in patient and control myoblasts. We also examined decay rates in patient and control cells that had been treated with siRNAs targeting DMPK to remove the toxic transcripts. Comparison of the four treatments identified 516 transcripts with altered stability that correlated with the expression of mutant DMPK mRNA. A majority of these (393 transcripts) are stabilized in the patient myoblasts compared to control cells. Overall, these data support the hypothesis that mRNA stability is affected in DM1. Further analysis will determine the mechanisms behind the changes in mRNA stability and how these changes may contribute to pathogenesis.

24/ INVESTIGATION OF GENETIC PREDISPOSITIONS TO COPY NUMBER VARIATION IN THE PARENTS OF AUTISTIC CHILDREN

Hailey N Conover and Juan Lucas Argueso

The first genetic causes of autism spectrum disorder (ASD) to be identified were de novo gene copy number variations (CNVs) in a child's genome that were not found in their parents. Up to 8% of ASD children carry a de novo CNV as a likely causal source for their symptoms. Increased CNV mutagenesis might be partly responsible for the recent rise in ASD cases, perhaps triggered by the interaction between genetic predispositions in the parents of ASD children and exposure to environmental mutagens. Data from genomic analyses of CNV-prone loci in affected patients and mechanistic studies in the yeast model system suggest that de novo recurrent CNVs arise from non-allelic homologous recombination (NAHR) between repeated DNA sequences. Existing common genetic variants in the human population may sensitize certain individuals to CNV mutagenesis by increasing NAHR, thus leading to a higher burden of de novo CNVs in their offspring. Using an innovative assay in yeast cells, I will test the hypothesis that inaccurate homologous chromosome synapsis during meiosis leads to increased rates of NAHR. I will investigate whether defects in genes conserved from yeast to humans that act in meiotic cycle control are candidates for predisposition to high CNV mutagenesis in humans. In the future, these results may support strategies for genetic counseling of prospective parents, and studies that will search for mutagens that can specifically perturb the meiotic NAHR pathway.

25/ EFFECT OF THE PROBIOTIC, ENTEROCOCCUS FAECIUM (SF-68), ON RESOLUTION OF ACUTE DIARRHEA IN YOUNG SHELTER CATS

Elena T Contreras, Meagan Chriswell, Michael R Lappin

Acute, non-specific diarrhea is common in shelter cats and leads to adoption delays, loss of shelter resources, and occasionally results in euthanasia. Oral supplementation of the probiotic *Enterococcus faecium* (SF68) (Nestle Purina FortiFlora™) lessens the incidence of diarrhea in shelter cat groups. The hypotheses in this study were that individual shelter cats with acute diarrhea would experience quicker resolution, less recurrence, and less severe diarrhea when fed SF68, compared to cats fed a standardized diet alone. A total of 26 shelter cats (4 months - 3 years) with diarrhea for at least two days were entered into the study and were randomly assigned to either the SF68 or placebo group. Cats were administered SF68 (n = 13) or placebo (n = 13) with food every morning for 10 days. Trained scorers masked to the treatment groups recorded a daily fecal score (FS) using a standardized system. Two cats that never responded were excluded from the analyses. Cats fed SF68 had an improvement in FS more quickly (median 2.0 days; range 2.0 – 4.0 days) than control cats (median 3.0 days; range 2.0 – 5.0 days; p=0.15), had resolution of diarrhea (FS < 4) more quickly (median 3.0 days; range 2.0 - 9.0 days) than control cats (median 4.0; range 2.0- 6.0 days; p=0.55); and had recurrence of diarrhea less frequently (3/11; 27.3%) than control cats (6/13; 46.2%; p=0.42). Percentages of stool samples with a FS >4 during Days 2-10 were 15% for placebo cats and 13% for SF68 cats; of these, very severe diarrhea (FS>5) occurred more frequently in the placebo group (4/15; 27%) versus the SF68 group (0/10; 0%; p=0.12). While none of the differences are significant, the numerical trends to date suggest feeding SF68 to cats with acute diarrhea may have clinical benefit.

26/ CONSUMPTION OF VIYO RECUPERATION – RENAL IN CATS WITH STABLE CHRONIC KIDNEY DISEASE AND ITS EFFECTS ON BIOCHEMICAL PARAMETERS

Crystal Cooley, Jessica Quimby, Laura Martin, and Michael Lappin

Viyo may increase appetite and water consumption. This pilot study evaluated effects of Viyo Recuperation™ – Renal (VRR) on biochemistry in cats with chronic kidney disease (CKD) and compared palatability in CKD cats with normal appetite versus decreased appetite. 12 cats with stable CKD (creatinine 2-10 mg/dl) were enrolled in two groups – 6 with normal and 6 with decreased appetite. Blood work, urinalysis, and blood pressure were measured prior to enrollment. 30 ml VRR was offered prior to meal daily for 14 days. Amount voluntarily consumed in 24 hours was measured. Owners scored appetite and water consumption daily. Weight and biochemistry were assessed on day 0, 7, and 14. Wilcoxon test compared results pre and post treatment. Prior to trial, median food consumption by cats with normal appetite was 100% (range 100-100%), and median food consumption by cats with decreased appetite was 50% (range 25%-100%), but there was no significant difference in biochemistry between groups. Of CKD cats with normal appetite, 5 of 6 consumed >70% of total 14 day volume of VRR, and a statistically significant decrease in serum phosphorus (P = 0.03) was detected after 14 days of Viyo ingestion. There were no differences in other biochemical parameters or weight. In CKD cats with decreased appetite, 3 of 6 cats consumed >70% of the total 14 day volume of VRR. There were no differences in biochemistries between cats that consumed < 70% or > 70% of the total 14 day volume of VRR. Overall, VRR was palatable to cats with CKD and decreased serum phosphorus levels in cats with normal appetite. Cats with CKD and decreased appetite were less likely to voluntarily consume VRR. Data collection continues to determine overall effects on appetite and biochemical findings in a greater number of cats. Funding provided by Viyo International.

27/ ACUTE LEUKEMIA IN DOGS: DIAGNOSTIC CRITERIA, TREATMENT AND OUTCOME.

Kaitlin Curran, Paul Avery, and Anne Avery

Introduction: Acute leukemia is recognized in dogs but specific diagnostic criteria is lacking. Although clinical outcome is assumed to be poor, there are no studies that have addressed outcome directly. The objectives of this study were to develop specific, objective criteria for the diagnosis of acute leukemia, and to evaluate a population of dogs with acute leukemia based on these criteria. Methods: Dogs were included if they met one of the following criteria: greater than 1000 /uL CD34+ cells based on flow cytometry; or had 20% or greater blastic cells in the peripheral blood or bone marrow and had bi-cytopenia or pancytopenia. Data were collected retrospectively from 47 dogs from one institution over a 10-year period. Signalment, clinical presentation, diagnostics, initial treatment specifics, best response and overall survival (OS) time were evaluated. OS estimates were calculated using the Kaplan-Meier method. Results: Forty-seven dogs met the inclusion criteria. Three dogs had cytologic evidence of large granulocytic lymphocytic leukemia and 44 dogs had evidence of acute leukemia. Of the acute leukemia population, 17 dogs had flow cytometry performed and 16 were CD34+. Thirty-nine dogs had some type of treatment and 25 were treated with chemotherapy. Median OS was 15 days (range 0-209). Treatment with chemotherapy significantly prolonged survival (p = 0.001; median OS with chemotherapy = 26 days; median OS without chemotherapy = 9 days). Severe (grade III or IV) anemia at presentation was not a significant prognostic factor, however, grade III or IV neutropenia at presentation did have a significant impact on survival (p = 0.02). CD34+ cases had similar survival outcome to cases diagnosed on clinical parameters. Conclusions: Both clinically and phenotypically defined acute leukemias have a guarded prognosis.

28/ INCIDENCE RATE AND EFFECTS OF PERSISTENT MATING-INDUCED ENDOMETRITIS (PMIE) IN QUARTER HORSE MARES

Katherine G DeHaan, Ryan A Ferris, Patrick M McCue

The goal of this retrospective study was to determine if a relationship exists between mare age and accumulation of intrauterine fluid post breeding and if this fluid affects pregnancy rates in a clinical setting. The hypotheses were: a) older mares will have a greater incidence of intrauterine fluid retention 24 hours post breeding and b) a reduction in pregnancy rates will occur in mares that retain intrauterine fluid for greater than 24 hours post breeding. Mares were managed at the Equine Reproduction Laboratory at Colorado State University. Inclusion criteria for the study were as follows: Quarter Horse mares, ovulation within 48 hours of breeding, and pregnancy or embryo flush result recorded. Treatments for intrauterine fluid were performed at the discretion of the attending clinician. Data was compared by a contingency table utilizing Fishers Exact test. Data was presented as the mean +/- SD. The overall incidence of PMIE was 36%. A significant ($p<0.05$) effect of age was observed on the incidence of fluid >24 hours (Table 1). A significant reduction in pregnancy rates was observed in mares that had intrauterine fluid 48 hours post AI compared to mares with normal or no fluid (34% vs 60%). There was no effect of intrauterine fluid on pregnancy rates within age groups (Table 1). There was a trend ($p=0.052$, 0.054, respectively) for an effect of age on conception rates for mares 16-20 and >21 years of age. In conclusion, older mares (> 16 years of age) have an increased incidence of PMIE subsequent to insemination and should be monitored closely following breeding. If intrauterine fluid is present at 48 hours post breeding, a reduction in pregnancy rates is observed. *Table 1 included in presentation

Keywords: conception rate, equine, intrauterine fluid, persistent mating-induced endometritis

29/ PROTEOMIC CHARACTERIZATION OF EXOSOMES RELEASED FROM HUMAN MACROPHAGES INFECTED WITH MYCOBACTERIUM TUBERCULOSIS

Gustavo Diaz, Nicole Kruh-Garcia and Karen Dobos

Exosomes are extracellular nanovesicles that are released by almost all nucleated cells. The composition of exosomes depends on the cell of origin and on the physiological status of the host. Exosomes have a great potential to be used as biomarkers since they can be isolated from virtually all human biofluids (serum, urine, etc). In our laboratory, it was recently demonstrated that exosomes isolated from *Mycobacterium tuberculosis* (*Mtb*)-infected patients contained mycobacterial peptides that could be potential biomarkers for tuberculosis. However, the highly complex and variable population of exosomes found in human serum could make the identification of the *Mtb* biomarkers very difficult, affecting drastically the sensitivity of potential routine tests. We believe that exosomes released from *Mtb*-infected macrophages exhibit a characteristic protein array that allows for a selective isolation/concentration of these exosomes, facilitating the detection of the potential *Mtb* biomarkers. We analyzed the protein composition of exosomes released from *Mtb*-infected macrophages (originated from THP-1 monocytes), comparing it with exosomes from uninfected cells. In addition, we evaluated the proteins that were localized in the exosomal membrane using a novel biotinylation strategy. Our proteomics mass spectrometry analysis identified six human proteins that were more abundant in the membrane of exosomes from infected cells (*Cathepsins S and D*, *Elongation Factor 1*, *Coronin, Myosin-9* and *Lamin A/C*). In addition, all *Mtb* proteins identified were found to be located in the lumen of the exosomes according with the biotinylation pattern, implying that these pathogen specific proteins are loaded into the exosomes during its intracellular processing. After validation of our candidate proteins (more abundant in exosomes from *Mtb*-infected cells and membrane-associated), our next step will be to develop affinity exosomes-separation experiments, to isolate/concentrate exosomes from complex matrices such as human serum.

30/ DETECTION OF SALMONELLA ENTERICA IN THE DAIRY ENVIRONMENT USING A COMMERCIALLY AVAILABLE LATERAL FLOW IMMUNOASSAY

Enrique Doster, Brandy A. Burgess, Justine P. Elam, Kristy L. Pabilonia, Nathan M. Slovis, Paul S. Morley

Salmonella enterica is one of the most common causes of health care-associated infections in veterinary hospitals. Its control is often dependent upon rapid detection; however, standard detection methods may take up to 3-5 days depending on the type of sample and test being employed. The purpose of this study was to describe the sensitivity and specificity of a rapid, point-of-care, commercially available lateral flow immunoassay (LFIs) for detection of *Salmonella enterica* in environmental samples. Environmental samples were collected from high traffic areas in dairy operations in Colorado and tested in parallel using standard aerobic culture and LFIs. Each environmental sample was pre-enriched in 90mls buffered peptone water for 18hrs at 43°C, then 1 ml was passed into 10ml of tetrathionate broth for 18hrs at 43°C, and plated on XLT4 for 18hrs at 43°C. Enriched cultures were also tested using LFIs. Overall, LFIs were not as sensitive as aerobic culture for the detection of *S. enterica* in environmental samples. Out of 190 samples, 106(56%) were culture-positive and only 38(20%) were test-positive by LFIs. In general, LFIs showed a limited ability to detect isolates that were serogroup C1 and K. Upon stratified analysis, among farms with isolates that were not C1 or K, 43 of 67 samples (64%) were culture-positive and 38 (57%) were test-positive by LFIs. The findings of this study suggest that detection rates for enriched culture and LFIs are comparable but may be farm-specific. Further testing is needed to determine the effect of strain and/or serotype on the utility of these test strips in clinical applications.

31/ ISOLATION AND CHARACTERIZATION OF PRIMORDIAL FOLLICLES FROM CANINE OVARIES

Kathleen M. Eddy, Dr. Terry M. Nett, Dr. Dan L. Baker, Dr. Jason E. Bruemmer, Dr. Terry R. Spraker, and Dr. Doug C. Eckery

Overabundant feral dog populations create several problems nationally and internationally, including transmission of disease to humans and livestock, livestock predation, and responsibility for numerous bite injuries. Means of population control have largely been restricted to methods such as lethal culls and spay/neuter clinics, which limit both the number of dogs treated as well as being cost prohibitive. While there are currently non-surgical sterilization choices available, many have limited efficacy and require supplemental applications. An option enabling permanent sterility of large populations with a single application to each individual is therefore an attractive choice. The finite population of ovarian primordial follicles represents the total reproductive potential of an individual; depletion of this store of follicles would result in permanent sterility. The objectives of this work are establishment of methods to isolate and culture primordial follicles from canine ovaries in conjunction with proteomic analysis determination of pathways and mechanisms that are essential for follicular growth and survival. The overall aim of this research is to identify compounds that can be utilized to cause primordial follicle depletion.

32/ IDENTIFICATION OF SUSCEPTIBILITY LOCI FOR SARCOMATOID CHANGE AND METASTASIS IN PULMONARY ADENOCARCINOMA

Elijah F Edmondson, Daniel M Gatti, Christina M Fallgren, Rishi R Rampersad, Mike M Weil

Pulmonary sarcomatoid carcinomas (PSCs) are a subset of non-small cell lung carcinomas with an aggressive clinical course and a high rate of metastasis. The sarcomatoid component of PSCs is thought to arise as the result of epithelial-mesenchymal transition (EMT) during carcinogenesis progression. In the present study, we have identified a spontaneous model for PSCs in a genetically heterogenous population of mice. Of 344 mice with pulmonary adenocarcinoma, 30 developed PSC (10 of which metastasized). Metastatic disease was significantly more common in mice with PSC ($p < 0.001$). To verify that the sarcomatoid component of these tumors indeed arose from the pulmonary adenocarcinoma, a subset of tumors were analyzed for Club Cell antigen (CC10) and Surfactant protein C (SFTPC). To determine how host genetic variation contributes to the development of this trait, each mouse was genotyped using dense single nucleotide polymorphism (SNP) arrays for quantitative trait loci (QTL) mapping. Three QTLs were identified which were associated with the transition to sarcomatoid carcinoma and metastasis. One QTL on chromosome 1 was narrow and contained a single protein coding gene: *Prox1*. Although overexpression of PROX1 has been shown to promote EMT in colon cancer, germline polymorphisms in *Prox1* that control the susceptibility to EMT have not been identified. Analysis of SNPs in *Prox1* between the eight founders used to create our mouse model identifies several SNPs in the 3'UTR of the mRNA product. These results identify multiple QTLs associated with the development of PSC and metastasis and indicate that alteration in PROX1 expression is a likely event during epithelial-mesenchymal transition in pulmonary adenocarcinomas.

33/WEST NILE VIRUS SURVEILLANCE IN FORT COLLINS, 2006-2013

Joseph R Fauver, Jessica A Schurich, Nathan D Grubaugh, Greg D Ebel, Lars Eisen, Chet G Moore

West Nile Virus (WNV) was first detected in Colorado in 2002 and caused a major epidemic in the eastern portion of the state in 2003. The virus subsequently spread to the rest of Colorado and is maintained on the Front Range through an enzootic cycle involving passerine birds and *Culex tarsalis* and *Cx. pipiens* mosquitos. The city of Fort Collins established an active mosquito surveillance program in 2003 that involves weekly mosquito trapping, virus testing, and data analysis. Current mosquito trap sites throughout the city have been maintained since 2006. To elucidate trends during years and between years, we use weekly historical trap data over the 8 year study period for (1) *Culex* abundance, (2) infection rate and (3) vector index. We also assessed if the city of Fort Collins is homogenous for these 3 entomological measures. In order to do this, 4 geographically and logistically relevant zones were established throughout the city. We hypothesized that Fort Collins is heterogeneous for these measures. This hypothesis was tested by comparing values generated from historical trap data for each quadrant using various statistical analyses. Our results demonstrate that clear patterns for *Culex* abundance, infection rate, and vector index can be established on a yearly basis. We also demonstrate that Fort Collins is heterogeneous for the 3 entomological measures assessed, and that the city should be evaluated on a zone by zone basis for WNV surveillance with particular focus on zones with demonstrated increased entomological risk measures.

34/ CHANGES IN LEUKOCYTE POPULATION SUBSETS IN GOATS EXPERIMENTALLY INFECTED WITH MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS

Darcy M Fletcher, Marcela Henao-Tamayo, Ann M Hess, and Torsten M Eckstein

Johne's disease, caused by M*ycobacterium avium* subspecies *paratuberculosis*, is a chronic infectious disease of the intestine in domestic and wild ruminants divided into four stages: (1) silent stage, (2) subclinical stage, (3) clinical stage, (4) advanced clinical stage. There are many studies on the immune responses in the clinical and advanced clinical stages, but there is a large knowledge gap on the first two stages. We report our findings on the leukocyte subset population changes during the first two and a half years of infection in the goat model. Peripheral blood was collected at weeks 1, 3, 5, and 8 and every four weeks thereafter from the infected and negative control goats. No significant changes in the overall granulocyte, lymphocyte, or monocyte populations were observed in the first year, but we detected an increase of the granulocytes and monocytes towards the end of year two. CD4+ T cell populations increase for the infected goats during the first year and normalized at the end of the two and a half years, while the CD8+ T cells showed the opposite trend. The gamma delta T cells during year one demonstrated differences between infected and uninfected goats with the infected having lower numbers of gamma delta T cells; this T cell subset was normal throughout the end of the two and a half years. We also analyzed the two antigen presenting cell markers MHCII and CD1. We noticed a short increase of both markers at the end of year one, but could not detect the same differences during year two and three. After year one there is a trend for an increase of granulocytes and monocytes in the peripheral blood of infected goats while the T cell subpopulation differences switch from CD4+ T cells and gamma delta T cells to CD8+ T cells.

35/ CYTOKINE ENZYME-LINKED IMMUNOSORBENT ASSAYS FOR THE DETECTION OF JOHNE'S DISEASE DURING PRE-CLINICAL STAGES IN EXPERIMENTALLY INFECTED GOATS

Allison B Genis, Darcy M Fletcher, Ann M Hess, and Torsten M Eckstein

Johne's Disease (JD) is a chronic gastrointestinal infection caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) that is prevalent in dairy cattle and other ruminant species. The disease is well characterized by asymptomatic and symptomatic phases, but the immunological changes that occur throughout the disease are not well understood. Furthermore, commercially available diagnostics seem to work during the clinical phase, but it would be advantageous for dairy farmers to know the infectious status of individual animals before the chronic diarrhea is present. A long-term study in experimentally infected animals is needed to define the time when cellular and humoral diagnostics would be useful. Using a caprine model, we infected nine goat kids with MAP (10^9 CFU) inoculated milk on three consecutive days. Ten other goats were used as a negative control. In addition to several serological diagnostic tests, an established cytokine enzyme-linked immunosorbent assay (ELISA) protocol was used to test for JD during the first two years of infection. Both IFN-γ and IL-10 antibodies were used as the sandwich detection antibodies for the assay. Para-LP-01, a major cell wall lipid antigen that is subspecies-specific, purified protein derivatives of MAP and of *M. bovis* were used as specific antigens. The assay was conducted every four weeks for two years of the study and statistically analyzed. While individual samples from inoculated goats were reactive at several time points, no clear trend or period of reactivity could be identified. Furthermore, there were no statistically significant differences in cellular response towards the lipid antigen and only a few time points with statistically significant differences for the bovis PPD or the Johnin PPD. The time point of detectability of this infection via cellular immune assays could not yet be determined, probably due the current stage of the disease for the nine infected goats.

36/ DEVELOPMENT OF AN IMMUNOCYTOCHEMISTRY MAST CELL TUMOR PROFILE

Meghan E Gibas, Brad Charles, EJ Ehrhart

Mast cell tumors (MCT) are a common neoplasm of dogs and exhibit large variability in biological behavior. As such, prognostic indicators are crucial in predicting the behavior of a particular MCT. Currently, histological grading, immunohistochemistry (IHC) for KIT and Ki67 proteins, and PCR for exons 8 and 11 cKIT mutations are the standard components of a MCT profile. This requires an invasive biopsy to obtain a tissue sample of the lesion in question. The purpose of this study was to determine if a similar profile could be performed on cytology samples obtained with a less-invasive fine needle aspirate, providing comparable prognostic capabilities. We developed an immunocytochemistry (ICC) protocol to stain KIT and Ki67 and completed PCR for exons 8 and 11 cKIT mutations on a single cytology slide for 61 MCT samples. Profiles were completed by analyzing the cellular distribution of KIT as Type I, II, or III and determining the number of cells expressing Ki67 of the total cell number. The results of these ICC profiles were compared to tissue profiles of the same tumors. This study shows that KIT Type II pattern is distinct and can be differentiated, with 100 percent of the ICC cases agreeing with the IHC results. Distinction between Type I or III patterns was less optimal and new evaluation parameters are currently being defined. Agreement between the mitotic index and Ki67 index was found for 70 percent of the cases. This pilot study has helped to establish working protocols and provide proof of concept that MCT profiles can potentially be performed using cytological samples. This valuable information will be expanded upon in a future study that will further explore the correlation between ICC and IHC for canine MCT profiles.

37/ SEROSURVEILLANCE FOR BRUCELLOSIS IN PACIFIC WALRUS (ODOBENUS ROSMARUS) IN ALASKA

Elizabeth W Goldsmith, Kimberlee B Beckmen, and Francisco Olea-Popelka

Brucellosis is a zoonotic infection of global importance caused by Gram-negative bacteria from the genus *Brucella*. We assessed brucellosis seroprevalence in Pacific walrus in Alaska with the Rose Bengal test and an indirect ELISA (iELISA), compared the results from these two tests for assessing seroprevalence in Pacific walrus, and identified walrus and sample characteristics of interest associated with positive results for the different test types. We analyzed 207 serum samples collected by the Alaska Department of Fish and Game between 1979 and 2013. We examined samples with two serologic tests, the Rose Bengal test and an iELISA. For the iELISA, we examined two cut-points, 74% positivity and 80% positivity. We analyzed data with proportions, Cohen's kappa, odds ratios, and chi-squared tests. We found that 8.7% (n=18) of Pacific walrus were seropositive by the Rose Bengal test. At the 74% iELISA cut-point, we determined that 18.8% of samples were positive (n=39) and 15.9% of samples were positive (n=33) at the 80% iELISA cut-point. We found that there was slight agreement between the Rose Bengal test and iELISA (74% cut-point k=0.14; 80% cut-point k=0.18). We identified both age class and collection year as risk factors of interest. We propose that further studies examining Pacific walrus tissues with PCR or bacterial culture are needed to establish an association between brucellosis seropositivity in Pacific walrus and infection with marine *Brucella* spp.

38/ VITAMIN D DEFICIENCY IN SLED DOGS

Jennifer L Hafferman, Arleigh Reynolds, and Kriya Dunlap

This study was performed to determine the vitamin D levels in sprint trained sled dogs located in Salcha, Alaska. Blood samples from 16 healthy sled dogs were collected: 8 physically fit and 8 sedentary. Physically fit sled dogs were dogs that participated in a uniform exercise protocol for a minimum of 2 months prior to sample collection. Sedentary sled dogs did not participate in an exercise protocol and their activity was limited to individual activity within their housing space. Five ml samples was collected in EDTA vacuutainer tubes and centrifuged on site. Plasma was collected to measure fasting 25-hydroxy-vitamin D with an ELISA kit manufactured by Enzo Life Sciences. Standard curves were run using standards within the kit. Analyses of these two groups were performed using a 2 sample t-test on GraphPad Prism Version 5.0. The parameters for the 2 sample t- test included: 2 tailed and 95% confidence interval. The mean Vitamin D level was 44.2 ng/ml for the exercised group and 63.22 ng/ml for the sedentary group. The results showed a trend of lower vitamin D levels within the exercised group when compared to the sedentary group. These results were not found to be statistically significant with a p value of 0.1679. There is a trend of lower vitamin D levels when comparing the exercised group to the sedentary group. Two possible outliers, one from each group, were identified for having values outside of the norm of their respective groups. Outlier analysis found both points to be on the extreme tail end of the standard deviation. With the two points removed experimentally a lower p value was achieved thus suggesting that more power and a higher "n" are needed for future studies. More research needs to be done on essential vitamin D levels in canines and canine athletes.

39/ RELATIONSHIPS BETWEEN INFLAMMATORY CYTOKINES AND LEUKOCYTE TELOMERE LENGTH IN HEALTHY ADULTS WITH AND WITHOUT A HISTORY OF COLORECTAL CANCER

Gregory Harbison, Erica Borresen, Genevieve Forster, Sangeeta Rao, Tiffany Weir, Susan M. Bailey and Elizabeth P. Ryan

Recent epidemiological studies suggest increased fiber intake from whole grain foods such as brown rice or dry beans reduce risk for colorectal cancer development and recurrence. We investigated the relationship between dietary rice bran (RB) and navy bean (NB) consumption by healthy adults with and without a history of colorectal cancer on plasma inflammatory cytokine levels and leukocyte telomere length (TL). The primary goal of this study was to determine relationships between cytokine levels (TNF-α, IFN-γ, IL-2, IL-4, IL-6, IL-8, IL-10, sCD40L, VEGF), telomere length, and dietary intake after 4 weeks. Twenty-one colorectal cancer survivors and eleven healthy volunteers were randomized by BMI, total caloric intake and sex to a control, RB, or NB diet group. No statistically significant differences were observed during interim analysis for cytokine levels or telomere length across dietary groups when compared to control and for changes from baseline. Mean telomere length was inversely associated with increasing age ($p<0.001$) as measured by interphase FISH. Age was not significantly associated for TL measured by qPCR. Further, TL measurement by qRT-PCR and interphase FISH did not display a strong correlation. Non-significant trends were observed in individuals with relatively long TL possessing relatively high levels of pro-inflammatory cytokines IFN-γ, IL-2, IL-4, IL-6, and IL-8, and low levels of pro-inflammatory TNF-α and anti-inflammatory IL-10. These results will be used for sample size and power calculations needed to assess significant changes in inflammatory cytokines or telomere length with a longer dietary intervention study. This work was supported by NIH NCI r21-CA161472-02 and the Norharvest Dry Bean Health Research Program.

40/ INVESTIGATION OF MECHANISMS OF MITOTIC RECOMBINATION IN YEAST: A MUTATIONAL AVALANCHE FOR SOME

Victoria Harcy and Juan Lucas Argueso

In non-allelic homologous recombination, a non-reciprocal translocation is indistinguishable at the karyotype level whether it originated from a reciprocal conical homologous recombination (CHR) event or from a non-reciprocal break-induced replication (BIR) event. Recently, data was published showing that exposure to methyl methanesulfonate (MMS) causes DNA base damage to long ssDNA tracts transiently formed during BIR resulting in a signature pattern of non-random, co-localized hypermutation, closely resembling mutation clusters found in cancer. In contrast, reciprocal CHR is not associated with extensive tracts of DNA synthesis, thus, should not lead to hypermutation. We plan to take advantage of this new finding to assign a mechanism of formation to the non-reciprocal translocations identified through genome stability assays. For this investigation, we will select for generation of a functional copy of the *LYS2* gene from two different mutant alleles. One allele is present in the native *LYS2* locus on chromosome 2 (Chr02) and is missing the 3' end of the gene, while the second allele is present on Chr04 and is missing the 5' end of the gene. Therefore, neither allele alone is sufficient to render the cells Lys+. The *LYS2* allele on Chr04 was synthesized such that it contains single nucleotide polymorphisms (SNPs) at regular 206 bp intervals along the gene. Cultures will be plated on selective medium lacking lysine to identify Lys+ clones carrying a recombination event between the two alleles. Using Sanger sequencing, we will map the site of genetic exchange at the recombination breakpoints. Incorporation of reporter genes conferring gene dosage-dependent tolerance to formaldehyde and copper will serve as a means to select for unbalanced translocations. We will sequence the genomes of Lys+ clones, determining the presence or absence of mutation clusters on Chr04, thus, resolving ambiguity of the mechanism that gave rise to these non-reciprocal translocations.

41/ IMMUNE REGULATION OF PD-L1 EXPRESSION ON CANINE TUMORS

Genevieve Hartley, Erica Faulhaber, Alita Caldwell, Jonathan Coy, Amanda Guth, Dan Regan, Denise Morovsky, Mohamad Morsey, and Steven Dow

The adaptive immune system regulates the intensity of T cell responses in order to prevent immune damage to the host through the expression of checkpoint molecules such as programmed-death 1 ligand 1 (PD-L1). However, this method of maintaining self-tolerance can also result in unwanted immune suppression if the proteins involved are dysregulated, as in the case of cancer. High expression of PD-L1 on tumor cells has been found to be associated with poor prognosis and tumor escape in both human and murine cancers. Little is known concerning the expression and regulation of PD-L1 by cancers in dogs. Therefore, we screened 14 canine tumor cell lines for PD-L1 expression and tested their responsiveness to immune stimuli, using flow cytometry and immunocytology. We found that PD-L1 was expressed constitutively on all of the canine tumor cell lines, though the level of basal expression was quite variable. We also found that PD-L1 expression was up-regulated on all the cell lines by exposure to IFN-γ, while individual tumor lines responded to varying degrees. Treatment of tumor cells with a TLR3 ligand also stimulated PD-L1 up-regulation. These data suggest most canine tumors express PD-L1 constitutively and that both innate and adaptive immune stimuli can further up-regulate PD-L1 expression, leading to greater inhibition of tumor immunity. Therefore, blockade of PD-L1 or PD-1 molecules in dogs with cancer may be effective in reversing immune-suppression and inducing a more robust T cell response.

42/ SURVEY OF WHOLE EXOME SEQUENCE DATA OF CANINE BLADDER TRANSITIONAL CELL CARCINOMAS

Belen Hernandez, Joe Brown, Susan Lana, Ken Jones, Rodney Page and Dawn L. Duval

Bladder cancer is a common cancer of humans and their canine companions. The majority of bladder cancers are papillary infiltrative transitional cell carcinoma (TCC). At diagnosis, the majority of TCCs are of intermediate to high grade. Similarities in risk factors, histopathology, sites of metastasis, and other common features indicate that canine TCC may serve as an excellent model for human TCC. To further assess the value of canine TCCs as a model for human TCC, we utilized whole exome sequencing to screen a panel of canine TCCs for cancer gene mutations contributing to the pathogenesis and progression of canine bladder cancer. Genomic DNA was isolated from 11 archived canine TCCs, 3 matched normal samples, and 1 canine TCC cell line. The Agilent Sure-select in-solution capture system designed for the canine genome was used to conduct whole exome capture. The captured DNA was sequenced using the Illumina HiSeq 2000 next generation sequencing platform. The CanFam3.1 canine sequence assembly was used as a reference to map and identify single-nucleotide polymorphisms, insertions, and deletions, with Freebayes software. Somatic mutations were characterized and compared to the human Cancer Gene Consensus (COSMIC). From the survey, 75 genes show nonsense, missense and insertion/deletion mutations that may be drivers or repressors in human cancer. The gene mutation spectrum is dominated by C:G> T:A transitions. Pathway analysis (Pathway Studio) identified DNA damage, genomic instability, and chromatin remodeling as some of the cellular pathways effected. Ten genes most frequently exhibiting potentially deleterious mutation were: *MITF, KDM6A, AKAP9,NSD1, BRCA2, ROS1, BRAF, C2orf44, NCOA4*, and *TPM3*. This mutation profile indicates that similar activating pathways drive both human and canine bladder transitional cell carcinomas, including members of the RTK/Ras/Raf pathway, histone modifying and chromatin remodeling enzymes.

43/ EARLY PRION TRAFFICKING IN DEER EXPOSED TO CHRONIC WASTING DISEASE

Clare E Hoover, Nikki T Buhrdorf, Davin M Henderson, Nathaniel D Denkers, Claudio Soto, Candace K Mathiason, Mark D Zabel, Edward A Hoover

Chronic wasting disease (CWD) is a uniformly fatal disease affecting cervid populations in 23 states. The infectious agent is a misfolded isoform of a normal cellular protein designated PrP^{CWD}. Deer are presumed to contact PrP^{CWD} through environmental exposure by aerosol (IN) and/or oral routes during natural foraging activities. Previous studies mimicking these exposure routes have detected PrP^{CWD} in the retropharyngeal lymph node at 1.5 months and tonsil at 3 months using the traditional assays of western blotting and immunohistochemistry. The timing and distribution of $PrPC^{WD}$ prior to these time points remains unknown. Here, white-tailed deer were exposed to CWD by either mucosal (PO/IN) or intravenous (IV) routes and sacrificed at 15 minutes, 1 and 3 days, and 1, 2 and 4 months post-inoculation with the purpose of determining the bio-distribution of PrP^{CWD} at each time point. At early post-infection time points, PrP^{CWD} is below the threshold of detection for traditional western blot or immunohistochemistry techniques. To overcome this limitation, tissues were assayed for PrP^{CWD} using several amplification techniques: real-time quaking induced conversion assay (RT-QuIC) and tyramide signal amplification immunohistochemistry (TSA-IHC). By combining these techniques we have detected PrP^{CWD} in the gastrointestinal-draining lymph nodes 3 days after PO/IN inoculation and in lung 15 minutes after IV inoculation. Moreover, PrP^{CWD} seeding activity was detected in the mandibular and retropharyngeal lymph nodes, at 1 month post-mucosal exposure, and immunohistochemical evaluation demonstrated PrP^{CWD} in follicular germinal centers and sinusoidal macrophage-like cells of lymphoid tissues by 2 months post-mucosal exposure. These data are the first demonstration of the rapid bio-distribution and early trafficking of CWD prions after mucosal exposure or IV inoculation in deer.

44/ VALIDATION OF THE HESKA ELEMENT POC BLOOD GAS AND ELECTROLYTE ANALYZER FOR USE IN ALPACAS AND GOATS

Katlin J Hornig, Stacey R Byers

Goats and alpacas are increasing in number as companion animals, and owners' expectations for veterinary care are also changing. Most of these animals are cared for by ambulatory veterinarians who do not have immediate blood testing capabilities. Instead blood samples are submitted to a laboratory and it can take 24-48 hours to obtain results. In the meantime, the veterinarian institutes medical therapies based on physical exam findings but these therapies may not be appropriate if there are severe derangements in hematology or electrolytes. The objective of this study was to compare the Heska Element POC Blood Gas and Electrolyte Analyzer (POCA) and a laboratory blood gas analyzer (BGA) to validate the POC analyzer and if the performance was acceptable, develop equipment specific reference intervals for these species. Jugular blood samples were collected from 48 adult alpacas and 41 adult goats using commercial heparinized syringes. Samples were gently agitated and stored in a cooler. Within 2 hours of collection, blood samples were again agitated and processed on the BGA and the POCA according to manufacturers' instructions. A manual hematocrit was measured in the CSU Livestock Medicine and Surgery Laboratory. Parameters evaluated included hematocrit, hemoglobin, glucose, creatinine, electrolytes, bicarbonate, lactate, and blood gas concentrations. Data was analyzed separately for goats and alpacas. Statistical analyses included descriptive statistics and comparison methods (Bland-Altman plots and Passing-Bablok regression). Preliminary results identified both proportional and systematic biases with most parameters however clinical significance varied. There were also species related differences between parameters.

45/ PRE-RIDE SERUM AMYLOID A IN ENDURANCE HORSES IS NOT PREDICTIVE FOR COMPLETION OF A 160-KM RIDE

Heidi C Immesberger, Karina S Cox, Jenifer Gold, Diana M Hassel, and Yvette S Nout-Lomas

Serum amyloid A (SAA), a major non-specific acute phase protein, is produced in the horse in response to inflammation, infection, and stress, such as extended exercise and prolonged transport. Horses competing in endurance events train over long distances and compete under difficult conditions, often after the stress of traveling. A previous study showed elevated pre-ride SAA concentrations correlated with inability to complete the ride in a small number of horses. Our hypothesis was that elevated pre-ride SAA concentrations would be associated with inability to complete a challenging 160-km ride. We obtained pre-ride serum samples from 75 out of 186 horses that competed in a 160-km ride in order to examine the relationship between SAA concentrations and ability to complete the ride. 107 horses successfully completed the ride and 79 did not finish the ride. In our study sample of 75 horses, 44 horses completed the ride (SAA 13.71 +/- 11.13ng/ml) and 31 horses did not finish (SAA 2.82 +/- 0.53ng/ml). Only four horses had SAA concentrations above the reference range (>10ng/ml), two of which completed the ride successfully (SAA 24.4ng/ml and 492ng/ml) and two of which did not complete the ride (SAA 16.3ng/ml and 11ng/ml). The remainder of the horses had SAA concentrations within the reference range (2.08 +/- 0.04ng/ml). In this study, there was no correlation between elevated SAA concentration and completion of this 160-km ride.

46/ POST-TRANSCRIPTIONAL MECHANISMS COORDINATE EXPRESSION OF ZINC FINGER PROTEIN MRNAS

Aimee L Jalkanen, Ashley T Neff, Ju Youn Lee, Bin Tian, Jeffrey Wilusz, and Carol J Wilusz

The C2H2 zinc finger proteins (ZNFs) are a vast 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. Our goal is to characterize the mechanisms, sequences and factors involved in C2H2 ZNF mRNA decay. Decay of most mRNAs initiates with removal of the poly(A) tail and can be regulated through association of proteins and/or microRNAs. Multiple ZNF mRNAs have unusually short poly(A) tails in HeLa, iPS and HFF cells suggesting that these mRNAs are metabolized differently. Interestingly, we also observe that ZNF mRNAs are predominantly nuclear. We used ultra-short metabolic labeling with 4-thiouridine (4sU) to select nascent mRNAs in HeLa cells and found that newly transcribed ZNF mRNAs also have short (A) tails, which suggests that poly(A) tail length is restricted during synthesis. We are currently developing C2H2 ZNF mRNA reporter constructs to determine the sequences and associated proteins and/or miRNAs involved in regulating the short poly(A) tail.

47/ REVERSAL OF PHENOTYPIC AND INTRINSIC ANTIMICROBIAL DRUG RESISTANCE IN MYCOBACTERIUM TUBERCULOSIS WITH 2-AMINOIMADAZOLE BASED SMALL MOLECULE ADJUVANTS

Albert Jeon, David Ackart, Brendan Podell, Juan Belardinelli, Mary Jackson, Roberta Melander, Christian Melander, Randall Basaraba

Tuberculosis (TB) granulomas harbor different populations of drug-tolerant or drug-resistant *Mycobacterium tuberculosis* (*Mtb*) that are difficult to treat with conventional antimicrobial drugs. Previous studies have demonstrated the ability of 2-aminoimidazole (2-AI) based small molecules to restore both phenotypic and genotypic antimicrobial drug susceptibility to a variety of different microorganisms. Using in vitro assays, we have shown that 2-AI compounds restore drug susceptibility by *Mtb* expressing phenotypic drug tolerance. To determine whether genotypic drug resistance can be reversed, the H37Rv strain of *Mtb* was treated with carbenicillin in the presence or absence of 2-AI compounds. 2-AI compounds rendered *Mtb* susceptible to carbenicillin and reduced the MIC from higher than 250 μM to 2 μM. Metabolic labeling with ^{14}C acetate and 14C propionate revealed that treatment of *Mtb* with sub-lethal concentrations (125 μM) of 2-AI compounds inhibits *Mtb* cell wall lipid biosynthesis, reducing disintegration per minute from 113930 (non-treated control) to 19548 (125 μM) by 5.8-fold. These data suggest that 2-AI based small molecules may be effective at reversing genotypic drug resistance, therefore not only potentiating the efficacy of existing first-line antimicrobial drugs but also revealing the possibility of treating TB with a new class of anti-TB drugs.

48/ HARNESSING THE ANTIMICROBIAL PROPERTIES OF ACTIVATED MESENCHYMAL STEM CELLS: A CLINICAL TRIAL IN DOGS WITH MULTI-DRUG RESISTANT INFECTIONS.

Valerie Johnson, Brianna Baca, Steve Dow

Background/Rationale: Antimicrobial resistance is one of the greatest challenges facing the medical community today and new interventions are needed. Recent studies have shown that mesenchymal stem cells (MSC) exhibit antimicrobial activity when activated by Toll-like receptor (TLR) ligands *in vitro*. We hypothesized that activated MSC could enhance the activity of conventional antibiotics in multi-drug resistant (MDR) infections effecting a cure for infections that have no current medical therapies available Methods: MSC derived from adipose tissue of dogs were expanded *in vitro* and activated with TLR ligands. These cells were assessed for antimicrobial activity in combination with common antibiotics *in vitro* using MDR bacteria obtained from clinical infections in dogs. The effects of activated MSC treatment *in vivo* were assessed in canine client owned animals with MDR infections evaluated at CSU Veterinary Teaching Hospital. Treatment was initiated with informed consent of the dog's owner. Three intravenous injections of activated MSC (1x10^6 cells/kg) were given two weeks apart and various techniques including culture and cytology were used to assess response to treatment. Results: Activation of MSC with TLR ligands significantly decreased bacterial counts when combined with antibiotics in MDR infection models *in vitro*. The two patients that have thus far been enrolled in the clinical trial both obtained complete resolution of their MDR infections when MSC treatment was combined with an antibiotic that the bacteria was resistant to according to *in vitro* susceptibility testing. Conclusions: Treatment with activated MSC combined with antibiotics may be effective to resolve MDR infections in dogs. This treatment shows great promise as an effective intervention for a serious health problem that currently has no promising therapeutics on the horizon. This could easily translate to a potential new therapy for humans with similar infections.

49/ MARKED TREMATODIASIS IN A POPULATION OF WILD-CAUGHT TRINIDADIAN GUPPIES

Jennifer Kopanke and Paula Schaffer

Wild-caught Trinidadian guppies (*Poecilia reticulata*) in a research laboratory were found to have an unusually high rate of morbidity and mortality for a month following capture. Reported clinical signs included emaciation, erratic swimming, external sores, and death in greater than 50% of the guppies. Ten affected guppies from three river drainages (Caroni, Marianne, and Oropuche) were sacrificed for histological assessment. Fish from all drainages sampled were affected by multifocal trematodiasis which was often associated with chronic-active inflammatory changes. Trematodes were identified in the coelom, pericardium, liver, and peri-renal tissues of many guppies. In one fish, multiple embedded trematodes were associated with development of pancreatic sarcoma. One fish from the Oropuche drainage had marked granulomatous meningitis of unknown etiology. Numerous fish also had microsporidia xenomas in skeletal muscle. Cumulatively, the findings suggest that infection with multiple infectious agents, especially visceral trematodes, was the most likely cause of morbidity and mortality in most fish. Fish from the Marianne and Oropuche drainages were treated once with 10 mg/L praziquantel in tank water for 5 hours, and fish from the Caroni and Oropuche drainages received salt treatment with Instant Ocean (3 tbsp/5 gallon tank) for 3 weeks and 1 week, respectively. Clinical signs and mortalities were reportedly reduced in all populations following treatment. The location of these trematodes in viscera suggests that they are of the platyhelminth group Digenea. Importantly, no digenean parasites have been previously described in wild Trinidadian guppies. Variations in parasite burden suggests that there could be differences in parasite-host ecology across the sampled drainages. In addition, investigators should be aware that wild-caught specimens used for experimental purposes may have multiple comorbid conditions that could compromise research outcomes.

50/ RELATIONSHIP BETWEEN ANDROGEN RECEPTOR (AR) AND LIN28A IN THE MOUSE PLACENTA

Makenzie S. Kurth, Elaine R. Cleys, Rachel C. West, Vanessa A. Enriquez, Thomas R. Hansen, Quinton A. Winger, Gerrit J. Bouma

LIN28A is a protein that binds RNA, including small non-coding RNA molecules called microRNA. LIN28A is known to be an important regulator of cell differentiation and maturation. LIN28A has been identified in placental and embryonic stem cells. It also appears to relate to tumorigenicity in several cancer types including breast, prostate, and ovarian cancer. LIN28A is highly conserved among species. However, expression varies by tissue and developmental stage. It is known that presence or absence of LIN28A affects the expression of other regulatory proteins via inhibition of the let-7 microRNA family, or by directly regulating target mRNAs. One target gene of LIN28A is the AR. Recently, it has been shown that increased LIN28A leads to increased levels of AR in prostate cancer. The regulation of AR by LIN28 appears to occur via let-7 microRNAs; high LIN28 inhibits let-7, which in turn leads to up-regulation of AR. Studies in our laboratory have focused on the role of LIN28 and androgen signaling in the placenta. Though we have information regarding the regulation of AR by LIN28 in prostate cancer, it is not known if this regulation occurs in the placenta. In order to begin to understand the relationship between LIN28A and AR in the placenta, we performed immunohistochemical analysis of mouse placental tissues of various gestational stages, to assess cellular localization of AR. AR is known to be mainly concentrated in the nuclei of cells and it is expected that this will be the case in mouse placental cells as well. We also will conduct IHC staining for LIN28A in mouse placental tissue. LIN28A has been localized to the cytoplasm and we expect to observe staining in the same placental cells that are positive for AR.

51/ BUILDING A VIRTUAL CAT: TOWARDS A PHYSIOLOGIC-BASED PHARMACOKINETIC (PBPK) MODEL FOR INVESTIGATING DRUG DOSING IN CATS

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

Background: 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. Predicting drug disposition in cats via extrapolation from canine-based pharmacokinetic models for drugs metabolized via such pathways is thus rarely appropriate, especially for drugs lacking an extensive clinical database. 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 felines as well as the availability of *in vivo* pharmacokinetic (PK) data for model validation. Methods: Biochemical parameters for ondansetron metabolism and tissue disposition were measured from time course incubations of microsomal preparations (n=3) isolated from freshly harvested feline organs. Incubations contained 1mM NADP, 0.25mg/ml (final) of microsomal protein, and a 0.3M potassium phosphate buffer (pH 7.4) at 37°C for 5 ondansetron dosing concentrations (50, 100, 250, 500, and 1000 ng/ml). Time course data were nominally collected for 0, 1, 3, 5, 10, 15, and 30 minutes, although incubations for selected doses were extended (up to 240 minutes) to capture longer-term data. Analysis of tissue drug concentration was performed using an LC/MS/MS method. Results: Enzyme kinetic parameters Vmax and Km were determined via initial rate data, yielding average values of 0.0942 uM/min (0.3767 units/mg protein) and 0.9851 uM, respectively. Ondansetron metabolic profiles showed expected decreases in parent drug and increases in two associated metabolites, hydroxyondansetron and N-desmethylondansetron. Conclusions: Experimental protocols for the characterization of feline metabolism and physiology parameters were developed, with successful *in vitro* demonstration of ondansetron metabolism. Comparison with available *in vivo* PK data represents a fundamental step toward development and validation of a PBPK model for ondansetron dosing in cats.

52/ ACTIVATED IMMUNE CELLS DEMONSTRATE DECREASED GLUCOSE RECEPTOR EXPRESSION AS POTENTIAL ANTIMICROBIAL MECHANISM IN EARLY STAGE MYCOBACTERIUM TUBERCULOSIS INFECTION

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

Nutrient deprivation by activated immune cells has been suggested as a potential antimicrobial mechanism during *Mycobacterium tuberculosis* infection. Thus far, evidence of this phenomenon has been limited to *in vivo* bacterial gene expression and there has been little investigation into host immune cell mechanisms of nutrient withdrawal, particularly glucose. While late-stage *M. tuberculosis* lesions have been shown by PET/CT scan to have a dramatic increase in glucose demand, early-stage *M. tuberculosis* infection may demonstrate the opposite effect. As the disease progression of tuberculosis in humans is most closely modeled by guinea pigs, both *in vitro* and *ex vivo* models were used to demonstrate metabolic demands of glucose by immune cells exposed to *M. tuberculosis*. Using a fluorescent glucose uptake assay, qRT-PCR, and flow cytometry, activated immune cells revealed a marked decrease in glucose uptake, glucose transporter 1 (GLUT1) mRNA transcription, and GLUT1 receptor expression during early infection. Results indicated a 54.71% decrease in GLUT1 expression within *ex vivo* infected lung immune cells as compared to those from naïve animals. Similarly *in vitro* bone marrow derived macrophages showed a 63.44% decrease in glucose uptake when infected and stimulated with the activating cytokine IFNγ after 60 hours. These results suggest that infection alters cellular metabolism by down-regulating transporter expression, ultimately leading to reduced glucose utilization.

53/ DOES FELINE FOAMY VIRUS CAUSE DISEASE IN DOMESTIC CATS?

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

Feline foamy virus (FFV) is a Retrovirus from the genus *Spumavirus* that has been historically regarded as apathogenic despite life-long infection in domestic cats. Because of this, FFV carries potential applications in vaccine vector and gene therapy development. In addition, current reports in primate HIV research show that foamy viruses (FV) can confound immunodeficiency virus (IV) infection studies; the development of a feline FV/IV co-infection model would allow the study of these interactions. To verify FFV apathogenicity and build on pre-existing reports about experimentally induced infections, we inoculated cats (n=4/group) with wild-type FFV or a chimeric FFV replicative virus ($TCID_{50}$ of 2.78E5 IU/ml) that contained the FIV *vif* gene replacing the FFV *bet* gene. Vif and Bet proteins counteract feline APOBEC3 cytidine deaminase restriction factors through different mechanisms and thus replacing these genes allows further study of A3 restriction during infection as well as a potential vaccine vector development. Blood, saliva, urine, and tissues were collected to determine viral presence and kinetics, immune response, tissue tropism, and histopathology. PBMC provirus was detected in wild-type FFV-inoculated cats as early as 21 days post-infection via nested PCR, and persisted throughout the study (176 days). None of the cats developed clinical disease and hematological and urinalysis results were unremarkable. One cat had significantly altered proviral kinetics compared to cohorts. Flow cytometric analysis revealed that leukocyte cell numbers and activation markers were not significantly different than control cats. Renal histopathology showed mild degenerative changes that may be attributed to FV infection. Based on these preliminary results, FFV can be assumed to be apathogenic in cats despite persistent infection, and thus may be a useful tool in vaccine and gene therapies as well as allow the development of a FV/IV co-infection model. Future analyses will evaluate FFV salivary excretion kinetics and tissue distribution.

54/ L-TYPE CA2+ CHANNEL KNOCK-OUT MODEL IN αT3-1 CELLS USING THE NOVEL CRISPR-CAS9 SYSTEM WITH GRNA

Naomi N. Lee, Heather J. Szerlong, and Gregory C. Amberg

Pituitary cell function is crucial in maintaining reproductive functions and requires multiple precise signaling events. The hypothalamic-pituitary-gonadal (HPG) axis cascade is dependent on gonadotropin-releasing hormone (GnRH). Following GnRH activation of G protein-coupled GnRH receptor stimulates the downstream events that increase Ca^{2+} influx by activating L-type Calcium channels and eventually leads to the LH surge. Although the events that lead to the LH surge are required to maintain HPG axis functions, the mechanistic basis of Ca^{2+} signaling in gonadotropes is unknown. We have hypothesized that activated GnRH receptors coalesce to form dynamic multi-protein signaling complexes necessary for colocalized calcium microdomains. We have planned to use the Clustered Regularly Interpaced Short Palindromic Repeats (CRISPR) and nickases to specifically target and knock out genes Cacna1c and Cacna1d that encode Ca_v 1.2 and Ca_v 1.3 respectively. We have studied to learn more about colocalization of L-type Calcium channels with GnRH activation from the knock out models we have designed. Subcellular Ca^{2+} signaling mechanisms are ubiquitous in many different cells and we anticipate that this modified pituitary cell model will identify molecular mechanisms of subcellular signaling events that are utilized in other cell types.

55/ EVALUATION OF BIOFILMS PRODUCED BY GRAM-NEGATIVE BACTERIA ISOLATED FROM THE EQUINE UTERUS

Kristen D Loncar, Patrick M McCue, Brad R Borlee and Ryan A Ferris

Biofilms are communities of bacteria encased in an extracellular matrix that provides resistance to the host immune system and antimicrobial agents. Chronic bacterial endometritis is a leading cause of subfertility in mares and the formation of biofilms is currently thought to be responsible for this issue. Therefore, anti-biofilm treatments with *N*-acetylcysteine, hydrogen peroxide, TRIS-EDTA, DMSO and Ceragyn® have been recommended for use in clinical practice. Unfortunately, very little is known about biofilms produced by bacteria associated with equine endometritis. Isolates of *E. coli, K. pneumoniae*, and *P. aeruginosa* from equine uterine cultures were obtained and in vitro biofilm formation was evaluated for each isolate in the MBEC biofilm assay. Preformed biofilms were challenged for 6 hours with media (negative control), 3.3% acetylcysteine, 100mM gallium nitrate, 20 mM 2-amino-2-hydroxymethyl-1,2-propanediol and 8 mM disodium EDTA dehydrate, 3.5 mM disodium EDTA dehydrate and 50 mM Tris, 1% hydrogen peroxide, 30% DMSO or 30% Ceragyn®. Biofilm biomass was measured by staining with crystal violet and quantified using a microplate reader. Results are presented as mean ± SEM for 5 replicates. The majority (~80%) of Gram-negative bacterial isolates from the equine uterus were able to produce a biofilm *in vitro*. No single anti-biofilm treatment evaluated in this study consistently disrupted a preformed biofilm in all isolates *in vitro*. Ceragyn® disrupted biofilms produced by *E. coli* and *K. pneumoniae* isolates but no single agent significantly disrupted *P. aeruginosa* biofilms. Results of this study indicate that intrauterine therapy to reduce or eliminate biofilms should be based on the bacteria cultured and, ideally, *in vitro* evaluation of the biofilm produced by the organism in response to an effective therapeutic agent. Keywords: Equine, Endometritis, Bacteria, Biofilm

56/ COMPUTED TOMOGRAPHY REVEALS HIGHER BONE DENSITY AT THE DISTAL ARTICULAR SURFACE OF RACEHORSES WITH THIRD METACARPAL FRACTURES

Alice B Loughridge, Ann M Hess, Tim D Parkin, Chris E Kawcak

Lateral condylar fractures of the third metacarpal (MC3) are common injuries sustained by racehorses. MC3 fractures are considered the end result of a chronic pathological process that affects the articular cartilage and subchondral bone. High intensity exercise modifies subchondral bone density, which initially, is a normal adaptation to joint loading. However, chronic loading may result in progression to a maladaptive state, whereby exercise-induced changes in bone density potentiate the risk of fracture. The goal of this study was to assess bone density patterns at the distal articulating surface of MC3 in the fractured and contralateral nonfractured limb of racehorses. Computed tomography images were obtained from 89 Thoroughbred racehorses euthanized in the United Kingdom. Third metacarpal bones were divided into 3 groups based on lateral condyle condition; fractured (FX), nonfractured (NFX), and paired condyles from racehorses euthanized for non-MC3 related causes (CTL). Density was assessed in 6 regions 1mm abaxial and axial to the fracture line in the FX group and 1mm abaxial and axial to the center of the lateral parasagittal groove in NFX & CTL groups. A mixed model ANOVA was performed to 1) assess differences in density related to age, sex and condition and 2) assess differences in density among regions of the lateral condyle. There was no effect of age or sex on density. For all regions, a higher density was observed in both condyles of racehorses that had sustained an MC3 fracture compared to controls. Fractured condyles had an increased heterogeneity in density among condylar regions compared to NFX & CTL condyles. Our data indicate that a characteristic pattern of bone density is present in horses with lateral condylar fractures of MC3. Identification of this pattern may provide a means for detecting pathological changes in bone density and assessing fracture risk in racehorses prior to injury.

57/ INVESTIGATION OF RADIOSENSITIVITY GENE SIGNATURES IN CANINE TUMOR CELLS

Junko Maeda, Coral Fronning, Colleen A Brents, Jared S Fowles, Douglas H Thamm, Takamitsu Kato

Individual tumors even with the same histological type exhibit different responses to radiation. Identification of radiosensitivity signatures using microarray analysis has been used to develop radiosensitivity predictive assays for human cancers. Since the gene expression profiling for canine tumor cell line panel of the Animal Cancer Center (ACC) of Colorado State University was available, we investigated radiosensitivity gene signature in the canine tumor cells. Clonogenic survival after exposure to gamma-rays was measured for the 27 canine tumor cell lines. In these cell lines, there was no significant correlation of SF2 (the survival fraction at 2 Gy of gamma-rays) with doubling time, number of chromosome and metacentric chromosome, while there was a statistically significant correlation between SF2 and plating efficiency. The basal gene expression profiles of the canine tumor cell lines were from Affymetrix GeneChip Canine Genome 2.0 arrays, obtained from the study by ACC. Using the PathwayStudio software, we analyzed the basal gene expression profiles to extract genes that are differentially expressed between radiosensitive and radioresistant cell lines. Absolute log ratio greater than 1 with a p-value less than 0.05 were used for the statistical significance. In the top 6 radiosensitive cell lines and the top 5 radioresistant cell lines based on the SF2 comparison, 387 differentially expressed genes were downregulated, and 179 were upregulated in radiosensitive cell lines. Among them, several genes in the list have a common function related to cell junction and adhesion, which was seen in the previous study for human cancer cell lines. However, the cell cycle and p53 signaling pathway identified as functions of radiosensitivity signature in human gene expression profiling were not identified in our study. Our results suggested that the identified genes which are related to cell junction and adhesion are candidates for a gene expression model to predict radiation treatment response.

58/ SEROLOGICAL EVIDENCE THAT TACARIBE VIRUS IS CIRCULATING AMONG BATS IN TRINIDAD AND TOBAGO

A Malmlov, J Seetahal, C Carrington, V Ramkisson, J Foster, V Munster, S Quackenbush, and T Schountz

Tacaribe virus (TCRV) is a bisegmented, ambisense, RNA virus within the genus *Arenavirus*. Arenaviruses are grouped into Old World lymphocytic choriomeningitis-Lassa virus complex and the New World Tacaribe complex viruses. TCRV is placed within the Tacaribe complex along with the South American hemorrhagic fever viruses: Chapare, Guanarito, Junin, Machupo, and Sabia viruses. The only isolates of TCRV were from 11 artibeus bats collected by investigators at the Trinidad Regional Virology Laboratory in the Republic of Trinidad in the 1950s. TCRV has not been isolated since, although serological data from the 1970s suggested it may circulate among Caribbean bats. Only one isolate remains, TRVL-11573, and it has been passaged in suckling mice and Vero cells. We sought to determine if TCRV is still circulating in bat populations in Trinidad through serological investigation. We developed an ELISA and western blot assay using His-tagged recombinant TCRV nucleocapsid antigen. Serum from *Artibeus jamaicensis* that had been experimentally infected with TCRV was used as a positive control, and serum collected from an uninfected *A. jamaicensis* used as a negative control. ELISA screen of bloods from 84 bats of various species captured in Trinidad identified several, mostly artibeus bats, as seropositive for antibodies to TCRV. Some of these were tested by western blot. Four were negative, eight were weakly positive, and five were strongly positive. These results suggest that TCRV or other arenaviruses continue to circulate among bats in Trinidad.

59/ BRAIN REGIONS INVOLVED IN SEASONALLY DEPENDENT A1AR INDUCED TORPOR IN ARCTIC GROUND SQUIRRELS

Kelsey M McClure, Carla Frare, Kelly L Drew

Discovering the mechanism behind hibernation and associated neuroprotective adaptations is expected to translate into potential therapies for a variety of conditions including stroke, cardiac arrest and traumatic brain injury. Hibernation is closely associated with sleep and is characterized by torpor bouts, metabolic suppression, and decreases in body temperature (T_b) and heart rate. The Arctic ground squirrel (AGS, *Urocitellus parryii*), a seasonal hibernator, provides a unique animal model due to the physiological extremes it reaches during hibernation. AGS have been shown to decrease body temperature to -3°C and heart rate to 3 beats/min; later recovering without incident. Previous research has shown that AGS injected with an A_1 adenosine receptor (A_1AR) agonist N^6-cyclohexyladenosine (CHA) will induce a torpor-like state in animals injected during the winter season. However, AGS injected with CHA during the summer season display only a slight and temporary reduction in body temperature and do not enter torpor. In the present study we injected CHA or a vehicle intraperitoneally to a group of 11 squirrels during the summer season and a group of 10 in the winter season. Body temperature (T_b) was measured every 30 minutes for 3 hours using abdominal transmitters. Animals were then euthanized and intracardially perfused with 4% paraformaldehyde and the brains were collected. Immunohistochemistry was performed to co-localize c-Fos (indicating active neurons after injection), galanin (a sleep active neuron) and orexin (an arousal inducing neuron) in the lateral hypothalamus. Within 2h of CHA administration there was a significant difference in T_b between summer and winter animals. Additionally, it is anticipated that more c-Fos will be co-expressed with galanin in the ventrolateral preoptic nucleus (VLPO) in winter animals indicating increased disinhibition of sleep active neurons. Alternatively, more c-Fos is expected to be co-expressed with orexin in the perifornial nucleus (pEf) in summer animals indicating increased arousal.

60/ EXPERIMENTAL INFECTION OF DEER MICE WITH MAPORAL HANTAVIRUS

Amanda McGuire, Joseph Fauver, Amber Rico, Tawfik Aboellail, Gretchen Hume, Kaitlyn Miedema, Sandra Quackenbush, Ann Hawkinson, and Tony Schountz

Hantavirus cardiopulmonary syndrome (HCPS) is a rodent-borne zoonosis caused by over a dozen New World viruses. The disease is characterized by a prominent inflammatory response without conspicuous damage to the microvasculature, the target organ of infection, suggesting HCPS is, in part, an immunopathology. Only two hantaviruses cause disease in small animal models, Andes virus (ANDV) or Maporal virus (MAPV) infections of Syrian hamsters (*Mesocricetus auratus*). ANDV causes HCPS and, thus, requires animal biosafety level-4 (ABSL-4) containment; however, MAPV is not a known human pathogen, permitting ABSL-3 precautions. We are interested in the differential immune responses of reservoir hosts and Syrian hamster HCPS models that could provide mechanistic explanations for persistence or immunopathology. While deer mice (*Peromyscus maniculatus*), the natural reservoir of Sin Nombre hantavirus (SNV), are experimentally susceptible to ANDV, both viruses require ABSL-4 containment. We therefore examined deer mice for susceptibility to MAPV. Following inoculation with MAPV, viral RNA was detected in multiple organs of all deer mice during the 56 day experiment. Deer mice generated both nucleocapsid-specific and neutralizing antibodies. Minimal to mild histopathologic lesions were present and primarily observed in lung, heart and liver. Cytokine gene expression at low to moderate levels was detected in spleens and lungs of infected deer mice, and deer mouse primary pulmonary cells propagated in endothelial cell growth medium were susceptible to MAPV. The deer mice appeared healthy for the duration of the experiment, suggesting that MAPV is not pathogenic in a heterologous reservoir host. These features resemble those observed in deer mice infected with SNV and suggest the course of infection with MAPV is substantially similar. The development of this model will permit direct comparison of two ABSL-3 rodent models with differential outcomes to MAPV infections, and may clarify how hantaviruses evade sterilizing immune responses.

61/ DO MESENCHYMAL STROMAL CELLS ABROGATE THE HOST IMMUNE RESPONSE IN MASSIVE CORTICAL ALLOGRAFT RECIPIENTS?

Kaitlyn L McNamara, Steven Dow, and Nicole Ehrhart

Massive bone allografts are utilized to reconstruct large bone defects after tumor resection or trauma, however, allografts are not are not matched for major histocompatibility antigens with the recipient. Previous studies have shown that the poor healing seen after mismatched large frozen cortical allograft transplants can be partly contributed to T cell recognition of graft alloantigens. This study's objective was to evaluate the cellular immune response against allograft bone versus autograft bone delivered as a vaccine, and to determine if the addition of adipose-derived mesenchymal stem cells (AD-MSCs) would dampen the immune response towards a bone vaccine. We developed a murine bone vaccine model to mimic the immune response seen following a limb-salvage procedure. C57BL/6 mice (n=68) received a vaccine, a vaccine with subcutaneous AD-MSCs, or a vaccine with IV AD-MSCs. T cell proliferation differences between treatment groups were determined using an ANOVA and a Bonferroni multiple comparison post-test. Evaluation of the AD-MSC immunomodulation of the cellular immune response revealed a suppressive effect on T cell proliferation in recipients of a vaccine with AD-MSCs. An unexpected observation was noted during analysis of the T cell proliferation following re-exposure to the bone antigens. Wherein one would normally expect re-exposure to an antigen via vaccine to enhance the immune response, we noted that T cell proliferation towards the allograft bone antigen was dampened in animals previously vaccinated with an allograft bone vaccine. This suggests that vaccinating against a total allograft bone antigen may decrease the immune response to the allograft. A future vaccine study investigating this potential mechanism is needed to determine if suppression of T cell proliferation in vaccinated animals is related to clinically improved graft incorporation. If successful, vaccination with an allograft bone antigen could yield a potential new therapeutic for increasing the success of a massive cortical allograft transplant.

62/ POTENTIAL TARGET FOR CANCER THERAPY: EXPRESSION OF CELLULAR PRION PROTEIN FOUND IN CANINE CANCER CELL LINES WITH UNIQUE GLYCOSYLATION PATTERNS

Sophia A Moore, Lisa Pang, Wilfred Goldmann, David J Argyle

Although the abnormal prion protein is known to cause mad cow disease, the role of normal cellular prion protein (PrP^C) remains unknown. Recent studies have shown an up-regulation of PrP^C in human cancer types such as colorectal and melanoma. Some studies have proposed multiple roles for PrP^C in cancer including anti-apoptosis, cell migration and differentiation. While PrP^C has been investigated in human cancers, it has not been studied in canine cancer. In this study, Western Blot analysis of seven canine cancer cell lines was performed using PrP^C specific monoclonal antibodies to visualize PrP^C glycosylation and cleavage patterns. A time course experiment was then conducted on the J3T canine glioma cell line treated with doxorubicin. Preliminary data show that all canine cancer cell lines express PrP^C, but some with unique patterns. For the first time it was shown that cancer cell lines, in this case all seven canine cell lines, express the PrP^C-C1 fragment, which has been described for healthy cells. Interestingly, the Lilly cell line appears to only have the diglycosylated C1 fragment. Future work using the Lilly cell line as a model may reveal the importance of this unique expression of PrP^C in tumorgenesis of inflammatory mammary carcinoma. Significantly, J3T cancer cells that were exposed to stress induced by doxorubicin had a different glycosylation pattern compared to the DMSO control. Further experiments are required, such as PNGase digestion and epitope mapping, to identify the PrP^C fragments affected by stress and their biological relevance. This is the first time PrP^C has been studied in canine cancer and it has been found to be up-regulated in all seven canine cancer cell lines. Having a canine model for PrP^C will allow us to explore the potential for PrP^C as a prognostic tool as well as a synergistic target for patients undergoing chemotherapy.

63/ IMPACT OF INSECTICIDE RESISTANCE ON THE IMMUNE RESPONSE OF AEDES AEGYPTI MOSQUITO

Miguel Moreno-García, Haoues Alout, Karla Saavedra-Rodriguez and William Black IV

The development and use of insecticides have played a key role in the control of populations of mosquitoes. Adverse effects of indiscriminate and excessive use had led to the selection of insecticide resistance, threatening the effectiveness and limiting the options for disease control. The mechanisms responsible for insecticide resistance are negatively correlated with larval survival, adult survival and longevity, sexual traits and behaviors, and fecundity. The negative effect of pleiotropy is commonly observed through the appearance of resource-based trade-offs. This project aims to compare the immune response of *Aedes aegypti* against a bacterial infection between the insecticide susceptible (Hunucma) and pyrethoid resistance strain (Vergel) in order to characterize the influence of insecticide resistance on *Ae. aegypti* response against bacterial challenge For this, we used permethrin to select mosquitoes during five consecutive generations. We measured immune response of mosquitoes (PO and and antimicrobial activity) of each selected (Vergel) and non-selected (Hunucma) strain after inducing an infection with *Escherichia coli* in each generation. For the three generations, the PO activity of susceptible strain (Hunucma) without infection is higher when compared with the resistant (selected) strain (Vergel). The antimicrobial activity showed differences at the first generation after insecticide selection, the Hunucma strain (without insecticide selection) showed the higher antimicrobial activity. However, this difference was not detected in the subsequent generations. Insect resistance selection has a negative impact in PO activity, but the mosquito has the capacity to enhance immune response response to fight against bacterial infection. Funding source: Fogarty International Training Grant

64/ DRY BEAN (PHASEOLUS VULGARIS) CONSUMPTION SUPPORTS LONG-TERM WEIGHT LOSS IN COMPANION DOGS

Nora Jean Nealon, Genevieve M Forster, and Elizabeth P Ryan

The objectives of this experiment were to determine how time and bean consumption influence nutrient utilization and apparent weight loss in overweight dogs on calorically-restricted diets. Dogs belonged to a 4-week, short-term (n=30), or a 26-week long-term trial (n=15). Participants were randomly assigned to diets containing 25% w/w black or navy bean powder, or a control diet (no bean powder). To evaluate nutrient utilization, a five-day fecal collection was performed at two weeks (short-term study) and twelve weeks (long-term study). From this, digestibility, metabolizable energy, and gross energy were calculated. For the long-term trial, a feed-to-loss ratio, comparing dry mater consumption to weight lost, was developed and used to evaluate apparent weight loss over consecutive four-week intervals. One-Way Analysis of Variance (Kruskal-Wallis Test) was used to observe differences between diets during one timepoint, and Two-Way Analysis of Variance was used to observe effects of a diet over time. On a dry matter basis, neither time nor diet altered digestibility, which remained high (median=79.83% short-term; 81.96% long-term). Metabolizable energy increased ($p<0.0001$) between two weeks (median=1980 kcal/kilogram) and twelve weeks (median=3689 kcal/kilogram), but was not different between diets at either timepoint. Total fecal gross energy decreased ($p<0.0001$) between two weeks (median=11.31 kcal/gram) and twelve weeks (median=5.04 kcal/gram), but was not different between diets at either timepoint. Feed-to-loss ratios generally decreased and varied over time ($p=0.0398$), but not between diets during a given timepoint. When used in long-term weight loss diets for dogs, cooked beans can be considered an effective and digestible nutrient source. Decreases in gross energy and increases in metabolizable energy suggest novel mechanisms by which beans may help to favorably modulate metabolism during caloric restriction. Furthermore, the feed-to-loss ratio represents a novel approach for evaluating apparent weight loss in companion animals.

65/ FECAL CORTISOL AND OTHER MEASURES OF STRESS BEFORE AND AFTER CLICKER TRAINING CATS IN TWO COLORADO ANIMAL SHELTERS

Lily Ngai and Rebecca Ruch-Gallie

In the United States, a common cause of feline euthanasia in animal shelters is stress-induced illness while waiting for adoption. The environments in many sheltering facilities frequently create stress, and the effects of this stress can increase susceptibility to disease, such as upper respiratory tract disease. Reducing stress may alleviate fear, anxiety and aggressiveness that can increase the potential for adoption and reduce stress-related illness. Although there are many systems used in shelters to mitigate stress, not all cats will respond positively, thus further methods of decreasing stress need to be investigated. Clicker training is a great technique to achieve this goal; it is a cost effective and a low stress method using positive reinforcement to shape behavior in many animal species. Currently, there are no published studies evaluating the effect of clicker training on shelter cat stress response. Our study examines the hypothesis that clicker training cats in an animal shelter environment decreases stress levels. The objectives of the study are to: i) determine fecal cortisol levels prior to and after clicker training, and ii) determine the effect of clicker training on stress-related behaviors. The study will be performed at two Colorado shelters and a total of 70 cats from both shelters will be enrolled in our study.

66/ USING METAGENOMICS TO UNLOCK THE ECOLOGY OF ANTIMICROBIAL RESISTANCE IN CATTLE PRODUCTION SYSTEMS

Noyes NR, Xiang Y, McArt JA, Linke LM, Magnuson RJ, Yang H, Dettenwanger A, Jones K, Boucher C, Belk KE and Morley PS

A complex ecology modulates the dynamics of antimicrobial resistance (AMR) within food production systems. Traditional microbiological methods provide only narrow glimpses into this ecology due to a reliance on phenotypic expression of resistance in cultured bacteria. Shotgun metagenomics can revolutionize research into food system AMR by providing access to all of the genetic material in samples, including all of the antimicrobial resistance determinants (ARDs). The goal of this pilot study was to investigate the feasibility and utility of metagenomics as an approach for better understanding AMR dynamics in beef production. Environmental fecal, soil and water samples (N=34) were collected from an organic and conventional dairy, a US and Canadian beef feedlot, and a ranch. Total DNA was extracted and sequenced on the Ion Proton. Sequence reads were aligned to a custom database of ARDs. Reads aligning to each ARD were summed and normalized by sample. Resistome abundance and diversity were compared across production and sample type, and associations were assessed using zero-inflated Gaussian mixture models. Sequencing produced 1.05B reads (mean = 30.8M reads per sample, range 14.9M to 48.9M). Alignment identified 248 unique ARDs across all 34 samples. The number of unique ARDs within a sample ranged from 0 to 125. No ARDs were found in the water or soil collected from pasture (N=4). ARDs conferring tetracycline resistance were most abundant, at 69% of all ARD-assigned reads (148,404/214,196). ARD abundance and diversity generally did not differ significantly between production and sample types. This project represents the largest assemblage of agricultural metagenomic sequence data identified, and provides proof-of-concept for use of metagenomics in AMR research. Initial results indicate a shared resistome of cattle production, however pasture-based systems harbor significantly fewer ARDs than more intensive systems. This pilot study highlights the unique insight that can be garnered from a metagenomics approach.

67/ONE HEALTH APPROACH TO REVIEW OF QUESTIONNAIRES FOR CLINICAL TRIALS OF BEAN-BASED DOG FOOD

Erin Nuckols, Lorann Stallones, Genevieve Forster, and Elizabeth Ryan

The human-animal bond has been associated with positive influences on human emotional well-being but less is known about the role of human-animal bond in physical health. The purpose of this study is to understand owner perceptions and behaviors regarding healthy weight, diet, and physical activity in their dogs. We hypothesize that a deeper understanding of dog health, particularly related to overweight and obese body types may influence positive behavior changes in humans. Questionnaires were completed by dog owners at baseline, mid- and end of study for two weight loss clinical trials. Information was obtained on diet and feeding patterns as well as routine activity of the dogs. Forty-five adult dogs were enrolled and randomized based on their body condition score, which is similar to a body mass index in humans. Dogs were distributed evenly between the diet groups in terms of age, weight, body condition score, and sex. The analysis examined responses to a series of question on dog behavior, diet, quality of life, and physical activity. Preliminary results show that owners whose dogs lost weight reported improved quality of life in their companion animal. We also found that many owners reported discrepancies between perceived and actual physical activity. This work demonstrates a knowledge-gap about obesity and owners perceptions about their overweight/obese dogs that may be related to the human-obesity epidemic. Dogs may be an incredible motivation to help improve human health and quality of life.

68/ THE REGULATORY ROLE OF CYCLIC DIGUANYLATE IN BURKHOLDERIA PSEUDOMALLEI MOTILITY AND BIOFILM FORMATION

Brooke Plumley, Grace Borlee, and Brad Borlee

Burkholderia pseudomallei is an emerging pathogen and the causative agent of melioidosis, a severe and often fatal disease that is endemic in Southeast Asia and Northern Australia. Infections caused by *B. pseudomallei* are difficult to eradicate with antimicrobial therapy. One recently described mechanism that contributes to persistence during infection is the ability to form a biofilm, which is regulated by the second messenger cyclic diguanylate (c-di-GMP). Elevated intracellular levels of c-di-GMP promote a biofilm mode of growth, while low levels of c-di-GMP promote a planktonic (free-living) state. Bioinformatics analyses identified 19 c-di-GMP metabolic genes that potentially alter c-di-GMP levels and bacterial behaviors. Our research is focused on two of these genes designated *cdpA* and *cdpB*. It is currently unknown how CdpA (a phosphodiesterase that degrades c-di-GMP) interacts with CdpB (a hypothetical protein of unknown function). We hypothesize that the proteins encoded by *cdpA* and *cdpB* work together to decrease cellular levels of c-di-GMP. A recent report has shown that CdpA decreases cellular levels of c-di-GMP, but the products of CdpA metabolism have not been fully characterized. Our prediction is that CdpA degrades c-di-GMP to pGpG and CdpB further degrades pGpG to GMP through a novel hydrolytic activity. To test this hypothesis, we created in-frame deletion mutants of *cdpA*, *cdpB*, and the double mutant. We found that Δ*cdpA*, Δ*cdpB*, and Δ*cdpAB* mutants are reduced in swimming and swarming motility as compared to the wild-type. Motility was partially restored to 75% of the wild-type when Δc*dpAB* was complemented with *cdpA*. Δ*cdpAB* was fully restored to wild-type levels when complemented with both *cdpA* and *cdpB*. Additional studies are underway to determine the role of *cdpA* and *cdpB* in biofilm formation, eukaryotic cell invasion, pathogenesis in murine models, and enzymatic degradation of c-di-GMP.

69/ IN VITRO EFFECTS OF PI3K/MTOR INHIBITION IN CANINE HEMANGIOSARCOMA

Alex A. Pyuen and Douglas H. Thamm

Hemangiosarcoma (HSA) is an aggressive, malignant tumor of blood vessels that accounts for nearly 2% of all canine tumors. Even with the current surgical and chemotherapeutic treatment regimens, median survival time is in the 6-month range. One possibility to increase the effectiveness of chemotherapeutic treatment of HSA would be to target a specific cellular pathway vital to the malignant cells. The PI3K/mTOR pathway is known to trigger a variety of cellular responses, including growth, proliferation, survival, and tumor progression, and thus is an excellent target for chemotherapeutic interventions. This study examined the in vitro effects of a novel agent (VDC-597) that is known to inhibit both PI3K and MTORC1 and 2. Following 72-hour growth inhibition and 24-hour migration assays, VDC-597 was shown to decrease both proliferation and migration in a HSA cell line. Additionally, VDC-597 was shown to increase the rate of apoptosis. The results of this study demonstrate the benefit of inhibiting the PI3K/mTOR pathway in reducing the aggressiveness of canine HSA in vitro.

70/ RETICULOCYTE HEMOGLOBIN CONTENT (CHR) DOES NOT DIFFERENTIATE TRUE FROM FUNCTIONAL IRON DEFICIENCY IN DOGS

Lauren B Radakovich, Kelly S Santangelo, Christine S Olver

True and functional iron deficiency can result in anemia. Current tests to assess iron status often do not allow differentiation between these entities, which is frequently vital for optimal treatment. Previous work suggested low CHr may be an early indicator of iron deficiency; however, other potential causes for low CHr were not investigated. This study aimed to correlate measures of inflammation to CHr values in dogs. We hypothesize that dogs with low CHr values have hematologic and/or biochemical evidence of inflammation. Animals with CHr values below reference intervals were included in the low CHr group, while dogs with normal or increased CHr were included in the control group. Hct, MCV, CHr, reticulocyte mean cell volume (MCVr), serum iron, total iron binding capacity (TIBC), percent transferrin saturation (% sat), C-reactive protein (CRP), ferritin, ceruloplasmin, total white blood cell (WBC), neutrophil, and monocyte concentrations were determined. Non-parametric tests were performed; median values and percentage of abnormalities between each group were compared. Relative to control dogs, animals in the low CHr group had higher median values for CRP, ferritin, ceruloplasmin, and WBC concentration (P£0.05) and lower median values for Hct and MCV (P£0.0001). Higher frequencies of abnormalities for CRP, ferritin, WBC, neutrophil and monocyte concentrations (P£0.02) were present in the low CHr group. Dogs with low CHr values often have evidence of inflammation. CHr may not be as useful in predicting iron deficiency as previously thought. Additional diagnostic tests are needed to differentiate true and functional iron deficiency.

71/ ENCEPHALITIC ALPHAVIRUS E1 GLYCOPROTEIN-LIPOSOME-NUCLEIC ACID COMPLEXES PROTECT MICE FROM LETHAL CHALLENGE WITH MULTIPLE ALPHAVIRUSES

Amber B Rico, Aaron T Phillips, Tony Schountz, Ann M Toth, Ann M Powers, Ken E Olson

Alphaviruses are globally distributed, mosquito borne pathogens that cause disease and death in vertebrates, including humans. Therapeutics to combat alphaviral disease are non-existent and only a handful of IND status vaccines are available. Of the available vaccines most are associated with a poor immunological response and a high rate of reactivity, and none protects against more than a single alphavirus species. We designed and tested novel alphavirus vaccines comprised of the E1 glycoproteins of Venezuelan equine encephalitis virus (VEEV) and Western equine encephalitis virus (WEEV). Immunization with cationic lipid nucleic acid complexes (CLNCs) and E1ecto (lipid-antigen-nucleic acid complexes:LANACs) provided significant protection in mice challenged with either VEEV, WEEV, or eastern equine encephalitis virus (EEEV) regardless of challenge route. LANAC immunized mice mount a strong humoral immune response lacking neutralizing antibody. Passive transfer of immune sera from immunized mice or rabbits to non-immunized mice confers protection to challenge, indicating that non-neutralizing antibody is sufficient for protection. Antibody from LANAC immunized mice sera additionally reduces Chikungunya and Sindbis virus replication *in vitro* suggesting broad alphavirus applicability for E1 based vaccines and therapeutics. In summary, our LANAC vaccines have both therapeutic and prophylactic potential and are able to offer protection against distinct alphavirus species irrespective of the route of infection.

72/ THE MYCOBACTERIUM LEPRAE SPECIFIC PROTEIN ML1419C FUNCTIONS AS A DIGUANYLATE CYCLASE TO PRODUCE CYCLIC-DI-GMP

Suwatchareeporn Rotcheewaphan, Brad R Borlee, Kristofor Webb and John T Belisle

Mycobacterium leprae is the causative agent of leprosy; a disease that continues to be a public health problem in several regions of the world. *M. leprae* infects the upper respiratory tract, skin and peripheral nerves, and can lead to immune mediated tissue damage. It was previously demonstrated that the *M. leprae* protein (ML1419c) induces antigen specific IFN-γ production by CD8+ T cells of leprosy patients and mice experimentally infected with M. *leprae*. This demonstrated that ML1419c is produced during infection, but the physiological function of this protein in *M. leprae* has not been defined. *M. leprae* cannot be cultured *in vivo*, and thus protein functions for this bacterium are typically studied in model systems. Bioinformatics analyses predicted that ML1419c functions as a diguanylate cyclase in *M. leprae*. Diguanylate cyclases possess conserved GGDEF domains and produce bis-(3',5')-cyclic dimeric guanosine monophosphate (c-di-GMP) from two molecules of GTP. The c-di-GMP molecule functions as a bacterial intracellular signaling molecule that regulates activities such as bacterial cell survival, virulence, cell differentiation, colonization, and biofilm formation. The production and function of c-di-GMP is best studied in *Pseudomonas aeruginosa*. Thus, this bacterium was used as a model organism to assess the function of ML1419c. The recombinant expression of ml1419c in *P. aeruginosa* altered colony morphology and motility. Moreover, ml1419c expression resulted in increased c-di-GMP production in *P. aeruginosa* cultures as compared to *P. aeruginosa* with the vector control. Although these data demonstrate the ML1419c functions to produce c-di-GMP, the role of this signaling molecule in *M. leprae* is unknown. Continued study of ML1419c function, including elucidation of the ligand for the PAS signaling domains located upstream of the diguanylate cyclase domain, will aid in defining the role of c-di-GMP as a signaling molecule in *Mycobacterium leprae* and in the pathogenesis of leprosy.

73/ CYTOSKELETAL ALTERATIONS OF EQUINE OOCYTES THAT FAILED TO CLEAVE AFTER ICSI: EVALUATION OF MATERNAL AND CELL AGING

Elena Ruggeri, Keith F DeLuca, Cesare Galli, Giovanna Lazzari, Jennifer G DeLuca, and Elaine M Carnevale

Assisted fertilization using intracytoplasmic sperm injection (ICSI) is used in equine clinical programs. However, not all oocytes cleave after ICSI and the age of the oocyte donor affects oocyte quality in mares and women. We hypothesized that age of the oocyte donor affects a/b tubulin asters and f-actin bubbles after sperm injection. Oocytes were retrieved from preovulatory follicles of mares 9 to 25 yr in a clinical program and injected with sperm. Between 24-51 h after ICSI, uncleaved oocytes (n=52) were fixed and incubated with a/b tubulin and human anticentromere antibody-CREST/ACA. Oocytes were then incubated with Alexa 488, 647 and 561-phalloidin, and Hoechst 33258. Images were acquired on an Olympus IX81 confocal microscope and analyzed with SlideBook software. Mean donor age was compared for oocytes with or without specific morphologies using Student's t-test. The incidence of specific morphologies was evaluated in two age groups, Young (9-13 yr) and Old (20-25 yr), using Fisher's exact test. Multiasters were present in 62% of oocytes that failed to cleave after ICSI. A greater number of multiasters were observed per oocyte in Young (P=0.03) than Old, and the mean age of the donor was lower (P=0.05) in oocytes with multiasters (17.88 versus 20.80 yr). Actin bubbles were observed in 71% of oocytes that failed to cleave after ICSI, and more actin bubbles (P=0.05) were present in oocytes from Old than Young. The area and perimeter within oocytes occupied by the actin bubbles was larger (P=0.05) in oocytes from Old than Young. This study demonstrates cytoskeleton remodeling in presumptive zygotes after ICSI and differences within oocytes from young versus old donors. We postulated that actin bubble formation was linked to advanced age of oocyte donors and multiaster generation was a sign of activation that occurs after ICSI, more prominent in the oocytes from young mares.

74/ THE EFFECT OF DOCOSAHEXAENOIC ACID (DHA) ON CARDIAC MYOCYTE ADIPONECTIN PROTEIN EXPRESSION

Samantha Salmon, Briana Trusiano, Melinda Frye

Introduction: Adiponectin (APN) is a cardioprotective circulating protein produced primarily by adipocytes. Recent literature suggests that cardiac myocytes may themselves generate APN, providing a local source in addition to the adipose-derived circulating pool. We recently demonstrated that myocardial APN protein expression was increased in association with dietary DHA supplementation. The present study aims to identify whether this represents increased endogenous production or enhanced uptake of circulating APN, and to characterize the relative contribution of cardiac myocytes and microvascular endothelial cells to endogenous myocardial APN generation. Method: Weanling male Wistar rats were anesthetized with 5% isofluorane. The heart was excised and perfused with heparinized Joklik solution, followed by collagenase digestion. Serial centrifugation was used to generate myocyte and endothelial cell isolates; myocytes were plated on laminin. Cells were maintained at 37° C and 5% CO_2/95% room air for 48 hours prior to treatment with 1, 10 and 60 μM DHA. APN was quantitated using a commercially available ELISA kit (Rat Total Adiponectin, R&D Systems) and expressed per unit of protein (DC Protein Assay, Bio Rad). Cell lysate and medium APN content from DHA-treated cells were compared to untreated controls. Results: Cell culture media contained detectable amounts of protein; however, myocyte lysates did not. Neither media nor cell lysates contained detectable APN. Conclusion: Supernatant data suggest that cardiac myocytes do not secrete APN in response to DHA; however, these data may reflect false negative results due to low protein yield. No conclusions can be generated in relation to intracellular APN due to lack of protein. Alternatively or in addition, DHA treatment duration may have been inadequate for eliciting APN generation. Further studies are needed to characterize the cardiac myocyte response to DHA.

75/ COMMUNITY-ACADEMIC PARTNERSHIP TO REDUCE CARDIOVASCULAR DISEASE RISK IN NORTHERN COLORADO CHILDREN WITH ELEVATED CHOLESTEROL

Katie Schmitz, Erica C. Borresen, Dustin Brown, NaNet Puccetti, GaryLuckasen, and Elizabeth P. Ryan

A recent report from the National Heart, Lung and Blood Institute Expert Panel recommends implementing healthier lifestyles in children, as childhood is considered an important window of opportunity to focus on cardiovascular disease (CVD) prevention. In Northern Colorado, the Healthy Hearts program (HH) hasidentified elementary school-age students with modifiable CVD risk factors since 1992. HH data showed that 15% of elementary school-age students screened in 2012-2013 had borderline elevated total cholesterol measures (170-200 mg/dL). Rice bran (RB) and navy beans (NB) have demonstrated efficacy for cholesterol regulation in adults, yet very little is known in children. The main objective of this project is to determine the feasibility of increased intake of cooked NB and/or RB in children with identified modifiable CVD risk factors. Additionally, this pilot study (n=40) is evaluating serum cholesterol changes. A randomized-controlled, single- blinded, 4-arm nutritional intervention trial was designed that included placebo (no NB or RB), 17.5 grams cooked NB/day, 15 grams RB/day, and a combination of 9 grams of NB/day and 8 grams RB/day. Twenty-three children have completed a 3-day food log each week on study (analyzed using NutritionistProTM). Dietary intake data at baseline confirms a western dietary pattern including low fiber, high sodium, and high fat intake. Interim analysis demonstrates that this dietary intervention supports increased fiber intakes as well as selected micronutrients. This pilot study has created a strong community-academic partnership focused on evaluating RB and NB efficacy to lower cholesterol in children. Adding RB, NB, or both represents an affordable approach to improve nutrition in children and may provide a non-pharmacologic approach for chronic disease prevention, as well as advance education programs on whole grains and legumes.

76/ PILOT INVESTIGATION OF PERIPHERAL BLOOD NATURAL KILLER CELL POPULATIONS IN COLORECTAL CANCER SURVIVORS

Zachary Schwartz, Erica Borresen, Dustin Brown, Elizabeth P Ryan

Natural killer (NK) cells play a vital role in antitumor immunity, and limited evidence exists for the peripheral blood immune phenotype of colorectal cancer (CRC) survivors. The present study set out to examine peripheral blood mononuclear cells (PBMCs) collected from ten CRC survivors enrolled in the BENEFIT study (NCT01929122). Participants were randomized by body mass index, total caloric intake, and sex. Preliminary immune marker analysis was completed on PBMCs from five participants that followed a navy bean powder diet (35g/day), and five participants that followed a control diet without the addition of navy bean powder. PBMCs were collected at baseline, 2-week, and 4-week time points and flow cytometry analysis was used to determine the relative abundance of monocytes, T cells, NK cells, and NKT cells, using relevant cell surface and activation markers. We observed a wide range of NK cell expression in both groups and across all time points, with NK cells representing 5.5%-38.2% of total PBMCs. All analysis was completed using FlowJo software program. These results suggest a wide range of variability in the relative NK immune phenotype of CRC survivors. Future studies could help further illuminate the immune phenotype of CRC survivors and contribute to the growing body of knowledge concerning diet modulation of immunity.

77/ ENVIRONMENTALLY INDUCED COPY NUMBER VARIATION IN DIPLOID STRAINS OF THE BUDDING YEAST SACCHAROMYCES CEREVISIAE

Rabab S Sharif, Juan Lucas Argueso

The human genome is structurally dynamic, frequently undergoing loss, duplication and rearrangement of chromosome segments. These rearrangements result in copy number variations (CNVs) that have been recognized as contributing factors in cancer and in autism spectrum disorder. We investigated CNV formation mechanisms in diploid budding yeast cells following induction by ionizing radiation exposure by employing two assays for CNV detection. Our first (broad spectrum) assay takes advantage of two genes, SFA1 and CUP1, that confer gene dosage-dependent tolerance to formaldehyde (FA) and copper (Cu), respectively. Cells carrying a single chromosomal insertion of this SFA1-CUP1 CNV reporter were exposed to two acute sub-lethal gamma radiation doses (50 Gy ,200 Gy), and plated on selective media containing high levels of FA and Cu. Our second approach was to use a narrow spectrum non-allelic homologous recombination assay. We constructed strains in which the 3′ portion of the URA3 gene was deleted from its native position on chromosome 5 (URA-) and a second copy of URA3 missing the 5’ end (-RA3) was introduced on chromosome 14. Cells were exposed to the same sub-lethal acute doses of IR above and plated on selective uracil-dropout and permissive plates. In both cases, radiation exposures induced significant increases in the CNV rate compared to uninduced cultures. To allow further understanding of the CNV mechanisms associated with IR, selected independent FA and Cu resistant and Ura+ clones were analyzed by PFGE karyotyping and array-CGH to characterize the spectrum of CNV-associated genome rearrangements. The majority of the CNVs were mediated through NAHR either between dispersed repetitive DNA sequences (e.g. Ty, delta) or between the URA- and -RA3 heteroalleles. In our first assay the primary mechanism of amplification was unbalanced translocations. In our second assay, both unbalanced as well as balanced translocations between Chr 5 and Chr 14 were recovered.

78/ OCCUPATIONAL RADIATION DOSES DURING FLUOROSCOPICALLY GUIDED PROCEDURES IN A HYBRID OPERATING ROOM

Omar H Shatila, Deirdre H Elder, Thomas E Johnson

Fluoroscopy is an imaging technique that uses x-rays to form real-time continuous images. Increasingly complex procedures can performed using this technique, without the need for invasive surgery. The trade-off will be increased patient and staff radiation doses. There are several factors that influence staff radiation doses. The closer personnel are to the patient, the greater their doses due to scatter radiation from the X-rays beam. Orientation with respect to the fluoroscopy equipment can also influence dose, as scatter is not evenly distributed around the device. In the example of transcatheter aortic valve replacement (TAVR), fluoroscopy is used to guide a catheter for replacing the aortic heart valve. This is a minimally invasive procedure that is used in place of open-heart surgery. Due to the complexity of the procedure, a significant amount of fluoroscopic imaging is used, resulting in relatively high patient and staff radiation doses. There is currently no occupational radiation dose data available in the literature for individuals working for the TAVR procedure. Radiation doses for personnel in specific roles will be measured following each procedure in the operating room. The average dose per procedure will be determined for each role and correlation with fluoroscopy equipment outputs will be analyzed.

79/ LONG-TERM BEHAVIORAL CONSEQUENCES OF FETAL GLUCOCORTICOID EXCESS

Olivia R Shoup, Krystle A Frahm, and Stuart A Tobet

Corticosteroid treatment in late gestation is used in several species to increase the success rate of preterm births and prevent potentially fatal birth defects including cardiovascular, respiratory, and thermoregulatory. Foals born to dams administered the synthetic glucocorticoid dexamethasone in late pregnancy were able to undergo precocious fetal maturation and be born with similar developmental maturity as foals born from a normal gestation length. However, studies in sheep and rodents have indicated that administration of late gestation corticosteroids may alter the development of the Hypothalamic-Pituitary-Adrenal (HPA) axis. Studies have also implicated increased fetal corticosteroid exposure in long-term consequences in humans, including depression and alterations in HPA axis function. The current study examined the occurrence of depression-like behaviors in early adulthood in offspring born to dams administered dexamethasone in late gestation. Pregnant mice were injected with either dexamethasone (0.1mg/kg) or vehicle once daily from Embryonic Day 11-17. Then starting in early adulthood (Postnatal day 50), offspring were administered a series of behavioral tests: the Open Field Test (OFT), Tail Suspension Test (TST) and Sucrose Preference Test (SPT). Each test was performed at least four days apart, and the resulting data were analyzed for signs of depression-like behavior. Statistical significance was determined using SPSS software, with data differences with a $p<0.05$ considered significant. Complications during the study resulted in varied findings with general trends towards depression-like behavior in offspring from treated dams. This indicates that fetal dexamethasone may cause long-term behavioral impacts in affected offspring. More OFT data must be analyzed to verify these conclusions. Additionally useful data could be gathered from long-term studies in more species to assess the use of synthetic glucocorticoids in preterm births and to weigh the consequences of long-term behavioral side effects in adulthood against the benefits of reducing occurrence of birth defects in premature young.

80/ DYNAMIC CHANGES IN HIGH AFFINITY YET POLYREACTIVE B CELLS DURING DEVELOPMENT OF HUMAN TYPE 1 DIABETES SUGGESTS PATHOPHYSIOLOGIC FUNCTION

Mia J. Smith, Thomas A. Packard, Shannon K. O'Neill, Carole Dunand, Min Huang, Lisa Fitzgerald-Miller, Rochelle Hinman, 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, surface phenotype, reactivity, and variable region genes 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 BND compartment. Antigen receptors expressed by these cells are polyreactive and have N-region additions, Vh usage, and charged CDR3 regions consistent with autoreactivity. Consistent with a potential early role in autoimmunity, these high affinity insulin-binding B cells are absent from the anergic compartment of some first degree relatives, and all pre-diabetic and new-onset (<1yr) T1D patients tested, but return to normal levels in individuals diabetic for >1 year. Interestingly, these changes were correlated by transient loss of the entire BND compartment, irrespective of autoantigen specificity. These findings suggest that environmental events such as infection or injury may, by disrupting B cell anergy, dispose individuals toward autoimmunity, the precise nature of which is specified by genetic risk factors, such as HLA alleles. Our results suggest a role for perturbation of B cell anergy in development of T1D, and perhaps other autoimmune diseases, and shed light on the role of autoreactive B cells in the pathogenesis of T1D.

81/ HEMATOLOGIC IMMUNE MARKERS AND THE RELATIONSHIP BETWEEN DISEASE, ENVIRONMENT, AND IMMUNOCOMPETENCE IN FRENCH MOUNTAIN UNGULATES

M Kate Smith, Gilles Bourgoin, Jean-Michel Jullien, Carole Toïgo, Mathieu Garel, Philippe Gibert, and Emmanuelle Gilot-Fromont

Understanding the susceptibility to disease is a major challenge in biological research, with practical implications for animal and human health. Historically, much of our understanding of the immune system and its responses to stressors has come from controlled laboratory assays. Increasingly, the field of ecoimmunology and the study of the variations of immune capacities of individuals in natural populations, those experiencing the stresses of environmental variation, social interactions, parasitism, etc., is emerging as the next frontier in studies of immunology and epidemiology. To this end, the Alpine chamois (*Rupicapra rupicapra*) and Pyrenean chamois (*R. pyrenaica*) have been selected as models to explore the relationship between environment, immunocompetence, and disease transmission. Both species are ecologically important in their respective mountain ranges, as well as culturally and economically significant. Samples from individuals were either obtained via live-trapping of the animals or from individuals killed by licensed hunters over the course of more than 3 years. Blood samples from three locations were analyzed for the body condition and immune system markers and for the presence of antibodies and/or antigens to specific diseases. Statistical analyses were then performed to determine if there was a significant difference in the type of immune investment by individuals previously or currently infected with a variety of parasites (singly or in multiples), by sex, by environment, by age, and by combinations of the variables.

82/ DENGUE VIRUS ALTERS CENTRAL CARBON METABOLISM AND INDUCES A WARBURG-LIKE EFFECT FOR SUCCESSFUL REPLICATION

J. Jordan Steel, Jay Kirkwood, Corey Broeckling, and Rushika Perera

Dengue virus infects around 400 million people each year, resulting in serious morbidity and mortality worldwide. There are no therapeutics or drugs currently available to stop or inhibit infection, leaving one third of the world's population at risk of contracting this untreatable disease. New targets for antiviral therapies are desperately needed and a better understanding of how viruses usurp cellular pathways may lead to the discovery of new mechanisms to inhibit infection. Viruses are obligate intracellular parasites that are completely dependent on cellular metabolism. Glycolysis and other central carbon metabolic pathways are especially critical to provide the energy and biomolecules needed to generate new virions. We have used pharmacological inhibitors to monitor the effect of different stages of central carbon metabolism on Dengue virus infection. Our results indicate a strict reliance on glycolysis for successful virus replication. Metabolic and proliferation diseases, such as cancer, induce a Warburg effect where glycolysis is significantly increased in order to provide the energy needed to support cell division. We hypothesize that Dengue virus is inducing a similar change in carbon metabolism during infection. We are working with the Proteomics and Metabolomics Facility (PMF) at CSU to develop cutting edge techniques to analyze the DENV induced flux of carbon metabolism. Through understanding the metabolic changes induced during infection, we hope to identify novel steps to interfere and inhibit dengue virus replication.

83/ PREDICTIVE MODELING INDICATES CLIMATE AND PHARMACEUTICAL VARIABLES HAVE AN INFLUENCE ON SHEDDING PREVALENCE OF DAIRY E.COLI O157:H7

Chloe Stenkamp-Strahm, Lindsey Linke, Roberta Magnusen, Sangeeta Rao, and Craig McConnel

Among the bacterial pathogens shed by cattle, *E.coli O157:H7* ranks highest among those causing human illness. Although risk factors for *O157:H7* colonization have been assessed in beef cattle, less is known about variables that significantly impact dairy cow colonization. Early lactation dairy cattle (n=1090) were sampled monthly over the course of one year on three dry lot dairies surrounding Fort Collins, CO. During each visit, cattle feces were assessed for the presence of *O157:H7* and multiple variables were measured (history of disease, pharmaceutical use, milk production, climate measures, etc). Regression analysis was performed using *O157:H7* outcomes and all collected variables. Humidity was shown to significantly increase chances of *O157:H7* shedding. Meanwhile, an increase in the number of days in milk, and administration of any pharmaceutical treatment decreased the likelihood of pathogen shedding. In the future, this information may be used to guide farm management strategies for the reduction and prediction of bovine *O157:H7* shedding.

84/ DETECTION OF ANTIGENIC PROTEINS OF FELIS CATUS GAMMAHERPESVIRUS 1

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

We recently identified and sequenced a novel herpesvirus of domestic cats, *Felis catus* gammaherpesvirus 1 (FcaGHV1). FcaGHV1 is a member of the gammaherpesvirus subfamily which also includes the human cancer-associated herpesviruses, Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV). As a first step toward developing a serologic assay to detect exposure to FcaGHV1, we sought to determine which viral proteins elicit an antibody response in naturally occurring infections of domestic cats. As a strategy to select potential antigens, we chose seven FcaGHV1 proteins which are conserved across the subfamily and antigenic in other gammaherpesviruses. To generate recombinant FcaGHV1 proteins, we cloned the seven FcaGHV1 genes into a mammalian expression vector and transfected into a feline cell line. We used immunofluorescent antibody staining to test reactivity of the fixed cells with sera from nine cats naturally infected with FcaGHV1. Our data indicate that capsid proteins ORF65 and ORF17.5 and tegument proteins ORF52 and ORF38 elicit antibodies during naturally occurring FcaGHV1 infection. This work provides a basis for developing a high throughput serologic screening assay for FcaGHV1 infection.

85/ EFFECT OF TOLL-LIKE RECEPTOR-INDUCED INFLAMMATION ON CHONDROGENESIS OF BONE MARROW-DERIVED MESENCHYMAL STEM CELL

Suwimol Tangtrongsup, John D. Kisiday

Healing of articular cartilage defects by mesenchymal stem cells (MSCs) may be hindered by proinflammatory mediators in the synovial fluid of diseased joints. While interleukin-1 beta (IL-1b) is one factor that can inhibit MSCs chondrogenesis, the effects of ligands that activate toll-like receptors (TLRs) are not well understood. Here we tested the effects of both proinflammatory mediators on adult equine MSC chondrogenesis. MSCs were encapsulated in agarose and cultured in chondrogenic medium containing 1 ng/ml IL-1b, 1 ug/ml lipopolysaccharide (LPS) to activate TLR4, or 1 ug/ml Pam3CSK4 to activate TLR2. The concentration of each proinflammatory mediator was based on previous studies in which the selected dose strongly activated IL-1b or TLR signaling. Control cultures were maintained in baseline chondrogenic medium. After 15 days, total accumulated glycosaminoglycan (GAG) was quantified as an indicator of chondrogenesis. Culture media were collected on day 3 and day 9 for evaluation of prostaglandin E2 (PGE2), which is secreted in response to inflammation. Data were analyzed by ANOVA and least squares means. In IL-1b, GAG accumulation was suppressed 98% compared to controls, while PGE2 secretion on days 3 and 9 were 15- and 138-fold higher than controls, respectively. LPS did not affect GAG accumulation, nor did it elevate PGE2 secretion on day 3. On day 9 LPS enhanced PGE2 secretion 2.8-fold relative to controls. Pam3CSK4 suppressed GAG accumulation 35%. PGE2 secretion in Pam3CSK4 cultures was not significantly different from controls on day 3, but was 10-fold higher than controls on day 9. Taken together, suppression of chondrogenesis was positively associated with increasing levels of inflammation. For the tested doses, IL-1b was more effective in inducing inflammation and suppressing chondrogenesis. In LPS cultures, the modest increase in PGE2 may suggest an enhanced level of inflammation that is not sufficient to influence MSC chondrogenesis.

86/ PALATABILITY AND CLINICAL EFFECTS OF AN ORAL RECUPERATION FLUID DURING THE RECOVERY OF DOGS WITH PARVOVIRAL ENTERITIS

Reut J Tenne, Elena T Contreras, Francisco Olea-Popelka, David C Twedt, Michael R Lappin, and Lauren A Sullivan

Dogs infected with canine parvovirus (CPV) can develop severe enteritis that requires supportive care until voluntary food and water consumption returns. An oral recuperation fluid (ORF, Viyo Recuperation™) containing essential nutrients may assist in the overall recovery from CPV. The hypothesis was that dogs with clinical signs of CPV would prefer an ORF to water and that dogs consuming the ORF would have a more rapid return to voluntary appetite and improved caloric intake during the initial recovery period. Twenty-eight dogs with no prior treatment intervention were enrolled following detection of CPV antigen in feces. Dogs were randomized to either an ORF or water group. The designated fluid was offered q12h throughout hospitalization, followed by offering the opposite fluid one hour later if the designated fluid was refused. All dogs received a standardized treatment protocol including intravenous fluids, cefoxitin (22 mg/kg IV q6h) and maropitant (1 mg/kg IV q24h). Beginning on Day 2, all dogs were offered a gastrointestinal diet q8h, staggered with the q12h fluid intake trial. Forty percent (40%) of CPV dogs preferred the ORF as their designated fluid when compared to those offered water as their designated fluid (31%). Dogs that consumed the ORF demonstrated a more rapid return to voluntary appetite [median 1.5 days (range 1-3), p=0.01] than those that consumed water [median 4.25 days (range 1.5-5.5)] or neither fluid [median 2 days (range 1.5-5.5)]. Additionally, dogs that consumed the ORF ingested a higher %RER [median 100% (range 61-100%), p=0.03], compared to dogs that consumed water [median 19% (range 9-100%)] or neither fluid [median 37% (range 3-100%)]. The results suggest that some dogs with CPV will voluntarily consume an ORF during the recovery phase of their illness and that consumption of the ORF may foster a more rapid return of voluntary appetite and improved caloric intake.

87/ IRON DYSREGULATION IS EVIDENT IN CANINE HEMANGIOSARCOMA: PRELIMINARY STUDIES

Sarah Therio, Lauren Radakovich, Matthew Truelove, Christine Olver, and Kelly Santangelo

As a micronutrient, iron plays a significant role in cellular survival and growth. Dysregulation of molecules responsible for iron import, export, and storage has been evidenced in human and canine cancers and is associated with a worsened prognosis. We hypothesize that iron dysregulation also occurs in canine hemangiosarcoma (HSA), evidenced by altered expression of iron regulatory proteins. To demonstrate this, we assayed neoplastic and non-neoplastic tissues with immunohistochemistry (IHC) for expression of the iron import protein, transferrin receptor (TfR), and iron export protein, ferroportin (FPN). From human studies, we know that TfR and FPN are expressed in normal endothelial cells. We expected increased levels of TfR and decreased levels of FPN in tumor cells compared to their normal counterparts. Via IHC, TfR and FPN staining were quantified among tissues from four different tumors, using a visual scoring system that characterized both number of immunopositive cells and color intensity. IHC results showed a 6-fold increase in TfR expression in HSA cells compared to normal endothelial cells, and mildly decreased FPN expression in HSA cells compared to normal endothelial cells. The results support the notion of iron retention in cancer, information that could prove useful for therapeutic design targeted at iron regulatory pathways, as well as prognostication based on tumor cell iron content. Follow-up studies will be conducted to characterize expression of additional iron regulatory molecules in HSA and other cancers, including lymphoma, osteosarcoma, and histiocytic sarcoma. Future work will include measuring tumor iron content with atomic absorption spectroscopy and measuring expression of additional iron regulatory genes with real time PCR to achieve a more global picture of iron regulation in cancer.

88/ IN VITRO EVALUATION OF THE EFFECTS OF PRE-CONDITIONING AND GENDER ON FELINE ADIPOSE-DERIVED MESENCHYMAL STEM CELL CYTOKINE PRODUCTION

Rebecca M Timmons and Tracy Webb

The use of mesenchymal stem cell (MSC) therapy to treat clinical disease may be optimized through selection and pre-conditioning methods that would enhance their immunomodulatory functions. We investigated the effects of several pre-conditioning methods and gender of the tissue donor on parameters of feline adipose-derived MSC *in vitro* that may be applicable to and optimize the clinical effect of MSC application in this species. Passage 3 feline MSC were generated from cryopreserved subcutaneous adipose tissue from 6 healthy cats, 3 males and 3 females. MSC were subjected to 6 different pre-conditioning agents or conditions for 24 hours: control, poly I:C, IFNγ, TGFβ, serum-free, and hypoxia. Supernatants from the MSC were harvested after 24 hours, aliquoted, and frozen for batch analysis. Levels of IL-6, IL-8, IL-10, and MCP-1 in the supernatants were determined by ELISA. Both pretreatment and gender of donor significantly affected measured cytokine levels in the 24 hour supernatants from feline adipose-derived MSC: IL-6 and IL-10 were affected by donor gender whereas all of the measured cytokines were affected by at least one of the pretreatments. Although further study is necessary, this initial study shows that specific pre-conditioning methods hold promise for tailoring and augmenting feline adipose-derived MSC immunomodulatory properties to treat specific diseases, and gender of donor cat may be significant in this species depending on the desired therapeutic outcome.

89/ INTRAUTERINE APPLICATION OF CIPROFLOXACIN IN THE MARE

David A. Trundell, Patrick M. McCue, Luke A. Wittenburg, Dan L. Gustafson, and Ryan A. Ferris

Increasing resistance of the most common equine uterine bacterial isolates to current antibiotics is becoming a concern for the treatment of mares with infectious endometritis. Ciprofloxacin has been shown to have good efficacy against aerobic gram positive and gram negative microbes, including those commonly isolated in the mare's uterus such as *Streptococcus equi* subspecies *zooepidemicus* and *Escherichia coli*. A related antibiotic (enrofloxacin) has been shown to cause severe reaction when administered as a uterine infusion. The adverse effects of enrofloxacin in the uterus have been attributed to the carrier arginine, which ciprofloxacin lacks. Thus ciprofloxacin is an attractive alternative antibiotic. The aim of this study was to determine concentrations of ciprofloxacin in the uterine lumen over a 24 hour period after intrauterine infusion. Six mares in estrus had intrauterine infusions of 600 mg of Ciprofloxacin Injection (10 mg/ml). Samples of fluid within the uterine lumen were collected at 0, 2, 4, 12 and 24 hours post infusion. The average ciprofloxacin concentration at 24 hours post infusion was 94.18 ± 93.87μg/ml (range 5.0 μg/ml to 229.4 μg/ml). Intrauterine concentrations of ciprofloxacin were maintained above published minimum inhibitory concentrations (MICs) for both *S. equi* subspecies *zooepidemicus* (1μg/ml) and *E. coli* (6μg/ml) throughout the 24 hour period. No adverse effects of ciprofloxacin infusion on uterine health were noted. In conclusion, ciprofloxacin appears to be a good alternative for treatment of infectious endometritis caused by susceptible organisms in the mare.

90/ THE EFFECTS OF DOCOSAHEXAENOIC ACID (DHA) ON CARDIAC MICROVASCULAR ENDOTHELIAL CELL ADIPONECTIN PRODUCTION

B. Trusiano, S. Salmon, M. Frye

Introduction Adiponectin (APN) exerts cardioprotective effects, including mitigation of pathologic myocardial hypertrophy. It is a hormone produced primarily by adipocytes, but there is evidence that it is also expressed by cardiac myocytes and aortic endothelial cells. Endogenous production by microvascular endothelial cells has not been described. Circulating APN production is enhanced with intake of DHA, an omega-3 polyunsaturated fatty acid found in fish oils. In this study, we aimed to describe the effects of DHA treatment on APN expression by cardiac microvascular endothelial cells. We hypothesized that coronary microvascular endothelial cells produce APN in response to DHA, possibly serving as an additional mechanism of DHA-associated cardioprotection. Materials and Methods Male weanling Wistar rats were anesthetized with 5% isofluorane, then heparinized via the pulmonary artery. The heart was extracted and digested with collagenase. Microvascular endothelial cells were collected via centrifugation, and cell viability and yield were determined using a hemocytometer. The endothelial cells were plated onto fibronectin and maintained at 37˚C and 5% CO_2/95% room air until 80% confluence was reached. Cells were treated with DHA at 1, 10 and 60μm for 48 hours, then collected using trypsin. APN was measured in lysates and media from DHA-treated cells and untreated controls using a commercially available ELISA kit (RatTotal Adiponectin, R&D Systems), and normalized to protein content (DC protein assay, BioRad). Results Neither endothelial cell supernatants nor lysates contained detectable APN. Conclusion The data suggest that microvascular endothelial cell APN production may not be a contributor to DHA-mediated cardioprotection. However, protein yield may not have been sufficient to allow for APN detection, or DHA treatment duration may not have been sufficiently long to influence APN expression. Further studies are needed to characterize the cardiac microvascular endothelial cell response to DHA.

91/ EFFECT OF AZAPERONE ON BLOOD PRESSURE IN IMMOBILIZED BULL ELEPHANTS IN KRUGER NATIONAL PARK, SOUTH AFRICA

Rachel K. Wanty, Peter Buss, Michele Miller, Marius Kruger, and Francisco Olea-Popelka

Immobilization of free-ranging mega-vertebrates, including elephants, has become an essential part of wildlife management. The drugs used for immobilization are often potent opioids, butyrophenone agonists and alpha-2 agonists, which can act synergistically and are not without their own set of risks. While great improvements have been made to these drugs and drug administration protocols, they can still induce a set of side effects, which may prove to be fatal in some circumstances. In this project we analyzed the effects of azaperone (a dopamine antagonist) on systolic, diastolic and mean blood pressures.

92/ MEASUREMENT OF PLASMA FIBRINOGEN IN DOGS WITH HEPATOBILIARY DISEASE

Sara A Wennogle, Allison M Bradley, Christine S Olver, and David C Twedt

Hypofibrinogenemia has been described in humans with advanced liver disease and dogs with chronic hepatitis (CH). Additionally, hypofibrinogenemia has recently been correlated with peri-operative bleeding during liver transplantation in humans. Plasma fibrinogen concentrations have not been compared among different categories of liver disease in dogs. The goal of this study was to retrospectively evaluate fibrinogen concentrations in dogs with various histologic types of liver disease. Fibrinogen was measured in stored citrated plasma from 41 dogs that underwent liver biopsy at Colorado State University from June 2013-November 2014. The fibrinogen assay was performed on the Destiny AMAX Plus analyzer and results reported in mg/dL. The reported normal reference range for this assay is 117 to 392 mg/dl. Based on the histological diagnosis dogs were grouped into one of the following categories: non-specific reactive hepatopathy/NSR (n=13); chronic hepatitis/CH (n=9); hydropic/vacuolar hepatopathy (n=8); cholangiohepatitis (n=4); neoplasia (n=4); or other (n=3). Groups were compared via one-way ANOVA with Tukey adjusted pairwise comparisons and significance was defined as $p<0.05$. Dogs with histologically confirmed CH had significantly lower mean fibrinogen concentrations than other histological categories: NSR ($p=0.004$), hydropic/vacuolar ($p<0.0001$), cholangiohepatitis ($p=0.0001$), neoplasia ($p=0.0009$) or other ($p=0.0328$). Mean fibrinogen levels were not different among other histological types of liver disease. In patients with hepatobiliary disease, hypofibrinogenemia is more likely to occur in dogs with CH than other types of liver disease. Plasma fibrinogen concentrations may be a useful indirect indicator of hepatic function and hypofibrinogenemia may also predispose a patient to peri-operative bleeding. Further studies are needed to better characterize hypofibrinogenemia in dogs with hepatobiliary disease.

93/ REGULATION OF HUMAN AND SHEEP TROPHOBLAST CELL DIFFERENTIATION BY LIN28B

Rachel C. West, Erin S. McWhorter, Russel V. Anthony, Gerrit J. Bouma, Quinton A. Winger

Lin28A and Lin28B are RNA-binding proteins that serve an essential role in the maintenance of embryonic stem cells. Both proteins work in tandem to directly inhibit the let-7 miRNA family and prevent stem cell differentiation. As cells begin to differentiate, Lin28A and Lin28B decrease, allowing mature let-7 levels to increase. Recently, abundant expression of both Lin28A and Lin28B has been reported in the trophoblast of the placenta; however, Lin28's role in the placenta has yet to be fully understood. Irregularities in placental cell proliferation and differentiation often lead to impaired trophoblast invasion, leading to pathologies such as preeclampsia and intrauterine growth restriction. As Lin28B is a potent regulator of cell differentiation in other cell populations, we hypothesized that Lin28B acts as a regulator of trophoblast cell proliferation in the placenta. To investigate the role of Lin28B in the placenta we generated a Lin28B shRNA knockdown in ACH3P cells using a lentiviral approach. The knockdown was confirmed in ACH3P cells using quantitative PCR and western blotting. The effect of the Lin28B knockdown was assessed using proliferation and migration assays. Lin28B was also analyzed *in vivo* on day nine sheep blastocysts. Nine days after breeding, hatched blastocysts were collected and treated with lentivirus containing Lin28B shRNA. After lentiviral incubation embyros were transferred into a recipient ewe and gestated until embryonic day 15. These findings *in vitro* and *in vivo* suggest a novel role for Lin28B in human and sheep placentation.

94/ COMPARING TRANSFECTION EFFICACY OF SUPERFECT TRANSFECTION REAGENT AND ELECTROPORATION IN MESENCHYMAL STEM CELLS

Morgan Woodard, Ruth Rose, Laura Chubb, and Nicole Ehrhart

Mesenchymal stem cells (MSCs) are known for their multipotent properties and have potential to treat a multitude of clinical diseases. In addition they play a role in immunoregulation and bone healing. Along with the interest in using MSCs in human and veterinary medicine there is a need to track their fate after implantation. Therefore, optimization of transfection with reporter genes such as luciferase is paramount. The purpose of our study was to compare the efficacy of two different methods of transfection in MSCs to determine which method was optimal. The MSCs were split into two transfection groups, one group was transfected with SuperFect (Qiagen©) and the other with electroporation. The electroporated cells were transfected at eight different voltage and time settings to determine the optimal settings for transfection with minimal cell death. Both the electroporated and SuperFect cells were imaged using the IVIS in-vitro bioluminescence imaging system to measure levels of luciferase expression. The optimum electroporation setting was found to be 140 volts for 60 milliseconds with the highest average expression of $2.09x10^4$ photons/second, however, there was no significant difference between electroporation settings ($p>0.05$). The SuperFect transfected MSCs on average expressed higher levels of bioluminescent expression ($8.69x10^4$ photons/second) than the electroporated cells (2.09×10^4) although this difference was not significant ($p>0.05$). Small sample size may have contributed to these findings. In future studies, we would like to improve upon the transfection technique to obtain stably transfected cells, determine how long they will contain signal, and to eventually determine the fate of the cells in vivo after local or systemic administration.

95/ EVALUATION OF A NOVEL CANINE ACTIVITY MONITOR FOR AT-HOME PHYSICAL ACTIVITY ANALYSIS

Jonathan M Yashari, Colleen G Duncan and Felix M Duerr

Objective: To validate a novel, affordable, smartphone-based activity monitor (Whistle) for at-home physical activity monitoring in dogs. Animals: 11 large breed, privately owned dogs. Procedures: Dogs wore a collar fitted with both the Whistle device and a previously validated accelerometer-based activity monitor (Actical) for a 24- hour time period. Owners were asked to resume normal daily activities. Total activity time obtained from the Whistle device (TAW) was compared to the total activity count from the Actical device (TAA). Activity intensity from the Whistle device (AIW) was calculated manually from screenshots of the activity bars displayed in the smartphone-application and compared to the activity count recorded by the Actical (AIA) in the same time period. Results: A total of 3740 time points were compared. There was a strong correlation between activity intensity of both devices for individual time points (Pearson's correlation coefficient 0.81, $p<0.0001$). An even stronger correlation was observed between the total activity data TAW and TAA (Pearson's correlation coefficient 0.925, $p<0.0001$). Conclusions and Clinical Relevance: Activity data provided by the Whistle activity monitor can be used as an objective outcome measurement in dogs. The total activity time provided by the Whistle application offers a simple, inexpensive, user-friendly and rapid method for obtaining at-home, canine, real-time physical activity data. Smartphone-based features associated with the application such as the ability to log data including administration of medications, feeding regime, clinical impressions and share photos provide further benefits for clinical studies.

96/ IRONED OUT: A NEW WAY TO DIFFERENTIATE ANEMIA ETIOLOGIES

Charles Yurkon, Lauren Radakovich, Kelly Santangelo, Christine Olver

Anemia is the major measurable outcome of derangements of iron metabolism and occurs through two major mechanisms: functional deficiency (sequestration of iron in macrophages) and absolute deficiency (depletion of total body iron stores). It is important to differentiate between these two mechanisms because they are treated differently, and parenteral iron administration may cause deleterious effects in human patients with anemia of chronic disease (ACD). Thus far, the field of veterinary medicine lacks a parameter to appropriately distinguish true iron deficiency anemia from ACD as most assays – particularly ferritin, a storage form of iron that is also a positive acute phase reactant – are influenced by the presence of inflammation. This makes diagnosing patients with concomitant absolute and functional iron deficiency exceedingly difficult. In humans, soluble transferrin receptor (sTfR) to ferritin ratio has shown promise in distinguishing iron deficiency anemia from ACD because sTfR expression is not influenced by inflammation. In this study, we take the first steps in determining if sTfR:ferritin ratio holds promise in veterinary medicine. We compared ferritin levels measured in our lab via ELISA with those measured at Kansas State University Veterinary Diagnostic Lab and show that they correlate well with a R^2 of 0.95. Additionally, we identified a human-based kit for detection of sTfR (Abnova) that appears to measure canine sTfR in a predictable manner based on reticulocyte concentration in blood. Finally, we show evidence for existence of either a prozone or steric hindrance effect interfering with the human-based sTfR ELISA when sTfR concentration is expected to be high. From this data and previous studies regarding sTfR cleavage from exosomes in canids, we hypothesize that transferrin receptor is likely to be exosome-associated in canids and are planning future studies to further explore this question.

97/ H7N9 INFLUENZA A VIRUS TRANSMISSION IN A MODEL WET MARKET

Amanda K Zellar, Angela Bosco-Lauth, Airn T Hartwig, Richard A Bowen

H7N9 influenza A virus is an emerging pathogen in China, causing human disease with high fatality rate. Wet markets are complex environments containing many species of animals offered for sale, along with wild or pest species. These animals interact in a variety of ways including direct contact, sharing of food and water, and indirect contact. Poultry in wet market settings are implicated in spreading the H7N9 virus to humans, and have been observed to shed virus at high titers and readily infect conspecifics through contact (Jones et al, 2014). Sparrows and other passerines commonly inhabit wet markets, and have been shown to shed virus at high titer and be capable of transmitting the virus to other sparrows (Pantin-Jackwood et al, 2014). Finally, rats are inevitably resident in wet markets, but their role in influenza virus transmission is not known. To better understand how interactions between these species may influence the spread of H7N9 through a wet market, several small scale experiments were performed, before the construction of a model wet market: a study to establish how readily free flying sparrows infect caged poultry, a study observing H7N9 pathogenicity in rats, and a study documenting persistence of H7N9 in water. Virus shedding was only observed in sparrows on day one post infection at or below 3.9 pfu/mL. Virus shedding was not detected in chickens at any time during the study. No signs of illness were observed in sparrows or chickens during the study. Rats became infected with H7N9 virus, but did not display signs of illness and shed virus at titers of up to 3.8 log pfu/mL. These studies contribute to our understanding of the potential risk of interspecific interactions in spreading H7N9 influenza and should be useful in informing further research aimed at controlling spread of this zoonotic pathogen.

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Regan	32		1:15, Salon V
Rico	74	71	
Rosenberg	36		3:30, Salon V
Rotcheewaphan	75	72	
Rout	36		3:45, Salon V
Ruggeri	75	73	
Salmon	76	74	
Schmitz	76	75	
Schwartz	77	76	
Sharif	77	77	
Shatila	78	78	
Shoup	78	79	
Sims	37		4:00, Salon V
Smith, B.	32		1:30, Salon V
Smith, Mia	79	80	
Smith, M. Kate	79	81	
Steel	80	82	
Stenkamp-Strahm	80	83	
Stutzman-Rodriguez	81	84	
Sullivan	37		4:15, Salon V
Summers	33		1:45, Salon V
Tangtrongsup	81	85	
Tenne	82	86	
Therio	82	87	
Thigeel	33		2:00, Salon V
Timmons	83	88	
Trundell	83	89	
Trusiano	84	90	
Tuttle	34		2:15, Salon V
Verma	38		4:30, Salon V
Wanty	84	91	
Warrit	34		2:30, Salon V
Wendland	35		3:00, Salon V
Wennogle	85	92	
Willingham	38		4:45, Salon V
West	85	93	
Woodard	86	94	
Yashari	86	95	
Yurkon	87	96	
Zellar	87	97	

NOTES:

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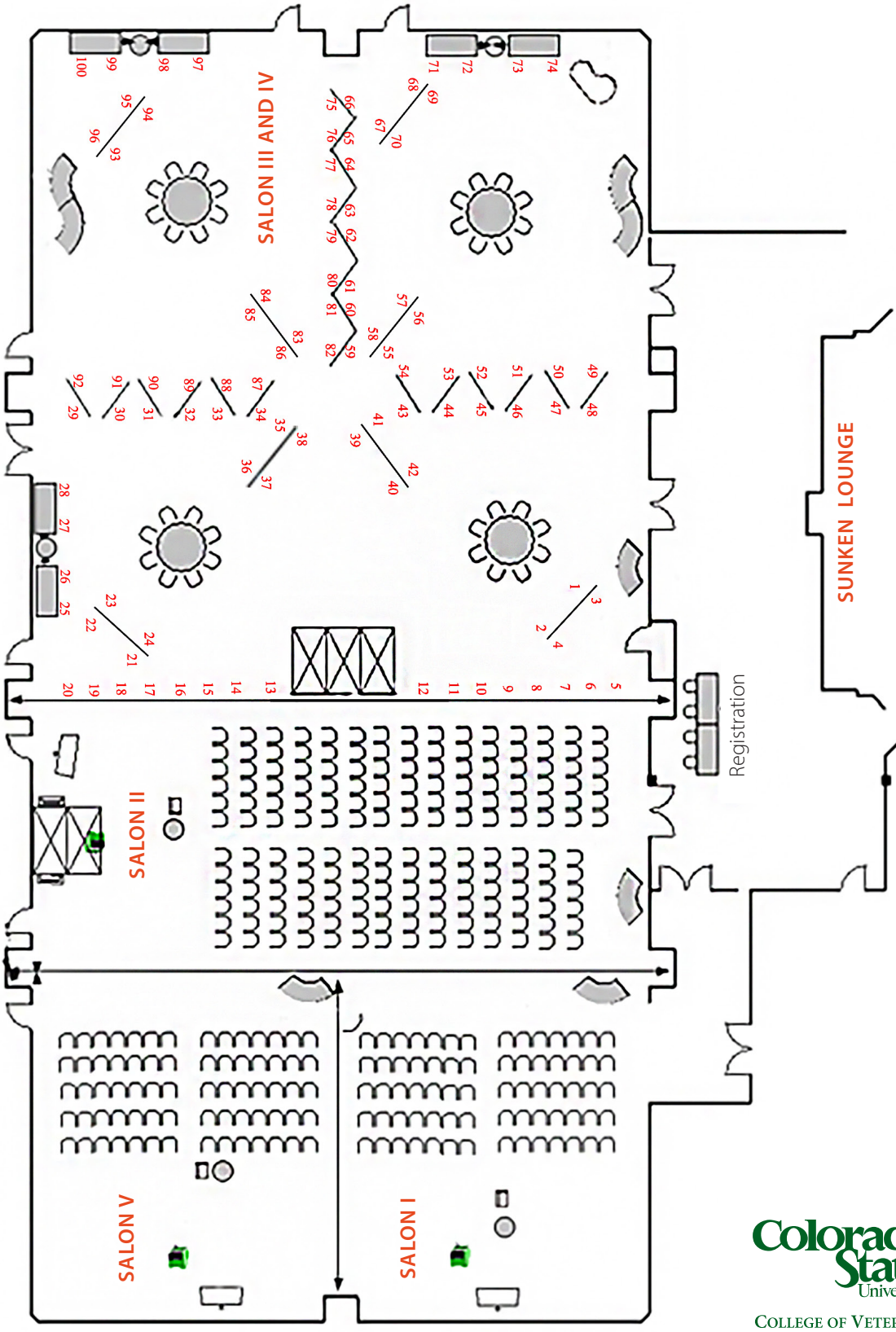
2015 CVMBS Research Day

FLOOR PLAN COLORADO STATE BALLROOM I HILTON FORT COLLINS



- Poster numbers shown in red on this floor plan correspond to those next to presenter names in the index, allowing attendees to find presenters and posters of interest.
- **Please walk through the back of Salon I to reach Salon V.**

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