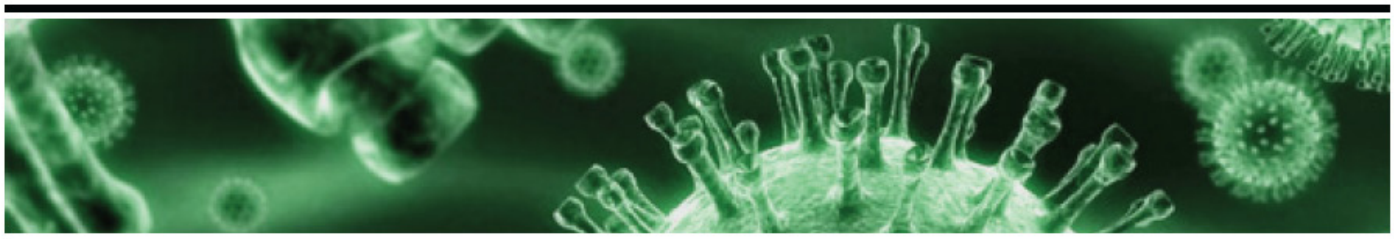
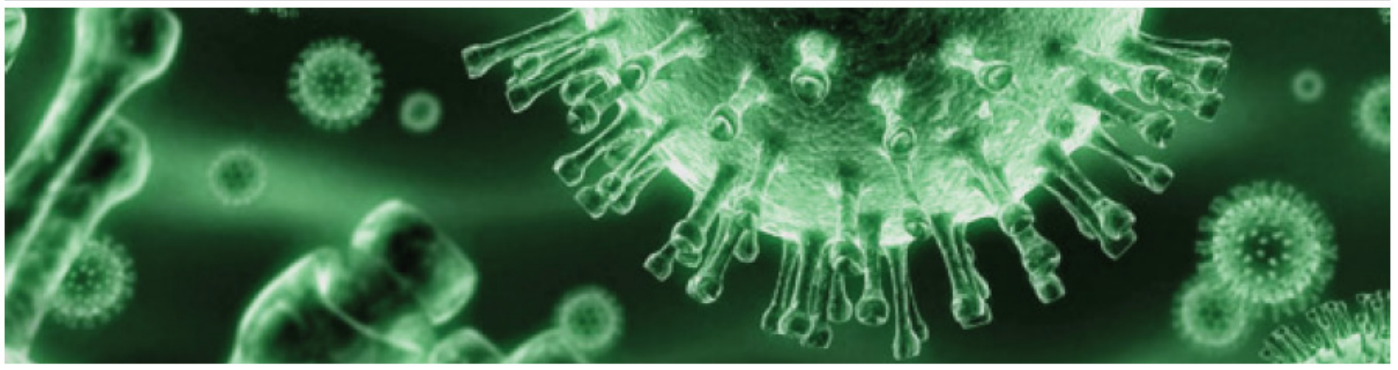


Colorado State University

13th Annual College of Veterinary Medicine and Biomedical Sciences Research Day Scientific Proceedings

The Hilton Hotel

January 28, 2012



Thank you moderators and judges!!

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CVMBS Research Day 2012

Schedule Of Events		Room
11:30-12:00	Poster set up	Salon III, IV
12:00	Opening remarks – Dr. Dawn Duval	Salon I
12:05	Pfizer Research Award Winner – Dr. Shane Hentges	Salon I
	"Transmitter Release in Neural Circuits Controlling Energy Balance and Reward"	
12:45	Break	
1:00-5:00	Oral Presentation I: Clinical Sciences	Salon I
1:00-5:00	Oral Presentation II: Basic Sciences	Salon V
1:00-5:00	Oral Presentation III : Basic Sciences	Salon II
1:00-3:00	Poster Session I Judging: Basic Sciences	Salon III, IV
3:15-5:00	Poster Session II Judging: Clinical Sciences	Salon III, IV
5:00-6:00	Social Hour, Remove Posters	Salon III, IV
5:30	Awards	Salon III, IV

Oral Presentation: - Please limit to a 12 minute talk with 1-3 minutes for questions and changeover. Oral presentations will be in Salons I, II, and V.

Poster Presentation: - Please hang your posters on Jan. 28 from 11:30-12:00 in Salons III and IV. Individuals presenting the poster must be in attendance to discuss their materials with judges as listed above.

PFIZER RESEARCH AWARD WINNER

CVMBS Research Day
Saturday, January 28, 2012

Dr. Shane Hentges, Ph.D.

"Transmitter Release in Neural Circuits Controlling Energy Balance and Reward"

Dr. Shane Hentges received her bachelor's degree in genetics and cell biology from Washington State University as well as her Ph.D. in neuroscience. She then completed postdoctoral training at the Vollum Institute at Oregon Health and Sciences University in Portland. Dr. Hentges is currently an Assistant Professor of Biomedical Sciences at Colorado State University. Her research is focused on understanding the central control of food intake and reward with the long-term goal of identifying targets for the prevention and treatment of eating disorders and other disorders of reward circuits such as drug abuse. Current studies are particularly focused on the release and signaling of neurotransmitters in relevant brain circuits. Experimental techniques used to address these issues include electrophysiology, circuit mapping, *in situ* hybridization, optogenetics, and transgenic animal models. Using these approaches, it is possible to determine how cells in complex circuits interact with one another and how these interactions become disordered. Understanding the connections in the complex brain circuits that control energy balance and encode the reinforcing nature of food and drugs is a key step towards identifying therapeutic interventions for eating disorders and other disorders involving the brain's reward circuits.

Salon I
The Hilton Hotel
Fort Collins, CO

Oral Presentations

SESSION 1: CLINICAL SCIENCE 1:00-4:45PM Salon I			
1:00	Allaband	Combined Vaccination and Antimicrobial Therapy for Treatment of Chronic Staphylococcal Infection	MIP
1:15	Brandes	Anti-inflammatory drugs will decrease the rate of endothelial cell infection with Equine Herpesvirus-1 in vitro	CS
1:30	Brundage	CT iodine contrast does not have a diagnostic effect on tumor glucose uptake for FDG PET-CT	EHRS
1:45	Carlsten	Pharmacokinetics of Vinblastine in Dogs with Mast Cell Tumors	CS
2:00	Chamberlin	Ultrasound-guided vascular access in dogs	CS
2:15	Griffin	Stereotactic Radiation Therapy for Intracalvarial Tumors in Dogs	EHRS
2:30	Holbrook	Acute Behavior of a Biologically Active Bone Graft Substitute for Spinal Fusion	EHRS
2:45	BREAK		
3:15	Kane	Expression of Cyclooxygenase-2 and Matrix Metalloproteinase-2 and -9 in Canine Atherosclerosis	CS
3:30	Moorman	Use of Inertial Measurement Units for Evaluation of Equine Motion	CS
3:45	Neary	The cross-sectional area of the longissimus dorsi muscle in pre-weaned beef calves is positively associated with the oxygen diffusion capacity of the lungs.	CS
4:00	Nolan	Intensity-modulated and image-guided radiation therapy for treatment of canine genitourinary carcinomas	EHRS
4:15	Sharpley	Color and Power Doppler Ultrasonography For Characterization Of Splenic Masses In Dogs	EHRS
4:30	Wilson	Arthroscopic Biceps Ulnar Release Procedure (BURP): Assessment of Regional Damage and Completeness of Release	CS

Oral Presentations

SESSION 2: BASIC SCIENCE
1:00-4:45PM

Salon V

1:00	Birkenheuer	The retroviral cyclin of walleye dermal sarcoma virus	MIP
1:15	Cadmus	Density, distribution and zoonotic disease risk factors associated with backyard poultry ownership in metropolitan Denver, Colorado	MIP
1:30	Cavarra	Socialization and training improves temperament and adoptability of laboratory Beagles	LAR
1:45	de Mooy	Mechanism and subpopulation specificity of mitochondrial Reactive Oxygen Species release in the post-ischemic hyperthyroid myocardium	BMS
2:00	DeFord	Early detection of chronic wasting disease in TG12 mice by neurological and behavioral assessment	NWRC
2:15	Dickson	CELF1-mediated mRNA decay regulates protein secretion and myogenesis and may be impaired in myotonic dystrophy	MIP
2:30	Enriquez	Molecular network involving LIN28 in ovarian cancer and secreted vesicles	BMS
2:45	BREAK		
3:15	Fox	Experimental Transmission of Bighorn Sheep Paranasal Sinus Tumors	MIP
3:30	Gates	Proline Rich 15 Regulates Trophoblast Proliferation and Differentiation	BMS
3:45	Haley	Sensitivity of protein misfolding cyclic amplification vs. immunohistochemistry in antemortem detection of chronic wasting disease.	MIP
4:00	Kumar	Nonspecific Induction of Gut Mucosal Immunity and Colonization Resistance against Salmonella by Rice Bran in Mice	CS
4:15	Le	Cardiac Mitochondrial Phenotype of the Tafazzin shRNA Mouse Model of Human Barth Syndrome	CMB

Oral Presentations

SESSION 3: BASIC SCIENCE
1:00-4:45PM

Salon II

1:00	Meyerett Reid	Host Factors Influence Prion Strain Adaptation	MIP
1:15	Neff	Global analysis reveals pathways of unique regulation of mRNA decay in human induced pluripotent stem cells	MIP
1:30	Penman	Equine Mesenchymal Stem Cells in vitro Differentiation Capacity: Differences Between Sternum and Ilium	CS
1:45	Podell	Alterations in the immunopathogenesis of tuberculosis associated with dietary-induced formation of advanced glycation end products	MIP
2:00	Reagan	Characterization of Semliki Forest fluorescent reporter viruses.	CS
2:15	Romero	Differential gene expression in corpora lutea from non pregnant and pregnant sheep	BMS
2:30	Sutherland	Post-Exposure Immunization Against Francisella tularensis Membrane Proteins Augments Protective Efficacy of Gentamicin in a Mouse Model of Pneumonic Tularemia	MIP
2:45	BREAK		
3:15	Troy	Liposomes Combined with TLR9 Agonist Produce Effective Mucosal Vaccine against Mycobacterium tuberculosis.	MIP
3:30	Venable	Hyaluronan cisplatin conjugate in five dogs with soft tissue sarcomas	CS
3:45	Wyckoff	Estimating Prion Binding Capacity of Soil	MIP
4:00	Lee	The PARN deadenylase regulates decay of a discrete set of transcripts in mouse myoblasts	MIP

Departmental Abbreviations

BMS: Biomedical Sciences
CMB: Cell and Molecular Biology Program
CS: Clinical Sciences
ERHS: Environmental and Radiological Health Sciences
MIP: Microbiology, Immunology, and Pathology

Poster Presentations

Session 1-Odd Numbered Posters 1:00-2:45PM
Session 2-Even Numbered Posters 3:15-4:45PM

#1	Antoniazzi	Endocrine Delivery of Interferon-tau into the Uterine or Jugular Veins at Different Concentrations Protects the Corpus Luteum from Prostaglandin F2 Alpha Induced Luteolysis
#2	Barnard	Role of Autophagy in Tumor Development and Metastasis.
#3	Beemer	Evaluation of Point of Care Glucose Meters in Alpacas
#4	Bender	Behavioral and Cognitive Differences of Mice Inoculated with Mouse Adapted Prion Strains
#5	Borresen	Increasing Consumption of Rice Bran and Navy Beans for Colon Cancer Control and Prevention: A Randomized-Control Pilot Investigation
#6	Bosco-Lauth	Mycobacterium bovis model of infection in goats
#7	Bradley	Intranasal Administration of a Modified Live Feline Herpesvirus 1 and Feline Calicivirus Vaccine Induces Cross Protection Against Bordetella bronchiseptica
#8	Brundage	Optimizing immunostaining of fibrillin-1 in canine tissue: Implications for quantifying changes in canine mitral valve disease
#9	Brundage	Normal canine brain glucose uptake and distribution using FDG-PET-CT
#10	Burgess	Rapid Salmonella detection in experimentally-inoculated equine feces and environmental samples using two commercially available lateral flow antigen detection systems
#11	Cerra	Evaluating Hypertension in the General Canine Population
#12	Chen	Pilot study evaluating the risk factors associated with zoonotic disease transmission in a goat contact area
#13	Christakos	Effects of platelet-rich plasma on chondrogenesis of bone marrow derived mesenchymal stem cells in dilute fibrin gels
#14	Clarke	Prevalence of select vector borne agents in owned dogs of Ghana
#15	Collins	Understanding Trends in Dog Ownership, Health, and Veterinary Care to Save Dog Lives
#16	Conway	Evaluation of the soft tissue attachments of the equine stifle using radiographic analysis
#17	da Silveira	MiRNA regulation of aromatase (CYP19) in equine granulosa cells
#18	Deogracias	Cancer stem cells in canine malignant melanoma and osteosarcoma have stable phenotypes and enhanced survival in the tumor microenvironment
#19	Dicken	Regulation of GABA and Glutamate Release from Proopiomelanocortin Neuron Terminals in Intact Hypothalamic Networks
#20	Dirsmith	Retrospective review of Northern fur seal (Callorhinus ursinus) placentas for Coxiella burnetii on St. Paul Island
#21	Doepker	The effects of intensive forest management on the prevalence of Hantavirus and gastrointestinal parasites in wild deer mice

#22	Dudek	Site-directed metabolic biotinylation of AMPA receptors may perturb protein-protein interactions with TARPs
#23	Emanuelli	Novel vaccination strategy for feline immunodeficiency virus
#24	Fenimore	Evaluation of novel treatments for shelter cats with suspected viral causes of upper respiratory disease
#25	Forster	Metabolomics as a novel tool to assess dietary modulation of the canine metabolome in response to navy bean consumption
#26	Fowles	Developing cross-species predictions of drug sensitivity for canine cancer
#27	Frank	Serological responses against antigenically distinct contemporary equine influenza virus strains (H3N8) induced by commercially available vaccines
#28	Gillette	Genetic polymorphisms in fabI in Burkholderia species and resistance to fabI inhibitors
#29	Goldrick	Ocular toxicity following stereotactic radiotherpay for canine nasal tumors
#30	Habenicht	Urinary Cytokine Concentrations in Normal Cats and Cats with Chronic Kidney Disease
#31	Halleran	Sheep placenta and developmental programming
#32	Halsey	The use of novel lymphatic endothelial cell-specific immunohistochemical markers to differentiate angiosarcomas in dogs
#33	Harms	Design and profiling of a series of AMPA receptor modulators
#34	Haugen	Disruption of advanced glycation end products by the antimicrobial drug, isoniazid
#35	Hodge	Detection of Salmonella spp. in the environment at agricultural fairs in association with poultry and waterfowl exhibitions
#36	Jalal	DNA strand break induced bystander effect (DBIBE) linked to gene mutations and telomere double strand break fusions in naïve cells.
#37	Jarvie	Proopiomelanocortin neurons in the arcuate nucleus have inhibitory and excitatory subpopulations
#38	Kalet	Transcriptome Analysis of Murine Osteosarcoma
#39	Khamsi	Studying the role of ADHFe1v3 and CCDC3 in canine osteosarcoma cell resistance to chemotherapy drugs
#40	Kihara	Effects of Synthetic Feline Facial Pheromone Use on Reducing Incidence of Upper Respiratory Tract Disease
#41	Kinner	Association of PECAM-1 and idiopathic pulmonary fibrosis
#42	Kitchen	Immunohistochemical detection of CWD prions in the CNS of Muntjac Deer
#43	Lagana	Characterization of FIV sequences in Bobcats (Lynx rufus)
#44	Lenberg	The effects of maropitant (Cerenia) on the clinical recovery of dogs with parvoviral gastroenteritis
#45	Lishnevsky	Comparative Analysis of Bleomycin In Pulmonary Disease Susceptible PECAM Deficient Mice
#46	Manzanares	Alternative Methods for Cryopreservation of Stallion Spermatozoa
#47	Marquez	The Analgesic Effect of Maropitant as a Pre-anesthetic Agent During and After Canine Ovariohysterectomy

#48	Martin	Vaccine-associated Leptospira antibody responses in client-owned dogs
#49	McMillan	A Rationale for Evaluating Livestock Contaminated with Radioactive Materials
#50	Meyers	The characterization of glutamate-gated chloride channels from Anopheles gambiae as insecticidal drug and vaccine targets
#51	Miller	Viral characterization of feline immunodeficiency virus in saliva and salivary tissues
#52	Monahan	The taste receptor T1R3 is expressed in at least 2 different cell populations in the mammalian hypothalamus
#53	Moon	A non-coding RNA produced by all arthropod-borne flaviviruses inhibits the cellular exonuclease XRN1 and modulates messenger RNA stability
#54	Morley	Identification of Methicillin-Resistant S. aureus (MRSA) of Animal Origin Using Bacteriophage Amplification and a Lateral-Flow Immunoassay
#55	Moser	Venous Lactate Measurement in Post Operative Colic Horses
#56	Moser	Evaluation of maxillary blockade via the infraorbital foramen approach ? A magnetic resonance imaging (MRI) study in equine cadavers
#57	Mosovsky	Effects of Interactions Between Antimicrobial Peptides and Antibiotics on Bacterial Killing
#58	Myers	Apoptosis in Normal and Coxiella burnetii Infected Placentas from Alaskan Northern Fur Seals (Callorhinus ursinus)
#59	Myrick	Chemically Induced Retinal Degeneration Model in Goldfish
#60	Nelson	Incidence of upper respiratory disease in cats at an emergency shelter.
#61	Niyom	Effect of Maropitant, an Antiemetic Neurokinin-1 Receptor Antagonist for Dogs and Cats, on the Sevoflurane Minimum Alveolar Concentration During Ovarian Stimulation in Cats.
#62	Pabilonia	Detection and isolation of pH1N1 influenza A virus from a privately owned small swine herd in Colorado
#63	Pennock	Presynaptic Gi/o-coupled receptors resist acute desensitization
#64	Phillips	Encephalitic alphavirus infection of outbred mice visualized using in vivo and ex vivo imaging.
#65	Porsche	Body condition score does not predict myocardial triglyceride content in canids
#66	Rauhauser	Computed tomography mapping of distal limb synovial structures studied in twelve horses
#67	Rezende	Plasma concentrations, behavioral and physiological effects following IV administration of dexmedetomidine in horses
#68	Rout	Transferrin receptor expression in serum exosomes as a marker of regenerative anemia in the horse
#69	Ruple-Czerniak	Isolation of Salmonella enterica from the environment in a large animal hospital
#70	Schuler	Potential effects of volcanic emissions (VOG) on respiratory health of free-ranging Mouflon Sheep

#71	Scofield	Equine endometrial concentrations of fluconazole following oral administration
#72	Sessions	Effect of equine metabolic syndrome on the intrafollicular environment and fertility
#73	Shapiro	A Novel Detection Method for Aerosol Reactivity
#74	Silva	Vaccination with attenuated Burkholderia for protection from acute and chronic melioidosis
#75	Soisson	LIN28A regulates human trophoblast syncytialization and preeclampsia diagnostic markers
#76	Spencer	Genes relevant to cardiomyopathy are differentially expressed in response to Western diet feeding alone and with DHA supplementation
#77	Sprenger	Developing an Animal Decontamination Protocol
#78	Stanton	Study of Spontaneous and Environmentally Induced Copy Number Variation and Possible Mechanisms
#79	Sullivan	The effects of omeprazole therapy on bacterial colonization of the pharynx in healthy dogs
#80	Swancutt	Endothelial cell apoptosis, in vitro and in situ, as a component of the tumor control mechanism induced by stereotactic radiation therapy
#81	Tarvis	Improving Rooster Sperm Cryopreservation: Effects of Alternative Cryoprotectants, Diluent and Straw Size on Cryosurvival
#82	Tighe	Anti-microbial activity of Fuzhuan tea, a fermented preparation of Camellia sinensis (L)
#83	Timmons	Osmotic Fragility and Flow Cytometric Determination of Lipid Peroxidation in Feline Erythrocytes
#84	Van de Motter	The evaluation of biochemical markers and an in vitro prion amplification assay for the diagnosis of CWD using cerebrospinal fluid.
#85	Walton	Combined Immuno-antimicrobial Therapy for the Treatment of Chronic Staphylococcal Infection
#86	Wiggans	Development of an indirect enzyme-linked immunosorbent assay for the detection of feline antibodies against Mycoplasma felis
#87	Wolf-Ringwall	Identification and characterization of metastasis-related microRNAs in osteosarcoma
#88	Wood	Development of microsphere immunoassays for the detection of domestic cat antibodies

Congratulations Again to 2011 CVMBS Research Day Winners:

Oral Presentations

First Basic	Anna Wykof
Second Basic	Kelly Carlsten
First Clinical	Karen Beckwith
Second Clinical	Joanna Virgin

Poster Presentations

First	F Sagawa
Second	Ajay Kumar
Third	Jared Fowles

2012 CVMBS Research Day Organizing Committee

Dawn Duval - Faculty Chair – Clinical Sciences
James Graham – Assistant Chair – Biomedical Sciences
KC Gates – Biomedical Sciences
Dawn Sessions – Biomedical Sciences
Valeria Scorza – Clinical Sciences
Valerie Moorman – Clinical Sciences
Donasian Ochola - Environmental and Radiological Health Sciences
Michelle Shave - Environmental and Radiological Health Sciences
Justin Lee - Microbiology, Immunology, and Pathology
Brady Michel - Microbiology, Immunology, and Pathology
Heidi Pecoraro - Alternate - Microbiology, Immunology, and Pathology
Ryan Pieterick - Alternate - Biomedical Sciences
Sue VandeWoude - CVMBS Associate Dean of Research
Aimee Oke – CVMBS Dean's Office

Veterinary Summer Scholars Program
College of Veterinary Medicine and Biomedical Sciences

The Veterinary Summer Scholars program was initially established through support from Merck-Merial to provide an opportunity for veterinary schools to expose students in their first and second years of veterinary medical school to biomedical research. With continued support from Merial, several other organizations, CVMBS and faculty mentors have contributed funds to provide summer stipends for program participants. The current Veterinary Student Scholars program gives veterinary students hands-on exposure to veterinary medical research to introduce them to potential research careers.

Twenty veterinary students from CSU and abroad to participated in the 2011 CSU Veterinary Summer Scholar program. Students spent the summer working in research labs, attending weekly research seminars and field trips to CSU, federal and state research facilities and concluded their summer research experience at the 2011 Merial NIH Veterinary Scholar Symposium in Orlando, Florida. Many of those projects are being presented today at the CVMBS Research Day.

2011 Summer Scholars Sponsors

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Royal Dick School of Veterinary Science, Edinburgh

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To view the research of students funded in 2011 or to apply for the summer 2012 program, please visit the website at:

http://www.cvmbs.colostate.edu/ns/students/veterinary_scholars_program/

We are excite to announce that the 2012 Merial NIH Veterinary Scholar Symposium will be hosted by Colorado State University at the Embassy Suites in Loveland Aug 2-5th 2012.

PVM Student Grant Program
Center for Companion Animal Studies
Department of Clinical Sciences

In 2006, the HESKA Corporation made a $20,000 donation to support research that involved PVM students. That year, the monies were used to support 5 excellent projects chosen from 9 that were submitted. With continued collaboration from the HESKA Corporation, the PVM student grant program was opened to other corporate and non-corporate donors. The amount of funding has continued to grow yearly and in 2011, $58,500 was raised and distributed to 28 different projects all of which involved a PVM student as a scientist. Many of those projects are being presented today at the CVMBS Research Day. Colorado State University offers thanks to all sponsors of this program and is looking forward to advancing the veterinary sciences with our partners in the years to come while concurrently involving PVM students in clinical research.

2011 PVM Student Grant Program Sponsors

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To view the grants funded in 2011 or to make a donation, please visit the Center for Companion Animal Studies website at:

http://csuvth.colostate.edu/veterinarians/research/companion_animals/student_projects.aspx

Oral Presentations

Session I ~ Salon 1
1:00-5:00PM

CLINICAL SCIENCE

Combined Vaccination and Antimicrobial Therapy for Treatment of Chronic Staphylococcal Infection

Celeste Allaband, Kelly Walton, Valerie Johnson, Lon Kendall, Steven Dow

Staphylococcus aureus (SA) infections, especially infection with MRSA strains, are a growing concern in both human and animal hospitals. Our lab has previously shown that combining immunotherapy with antimicrobial therapy can significantly improve the effectiveness of antibiotic therapy for acute bacterial infections. Therefore, we hypothesized that vaccination against SA could be used to improve the effectiveness of antimicrobial therapy for chronic SA implant infections. To test this hypothesis, we developed a mouse model of chronic SA implant infection, using SA-infected polypropylene mesh implanted s.c. in ICR outbred mice. A luciferase-expressing SA strain (XEN36) was used for these studies, which allowed us to quantitate bacterial infection using in vivo imaging (IVIS system) of infected mice every 2-3 days for 2 weeks. The SA vaccine was prepared from SA biofilm cultures, and was administered s.c. twice before infection and repeated once after infection. Mice treated with antibiotics received amoxicillin-clavulanic acid by drinking water (0.150 mg/mL). There were 4 treatment groups of n = 5 mice each: a. Untreated control; b. Antibiotic treated; c. Vaccinated; d. Vaccinated plus antibiotic treatment. In vaccinated only mice, bacterial burden actually increased significantly initially, then returned to near control values by the end of the experiment. Mice treated with antibiotics only had a slow decline in bacterial burden. In the group treated with antibiotics plus SA vaccine, bacterial burden was lower at all time points. We concluded that the biofilm infection model was a useful animal model to investigate the effects of chronic antimicrobial therapy of SA infection. Vaccination against SA appeared to improve the effectiveness of antibiotic therapy for chronic SA biofilm infections. Additional studies are required to determine the optimal timing and duration of vaccination for optimal enhancement of antimicrobial therapy for chronic SA infections.

Anti-inflammatory drugs will decrease the rate of endothelial cell infection with Equine Herpesvirus-1 in vitro

Kathleen M. Brandes, Laura V. Ashton, Luke Wittenburg, Francisco Olea-Popelka, Lutz S. Goehring

Central nervous system (CNS) endothelial cell (EC) infection with EHV-1 is the most likely cause of EHV-1 Myeloencephalopathy(EHM). Cell-associated viremia in peripheral blood mononuclear cells (PBMC) transports virus to the CNS EC; however, EC infection likely requires a cell-to-cell contact between EC and PBMC. Intercellular contact is thought to be facilitated by inducible adhesion molecules. We hypothesize that anti-inflammatory drugs will decrease the interaction between PBMC and EC, and, therefore, will decrease EC infection. Carotid artery EC monolayers were exposed to either in vitro EHV-1 infected (strain Ab4; MOI 0.01) PBMC or with a viral suspension (control). Infected PBMC were incubated 24 hrs with media alone (NTx), or with equine therapeutic plasma concentrations (1xTx) or 10x concentrations (10xTx) of one of the following drugs: flunixin meglumine, firocoxib or dexamethasone. Virus suspension (EHV-1 Ab4; 100 PFU/mL) or NTx/Tx PBMC in media containing EHV-1 neutralizing(VN) antibody (titer 1: 200) were added to monolayers for 4 hours. Then, monolayers were washed 3x times, and media NTx, 1xTx or 10xTx -various drugs- were re-applied for additional 48 hrs. All media contained a VN antibody titer of 1:200.Monolayers were stained with crystal violet solution to allow a plaque count. Generalized linear and latent mixed models were used to assess differences in plaque counts. Statistical significance was assumed with a p-value of 0.05. In the cell contact model all drugs significantly decreased plaque counts at 1xTx and at 10xTx ($p<0.001$); however, there was no statistical difference between the effects of 1xTx and 10xTx($p=0.163$) on plaque reduction.With virus-in-suspension inoculation there was no statistical difference between groups. These results provide a rationale for the use of anti-inflammatory drugs during early phases of EHV-1 infection. Moreover, the use of anti-inflammatory drugs during viremia may aid in preventing EC infection in vivo.

CT iodine contrast does not have a diagnostic effect on tumor glucose uptake for FDG PET-CT

Cord Brundage, Elissa Randall, Billie Arceneaux, Jeff Stewart, Susan Kraft

18-fluorodeoxyglucose positron emission tomography–computed tomography (FDG PET-CT) is a highly sensitive means of detecting and staging cancer. CT scans from human cancer PET-CT are used only for anatomy and PET attenuation correction; a diagnostic CT scan is done separately. In veterinary medicine, a diagnostic pre- and post-contrast CT scan done simultaneously with PET-CT would economize on anesthesia time as well as cost. Contrast media may significantly alter the attenuation correction, and therefore the PET FDG standard uptake values (SUV). To evaluate the effect of CT contrast enhancement on SUV values, PET scans from canine (n=11) and feline (n=13) cancer patients were attenuation corrected 3 ways using the CT scan obtained prior to, immediately following, and one hour after iodine contrast media injection (Pre, Post, and Delayed scans respectively). Region of interest analysis was used to quantify CT Hounsfield units (HU) and attenuation corrected standard uptake values (SUV) for specific normal tissue regions (ascending aorta, spleen, right and left epaxial muscles, liver lobes, renal cortices, pelvic cortical bone, and marrow) as well as tumors (Philips workstation). For tumors and renal cortices, tissues which typically strongly enhance, average SUV's derived by attenuation correction using the 3 different CT scans did vary significantly, but only slightly (greatest difference between means was 2%). For the rest of the tissues, there were no statistical differences in PET SUV values regardless of CT attenuation correction. The effect of contrast enhancement and CT scan used for attenuation correction is therefore inconsequential for diagnostic sensitivity of PET images, and should have minimal bearing on the use of SUV quantification when using PET longitudinally to evaluate response to therapy. This research was supported by a Morris Animal Foundation Veterinary Student Scholars Grant, the Department of ERHS and the Animal Cancer Center.

Pharmacokinetics of Vinblastine in Dogs with Mast Cell Tumors

Kelly S. Carlsten, Daniel Gustafson, Luke Wittenburg, Douglas Thamm

Purpose: Vinblastine is commonly used for the treatment of canine mast cell tumors. The pharmacokinetics of vinblastine has been evaluated in humans, but have yet to be investigated in dogs. Vinblastine is typically well tolerated; however, significant interpatient variability in toxicity is appreciated clinically. At standard doses some dogs develop neutropenia. The goal of this study is to evaluate the interpatient variability in the pharmacokinetics (PK) of vinblastine in dogs. Materials/methods: 6 client-owned dogs with a confirmed mast cell tumor were enrolled in this study. All dogs had an acceptable health status and adequate blood work. Dogs received vinblastine at 2.5 mg/m2 intravenously (IV). Serum was collected at times 0, 5, 10, 15, 30, 45 minutes, and 1, 1.5, 2, 4, 6, and 24 hours post drug administration. Serum was evaluated for vinblastine concentration using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and analyzed using non-compartmental analysis. Neutrophil count was assessed pre-treatment and one week post treatment (nadir). Results: The mean values were as follows: area under the curve (AUC) was 2102.1 ng/mL*min, t½a was 1.3 min, t½ß was 111.2 min, maximum concentration (Cmax) was 273.9 ng/mL. Plasma concentrations sharply decreased within the first 10 minutes indicating rapid distribution. Interpatient variability in PK parameters was substantial. Four dogs had detectable levels at 24 hours. No association was found between AUC or Cmax and remaining neutrophil fraction at day 7. Conclusions: Our data suggest that vinblastine administered IV to dogs has rapid distribution and prolonged elimination with substantial interpatient variability. We plan to enroll additional dogs to verify these findings.

Ultrasound-guided vascular access in dogs

Scott C. Chamberlin, Lauren A. Sullivan, Pedro Boscan

Purpose: To describe the technique and determine the feasibility, success rate, perceived difficulty and time to vascular access using ultrasound guidance for jugular vein catheterization in a cardiac arrest dog model.
Materials/Methods: A total of 27 jugular catheterizations were performed using ultrasound guidance in nine Walker hounds post cardiac arrest. Catheterizations were recorded based upon the order in which they were performed and presence/absence of a hematoma around the vein. Time (minutes) until successful vascular access and perceived difficulty in achieving vascular access (1-10) were recorded for each catheterization.
Results : Mean time to vascular access was 2.9 minutes (95% confidence interval 1.6-5.2 minutes) for catheterizations without hematoma, versus 5.8 minutes (95% confidence interval 1.9-17.7 minutes) for catheterizations with hematoma (P = 0.2). Median perceived difficulty for all catheterizations was 2 out of 10 (range 1-8). A learning curve was evaluated by comparing mean time to vascular access and perceived difficulty in initial versus subsequent catheterizations. Mean time to vascular access (with or without hematoma) was 4.2 minutes (95% confidence interval 1.6-11.2 minutes) in the initial 13 catheterizations versus 3.9 minutes (95% confidence interval 1.4-11.5 minutes) in the subsequent 14 catheterizations (P = 0.9). Median perceived difficulty (with or without hematoma) in the first 13 catheterizations (3.5 out of 10, range 1-8) was significantly greater (P = 0.03) than median perceived difficulty in the subsequent 14 catheterizations (2 out of 10, range 1-7).
Conclusions: Ultrasound-guided jugular catheterization is associated with a moderate learning curve but is successful in obtaining rapid vascular access in dogs. Further prospective studies are warranted to confirm the utility of this technique in a clinical setting.

Stereotactic Radiation Therapy for Intracalvarial Tumors in Dogs

Lynn Griffin, Micheal Nolan, James Custis, Susan LaRue

Stereotactic radiotherapy (SRT) involves large doses of radiation in a small number of treatments delivered in a precise fashion to a defined target. SRT has been performed in humans for treatment of intracalvarial tumors for the past 5 decades. The objective of this retrospective study is to assess toxicity and survival in dogs treated with SRT for intracalvarial tumors. MATERIALS AND METHODS: SRT was used to treat intracalvarial tumors in 40 dogs at Colorado State University between July 2008 and June 2011. Medical records were retrospectively evaluated for oncologic history, toxicity data and overall survival. Diagnosis of tumor type was presumptive based on imaging characteristics. 24 to 30 Gray (Gy) in 3 to 6 fractions were delivered on consecutive days. Statistics were descriptive; survival outcomes were evaluated using Kaplan-Meier analysis. Overall survival was based on death due to any cause RESULTS: Of the 40 dogs treated the following tumor types were represented; 2/40 choroid plexus tumors, 3/40 glial cell tumors, 1/40 nerve sheath tumors, 3/40 unknown tumor types, 12/40 pituitary macroadenomas, 19/40 meningiomas. Median overall survival time (MST) was 594 days. Dogs with meningiomas had an increased survival time of 594 days compared with other tumor types; this was not statistically significant. DISCUSSION: Previously reported survival times for dogs with intracalvarial tumors treated with radiation alone range from 250-699 days. In these studies duration of treatment was 15 to 41 days with doses per fraction ranging from 2.5 to 4 Gy. CONCLUSION: SRT is an effective means of treatment with minimal side effects for dogs with intracalvarial tumors. Outcome data appears to be comparable to that in dogs treated with fractionated radiation therapy. Duration of treatment and number of anesthetic episodes are greatly reduced in comparison to conventional fractionation.

Acute Behavior of a Biologically Active Bone Graft Substitute for Spinal Fusion

Ellen Holbrook, Jeremiah Easley, Howard Seim, Dana Ruehlman

Purpose: There is a significant need for biologically active bone graft substitute products that stay in their intended location long enough to promote bone healing. Numerous products have been developed, none of which are perfect for their intended use and further testing is required for improvements in this field.
Materials/Methods: Three different reinforced forms of a biologically active bone graft substitute will be compared to a control (the current commercially available product). Six sheep will be used for the study in a 2-level instrumented/uninstrumented posterolateral lumbar fusion (PLF) at L2-L3 and L4-L5. Implants will be placed on the lamina and transverse processes bilaterally. Handling properties of all products will be evaluated. Immediately post-op, CT scans will be made of the lumbar spine and again 3 weeks later at sacrifice date. Product thinning as well as migration/movement of the test products will be determined by CT scans and gross necropsy examination by two separate evaluators. No statistical analysis was performed for comparison between products.
Results: A modified fiber-reinforced form of the commercially available product outperformed all other products. Prior to implantation, the fiber-reinforced product was strongest. Very little thinning and absolutely no migration occurred. Good vascularization and ground substance layer were evident on gross necropsy.
Conclusions: The current study concluded that the biologically active fiber-reinforced bone graft substitute out-performed the others in vivo. In its dry state, it is not ideal because it is difficult to handle. However, once hydrated, this sample conforms to the implantation site and is structurally sound during manipulation. Further studies evaluating the different formulations of the fiber-reinforced products are needed prior to human clinical trials.

Expression of Cyclooxygenase-2 and Matrix Metalloproteinase-2 and -9 in Canine Atherosclerosis

Nathaniel Kane, EJ Ehrhart, Brad Charles, Colleen Duncan

Atherosclerosis is a common disease in humans and is seen in several animal species. While there are similarities between man and dog with respect to atherosclerosis, there are also differences—this disease is frequently clinically appreciated as an acute cardiovascular insult in humans, while in animals it is often an incidental finding on necropsy. Extensive research has focused on the inflammatory and structural remodeling characteristics of human atherosclerosis. However, far less is known about the pathogenesis of this naturally occurring disease in animal species. Cyclooxygenase-2 (COX-2) and matrix metalloproteinases 2 and 9 (MMP-2, MMP-9) are known to play a role in the pathogenesis of human atherosclerosis. The objective of this study was to identify expression of COX-2, MMP-2 and -9 in canine atherosclerotic lesions using histopathologic and immunohistochemical (IHC) evaluation. Tissues were collected from eight dogs of varying age, sex, and breed, identified through retrospective analysis of cases presented to a single university veterinary diagnostic laboratory. Individual samples from each dog were stained using hematoxylin and eosin (H&E) for standard histopathologic interpretation, and then stained using IHC for COX-2, smooth muscle actin (SMA), integrin beta-2 (CD-18), MMP-2, and MMP-9. Histologic features and IHC stains for SMA and CD-18 revealed lesion architecture consistent with that seen in humans; specifically, macrophage infiltration and smooth muscle proliferation throughout the tunica intima. COX-2 was not expressed in any of the affected vessels. MMP-2 and MMP-9 were increasingly expressed by macrophages and smooth muscle cells with lesion severity, consistent with the pathogenesis seen in humans. These findings suggest that canine atherosclerosis lacks the same inflammatory COX-2 driven cascade, but shares similar structural remodeling characteristics involving MMP-2 and -9 as those present in humans affected with this disease.

Use of Inertial Measurement Units for Evaluation of Equine Motion

Valerie J Moorman, Raoul F Reiser, C Wayne McIlwraith, Chris E Kawcak

Subtle lameness is one source of poor performance in athletic horses. While stationary force platform and 3-D optical systems are considered gold standards for diagnosis of subtle lameness, these methods are restricted to laboratory settings due to cost, expertise, and time for data collection. Thus, horse-mounted motion analysis systems are being developed for on farm diagnosis of lameness. Inertial measurement units (IMUs) are ideal for this purpose, as they are relatively inexpensive, easy to use, and can provide data during multiple consecutive strides in a short period of time. The central hypotheses of this investigation were that the IMU would provide accurate distal limb kinematics compared to a 3-D optical system, as well as provide accurate peak vertical ground reaction forces (pVFs) compared to a stationary force platform. Six normal horses were examined at the walk and trot over hard ground. Kinematic and kinetic data were collected simultaneously from the 3-D optical, force platform, and IMU systems. Linear and angular kinematic variables were extracted from both the 3-D optical and IMU systems. A duty factor was calculated from the IMU data, from which the pVF and normalized pVF were determined, which were then compared to force platform data. Paired t-tests, Pearson's correlation coefficients, and root mean squared errors were used to test the accuracy of the IMU kinematic and kinetic data. Sagittal plane kinematics had overall high correlations ($r>0.8$) and low error rates (0.86) and were not significantly different ($p>0.14$) between the force platform and IMU. From this data, the IMU appears suitable to examine sagittal plane kinematics and pVFs in normal horses over hard ground at the walk and trot. This system should be examined further to determine its ability to diagnose lameness in horses in both experimental and clinical settings.

The cross-sectional area of the longissimus dorsi muscle in pre-weaned beef calves is positively associated with the oxygen diffusion capacity of the lungs

Joseph M. Neary, Franklyn B. Garry, Kraig Peel

Purpose: Per unit of basal oxygen consumption the alveolar surface area of domestic cattle is less than half of the mammalian average. This means that a high demand is placed on the bovine pulmonary system. Since muscle growth has a high demand for oxygen one hypothesis is that calves with poor pulmonary oxygen diffusion experience sufficient systemic hypoxemia to limit musce growth. Live animal total muscling can be predicted from the cross-sectional area of the longissimus dorsi muscle ultrasonically. We hypothesized that the longissimus dorsi diameter of calves is positively associated with the pulmonary oxygen diffusion capacity or, negatively associated with the alveolar-arterial (Aa) oxygen gradient, when controlling for confounders.
Methods: 60 beef calves, 102-280 days old, from two ranches 1466m and 2008m above sea level were studied. Calves were restrained, weighed and sampled in a squeeze chute. Coccygeal arterial blood was analyzed using a handheld blood-gas analyzer. Pulmonary arterial pressure (PAP) was measured by passing a catheter through a needle inserted into the jugular vein through the right atrium, into the right ventricle, and then into the pulmonary artery. The cross-sectional area of the rib-eye was obtained ultrasonically between thoracic vertebrae 12 and 13.
Results: Generalized estimate equations were used to account for repeat measures on calves. Mean PAP was not significantly associated with muscling ($p=0.27$). For every 1 mmHg reduction in the Aa oxygen gradient the rib-eye area increased by 0.18 cm2 ($p<0.001$) when controlling for weight ($p<0.001$), ranch effects ($p=0.002$) and age ($p<0.001$).
Conclusions: Cross-sectional area of the longissimus dorsi muscle in pre-weaned beef calves between 3 and 10 months old was positively associated with pulmonary oxygen diffusion capacity. These findings highlight the importance of calf pulmonary health.

Intensity-modulated and image-guided radiation therapy for treatment of canine genitourinary carcinomas

Michael W. Nolan, Lori Kogan, Lynn R. Griffin, James T. Custis, Fred Harmon, Barbara Biller, Susan M. LaRue

Purpose: External beam radiation therapy can be used to treat pelvic tumors in dogs, but the utility of this treatment modality is limited by associated late complications. The objective of this retrospective case series was to assess local tumor control overall survival and toxicity following intensity-modulated and image-guided radiation therapy (IM/IGRT) for treatment of canine genitourinary carcinomas (CGUC).
Materials/methods: Medical records of patients that were treated with IM/IGRT for CGUC were reviewed. Toxicity data was analyzed using descriptive statistics; actuarial statistics were employed to describe survival data. Twenty-one dogs, having primary disease of the urinary bladder (9), urethra (2) or prostate (10), had the intent to complete a course of IM/IGRT between 2008 and 2011. Disease was confined to the genitourinary system in 16 dogs at the time of initial staging; 4 patients had nodal metastases and one had distant metastases. The majority of patients were treated with adjuvant chemotherapy and NSAIDs. The radiation dose ranged from 54-58 Gy, delivered in twenty daily fractions.
Results: A majority of patients developed mild to moderate and self-limiting acute gastrointestinal toxicity; fewer than 25% developed acute urinary tract or integumentary toxicity. Late radiation toxicity was also limited. There was a 60% subjective response rate, and 90% of patients experienced potential clinical benefit. The median event-free survival was 317 days; overall median survival time was 654 days. Location of the primary tumor had no demonstrable effect on either local tumor control or survival.
Conclusions: IM/IGRT is generally well-tolerated and provides an effective treatment option for locoregional control of CGUC. As compared with previous reports in the veterinary literature, inclusion of IM/IGRT in multimodal treatment protocols for CGUC results in superior survival times.

Color and Power Doppler Ultrasonography For Characterization Of Splenic Masses In Dogs

Jenelle Sharpley, Angela Marolf, Jean Reichle, Annette Bachand, Elissa Randall

Purpose: Splenic masses are a common ultrasonographic finding in dogs. Benign and malignant splenic masses can have similar B-mode imaging features, making ultrasound sensitive but not specific in their diagnosis. The goal of this study was to find Doppler characteristics of vasculature within and adjacent to a splenic mass which would distinguish a benign versus malignant lesion. The hypothesis was that malignant splenic masses will have altered vascular patterns compared with benign masses. Methods: This was a prospective study of dogs with an ultrasound finding of a splenic mass with histologic or cytologic diagnoses. Multiple Doppler cineloops evaluating the vasculature within the mass and in adjacent normal splenic parenchyma were obtained. Cineloops were reviewed independently by a radiologist and a resident unaware of the final diagnosis. If a discrepancy occurred, cineloops were re-evaluated adding another radiologist to reach a consensus opinion. Categories evaluated included presence of peritoneal effusion, presence of a large aberrant or tortuous vessel within the mass, relative blood flow within the mass compared with normal parenchyma, and path of vessels in the adjacent parenchyma entering into the mass. Fisher's exact test was used. Results: Twenty-six dogs were included. There were 10 malignant and 16 benign masses. Peritoneal effusion was significantly associated with malignancy ($p=0.0038$). Presence of an aberrant or tortuous vessel within the mass was borderline significant ($p=0.0549$). There was no significant difference in any of the Doppler blood flow evaluations. Conclusions: Ultrasonographic findings of a splenic mass and peritoneal effusion may indicate malignancy. The presence of an aberrant vessel within a splenic mass could suggest malignancy; however, further investigation to determine true significance is needed. Doppler evaluation of blood flow of splenic masses cannot distinguish between benign and malignant lesions.

Arthroscopic Biceps Ulnar Release Procedure (BURP): Assessment of Regional Damage and Completeness of Release

David M. Wilson and Ross H. Palmer

Introduction: The purposes of this study were to refine and describe a technique for arthroscopic BURP and to assess the influence of arthroscopist experience on the completeness of release and regional damage associated with the procedure. Materials and methods: Twenty elbows from 10 canine cadavers were assigned to either experienced- or inexperienced arthroscopist groups. Using conventional portals, each surgeon sought to identify the medial collateral ligament (MCL) and biceps tendon (BT) before attempting a complete release using a meniscal push knife. Each surgeon assigned a “visual control” score (1-5) that described the degree of BT and MCL visualization during the procedure. In addition, each surgeon was required to predict the degree of unintentional damage. Following arthroscopic BURP, each elbow was dissected to determine the percent tenotomy completion and the amount of regional damage (MCL, median nerve, etc.). Statistical analyses assessed for differences in the visual control score, the rate of complete tendon release, and presence of regional damage between experienced- and inexperienced arthroscopists. Results: A complete BURP was performed in 85% of the elbows using a meniscal push-knife oriented proximal-to-distal and “saddled” over the proximal BT margin immediately caudal to the MCL. There was an association between visual control score during the procedure and tenotomy completeness ($p < 0.01$), though there was no association between visual confirmation of a complete release and tenotomy completeness ($p = 0.47$). Iatrogenic partial thickness damage to the MCL was noted in 10% of elbows. Surgeon experience did not influence visual control score, iatrogenic regional damage, or the ability to perform a complete BURP. Conclusion: In canine cadavers, arthroscopic BURP can be performed safely and consistently using conventional arthroscopy instruments and portals by experienced and inexperienced arthroscopists.

Oral Presentations

Session II ~ Salon V
1:00-5:00PM

BASIC SCIENCE

The retroviral cyclin of walleye dermal sarcoma virus

Claire Birkenheuer, Sandra L. Quackenbush, and Joel Rovnak.

Purpose: Walleye dermal sarcoma virus (WDSV) is a complex retrovirus causing seasonal dermal sarcomas in walleye fish. The cyclic nature of the tumors is due to regulated expression of three accessory proteins encoded by virus. These proteins are produced by open reading frames, ‘a’, ‘b’, and ‘c’. Open reading frame ‘a’ encodes a retroviral cyclin (RV-cyclin) that is one of only two viral proteins produced during tumor development. It has two main functional domains, a cyclin box and a transcriptional activation domain. The cyclin box binds cyclin dependent kinase 8 (CDK8) and the activation domain binds TATA binding protein associated factor 9 (TAF9). CDK8 and TAF9 are key transcription factors, and studies of the RV-cyclin are providing insights into how these factors contribute to oncogenesis.
Methods and Results: We hypothesize that RV-cyclin alters host gene expression by enhancing transcriptional initiation and elongation through interaction with CDK8 and TAF9. Here, by quantitative reverse transcription PCR (Q-RT-PCR), we show RV-cyclin increases transcripts from cell cycle and serum response genes shortly after entering the nucleus. Furthermore, RV-cyclin enhances transcription of the serum response genes 60 min after serum stimulation in cells stably expressing RV-cyclin as measured by Q-RT-PCR. Additionally, mutants of RV-cyclin, which do not bind to CDK8 or TAF9, do not increase transcript levels of these genes. CDK8 functions specifically in the elongation of serum response gene transcripts. Chromatin immunoprecipitation experiments show the 3’ end of the cyclin D gene has an increased occupancy by CDK8 in cells expressing RV-cyclin compared to the control.
Conclusions: This data suggests that the RV-cyclin is playing a role in transcription elongation, and supports the hypothesis that RV-cyclin's role in tumor development is to alter expression of regulatory genes by directly interacting with the transcriptional machinery.

Density, distribution and zoonotic disease risk factors associated with backyard poultry ownership in metropolitan Denver, Colorado

Kyran J. Cadmus, Ryan S. Miller, Matthew Farnsworth, Katherine E. Slota, Sarah M. Millonig, Kimberly Forde-Folle, Richard A. Bowen, Kristy L. Pabilonia.

Purpose: Backyard poultry flocks are not well characterized, and prevalence of these flocks in the U.S. is unknown. Backyard flocks have been involved in economically significant nationwide avian disease outbreaks and flock owners are considered at high risk for infection with zoonotic poultry diseases. This study was conducted to determine backyard poultry flock prevalence and distribution, understand flock characteristics and human-bird interactions, and to identify risk factors for introduction of avian zoonotic diseases in a typical urban-suburban area. Materials/Methods: A cross-sectional survey was conducted of census block groups in urban and suburban areas of Denver, Colorado. Flock distribution data were compared to U.S. Census demographic data to determine potential predictive characteristics for poultry ownership. Results: Poultry flock prevalence in the Denver metropolitan area was 2.2% overall (1.55% urban, 4.40% suburban). Egg laying chickens were the most common birds kept (79%). The most common reason for owning birds was food production for the family (50.7%). Bird movements were rare (13% of flocks); however, all went to places where other birds were located. Flocks raised for show were more likely to be large flocks ($p=0.001$), more likely to travel ($p=0.007$), and were the only flocks that traveled out of state. Demographic variables related to poultry ownership included married childless couples, single females, and housing values above $200,000. Poultry were more likely to be found in census block groups having larger lot sizes and lower population per square mile. Conclusions: Our study identifies risk factors for introduction of avian diseases to backyard flocks, and describes characteristics of poultry ownership in a typical urban-suburban area. This information may be useful for targeted surveillance and public education efforts in the event of an avian infectious or zoonotic disease outbreak.

Socialization and training improves temperament and adoptability of laboratory Beagles

Jennifer Cavarra, Lon V. Kendall, and Jennie Willis

Purpose: Socialize and train laboratory research dogs to improve their well-being and handlablity while in study, and prepare dogs for adoption as pets at study conclusion. This typically fulfills the goal of minimizing the number of animals euthanized, and funding organizations prohibition of the euthanasia of these animals. Some of the difficulties in working with and adopting research dogs are related to a lack of human socialization as well as rearing and living in a kennels as part of a study. New owners are challenged in establishing relationships with their dogs and in training a study dog raised in a kennel situation to perform basic tasks such as walking on a leash. Methods: A socialization program was developed to increase human interactions with research dogs and train the dogs in commands useful for husbandry and to prepare them for adoption. After obtaining consent from the investigators and the IACUC, undergraduate students were paired with a dog for regular training sessions at least 20 minutes 5 days a week. Through workshops and monthly meetings with experienced behaviorists, students were taught positive reinforcement methods for dog socialization and training. Students were instructed on standardized techniques to use with the dogs including approach, entering and exiting the kennel, sit and walking on a leash. Results: In order to determine the effectiveness of the program, temperament assessments were conducted prior to implementing the program and 3 months later. The assessment tested dogs on18 elements of handling and human interaction. Compared to the initial assessments performed, all the dogs had an improved temperament score. Additionally, all dogs were adopted into homes. Conclusion: The socialization program enhances the dog's well-being while on study by providing human interactions and training. This program provides dogs that are easier for investigators to handle, and improves adoptability.

Mechanism and subpopulation specificity of mitochondrial Reactive Oxygen Species release in the post-ischemic hyperthyroid myocardium

Anthony B. de Mooy, Catherine H. Le, Victoria Harcy, Adam J. Chicco

Hyperthyroidism (HT) augments release of reactive oxygen species (ROS) from cardiac mitochondria following myocardial ischemia/reperfusion (I/R). ROS is a collective term that includes oxygen radicals and in addition, nonradical derivatives of oxygen. ROS are normal metabolic byproducts; however, when ROS production exceeds the quenching capacity of cellular antioxidants, this can damage cardiac tissue through oxidation of DNA, lipids, and proteins. Cardiac muscle contains two structurally distinct mitochondrial subpopulations. The subsarcolemmal mitochondria (SSM) are located just beneath the sarcolemmal membrane while interfibrillar mitochondria (IFM) are located within the myofibrils. Purpose: The present study examined the mechanism(s) of this phenomenon and determined whether SSM and IFM are differentially affected. Methods: Male SD rats received 10 daily i.p. injections of thyroid hormone (30mcg T3/kg; HT) or vehicle (CON) before hearts were excised and exposed to a 20/25 min global I/R protocol ex vivo. Following I/R, ROS release was assessed in freshly isolated SSM and IFM using the Amplex Red fluorometric assay with a variety of substrate and inhibitor combinations to examine sites and mechanisms of release. Results: ROS release from SSM exceeded IFM in CON and HT hearts by 25-50% following I/R ($P < 0.01$). Surprisingly, HT augmented ROS release from SSM but decreased ROS release from IFM ($P < 0.05$ for both). Blocking electron flow from respiratory complex 1 to 3 abolished the effect of HT on SSM but not IFM. Inhibition of uncoupling proteins with GDP abolished the HT-induced reduction in IFM but had little effect in SSM. Maximally uncoupling mitochondria with an uncoupling protein, FCCP, abolished effects of HT in IFM and SSM. Conclusions: Collectively, results indicate that 1) complex 3 in SSM is the primary source of mitochondrial ROS release following I/R in HT, and 2) enhanced activity of uncoupling proteins limits ROS release from IFM.

Early detection of chronic wasting disease in TG12 mice by neurological and behavioral assessment

S Michelle DeFord, Tracy A Nichols, Cynthia A Smeraski, Tara Caminesch-Ruby, Terry R Spraker, Qingzhong Kong and Kurt C VerCauteren

Clinical and behavioral signs are well documented in terminal stages of prion disease. However, little is understood about changes during earlier stages of prion infection. In this study, behavior and neurological function was evaluated in TG12 cervidized mice after intracerebral inoculation of chronic wasting disease (CWD)-terminal elk brain homogenate. A neurological test battery examining numerous measures ranging from basic neurological reflexes and function (tested 1-2 times weekly) to complex, integrated brain and nervous system function (e.g., coordination, learning/memory; tested at 3 time points) was used to be able to detect even subtle neurological and/or behavioral changes. Neurological exam results indicated significant pre-clinical phase [days post-inoculation (DPI), 26-60] differences in palpebral reflex [cranial nerve (CN) VII], thoracic limb and tail muscle tone, thoracic limb function, mentation as well as coat changes. During the early-clinical phase (DPI 67-109), CWD mice had significantly lower body mass compared to controls, lower body condition scores, less spontaneous vibrissa movement (CN VII), decreased thoracic limb muscle mass and progressive increase in tail tone. Several neurological measures were significantly altered in the clinical phase (DPI 117-138), generally: mentation, muscle mass and tone, proprioception, strength, coordination, CN VII function, tactile sensation, hind-limb clasping. Hippocampal task performance was inconclusive as neurological results indicate control and CWD mice had low visual ability. Overall, results suggest detectable neurological dysfunction as early as DPI 26, with consistent effects in mentation, CN VII, muscle tone and mass, coat condition, progressing to severe changes in all these measures by the clinical phase.

CELF1-mediated mRNA decay regulates protein secretion and myogenesis and may be impaired in myotonic dystrophy

Alexa M. Dickson, Carolina M. López, Jerome E. Lee, Jeffrey Wilusz, Carol J. Wilusz

The function of the CELF1 RNA-binding protein is profoundly disrupted in type 1 myotonic dystrophy (DM1) and in other neuromuscular diseases. In DM1, aberrant expression of CELF1 is directly linked with erroneous splicing of several clinically relevant mRNAs, such as the insulin receptor (IR) and the chloride channel (CLCN1), and is thought to be responsible for a significant proportion of the disease pathogenesis. Despite the fact that CELF1 is also a critical regulator of mRNA stability, it is not currently known whether mRNA stability is affected in DM1.
We have previously shown that CELF1 is associated with GU-rich elements in the 3' untranslated regions (UTRs) of a large number of transcripts in C2C12 muscle cells. We have now further investigated two subsets of CELF1-targeted transcripts that encode proteins with roles in protein secretion and in myogenesis. Several of these mRNAs are stabilized following CELF1 knockdown. Moreover, CELF1 knockdown myoblasts exhibit enhanced secretory capabilities and demonstrate defects during differentiation into myotubes.
In order to examine whether mRNA stability is impacted by the changes in CELF1 function in DM1, we have developed a set of conditionally immortalized DM1 patient myoblasts. Our results indicate that transcripts targeted by CELF1 are significantly more stable in DM1 cells than in normal controls. We are currently examining whether this increased stability is directly linked to disrupted CELF1 function and whether additional CELF1 target mRNAs are similarly affected. Taken together, these results suggest that altered mRNA stability may be an important contributor to DM1 pathogenesis.

Molecular network involving LIN28 in ovarian cancer and secreted vesicles

Vanessa A. Enriquez, Juliano C. da Silveria, Monique A. Spillman, Quinton A. Winger, and Gerrit J. Bouma

Ovarian cancer is the 5th most deadly cancer among women in US due to the lack of early diagnostic markers, late diagnosis, and persistence of dormant, drug-resistant cancer cells. Nearly 90% of ovarian tumors arise from epithelial cells lining the surface of the ovary, and recent studies reveal human ovarian cancer cells express distinct miRNA signatures. Importantly, the stem cell factor LIN28, a known regulator of miRNA function, is expressed in cancer cells. LIN28 is a RNA binding protein that negatively regulates let-7 miRNA expression thereby inhibiting cell differentiation. Tumor cells release cell-secreted vesicles called exosomes. Exosomes are endosome-derived vesicles (40-100nm) containing bioactive materials, including miRNAs that are released into the bloodstream. We hypothesize that the LIN28-let-7 miRNA regulatory loop controls ovarian cancer development and metastasis. Our objectives were to: 1) Determine LIN28 levels in IGROV-1, OV420, and SKOV3 human epithelial ovarian cancer (EOC) cells. 2) Identify miRNAs in these three EOC cells. 3) Demonstrate that EOC cells secrete exosomes containing oncogenic factors that can be taken up by target cells. Our qPCR and Western blot data revealed LIN28 levels were highest, whereas let-7 miRNA levels were lowest in IGROV-1 cells using a Students T-test ($P < 0.05$). Furthermore, RT-PCR demonstrated IGROV-1 cell-secreted exosomes contain LIN28 and MYC. In vitro transfer of GFP tagged exosomes isolated from IGROV-1 cell culture medium to HEK293 cells indicate uptake of exosomes by HEK293 cells. Moreover, a significant increase in LIN28 levels in HEK293 cells was observed following exosome uptake. Our data revealed that a potential regulatory role of LIN28-let-7 miRNA exists in ovarian cancer cells that may play a role in ovarian cancer metastasis. This research supported by NSF Bridge to the Doctorate Fellowship Recipient Award #0603176 and the American Cancer Society Institutional Research Grant #57-001-50.

Experimental Transmission of Bighorn Sheep Paranasal Sinus Tumors

Karen A Fox, NM Rouse, LL Wolfe, IK LeVan, AJ Marolf, SK Wootton, TR Spraker, MW Miller, SL Quackenbush

Purpose: Paranasal sinus tumors have recently been described in Rocky Mountain bighorn sheep. Cases have been diagnosed in sheep from multiple wild herds, and preliminary data suggest a prevalence of 50% or greater in some affected herds. The etiology of this disease remains undetermined, although an infectious cause is suspected. To determine the transmissibility of bighorn sheep sinus tumors, an experimental infection study was performed.
Materials/Methods: Four bighorn sheep and four domestic sheep were intranasally inoculated with material derived from a bighorn sheep sinus tumor and associated exudates. The material was homogenized, clarified by centrifugation, and the resulting supernatant was passed through a 0.45 micron filter, producing a cell-free filtrate. One additional domestic sheep and one bighorn sheep served as negative control animals and were inoculated with sterile saline. All animals were observed for 18 months post-inoculation, with computed tomography (CT) scans completed at 9 months and 18 months post-inoculation. Full post-mortem examinations were performed at the termination of the study.
Results: As early as 9 months post-inoculation, CT scans demonstrated the presence of tumors within the sinuses of both domestic and bighorn sheep from the treatment groups, with no evidence of tumors within the negative control animals. Histologically, the tumors possessed characteristics of both bighorn sheep sinus tumors, as well as enzootic nasal tumors of domestic sheep and goats. Preliminary molecular diagnostics suggest that bighorn sheep sinus tumors are caused by a virus similar to enzootic nasal tumor viruses of domestic sheep and goats. Further investigation of a viral etiology for these tumors is ongoing.
Conclusions: The results of this experimental transmission study suggest that bighorn sheep sinus tumors are caused by an infectious agent, and that this disease is transmissible to both bighorn sheep and domestic sheep species.

Proline Rich 15 Regulates Trophoblast Proliferation and Differentiation

Katherine C. Gates, Jeremy D. Cantlon, Russell V. Anthony

Proline rich 15 (PRR15) is a small nuclear protein expressed by the trophoblast during early gestation in several mammalian species, including humans, mice, cattle, and horses. Immunohistochemistry localized PRR15 to the trophectoderm and extraembryonic endoderm of gestational day 15 ovine conceptuses. The expression profile in the sheep conceptus during pregnancy revealed a peak in expression at day 16 of gestation. This coincides with a halt in elongation of the conceptus, and the initial period of apposition to the uterine epithelium. Lentiviral-mediated knockdown of PRR15 in ovine trophectoderm at the blastocyst stage led to embryo demise by gestational day 15. This provides compelling evidence that PRR15 is a critical factor during this critical window of development. The aims of these experiments were to determine the effect of PRR15 knockdown on trophoblast gene expression, as well as trophoblast proliferation and survival in vitro. The human first trimester trophoblast cell line, ACH-3P, was infected with control lentivirus (LL3.7) and virus expressing shRNA to target PRR15 mRNA for degradation, resulting in a 68% decrease in PRR15 mRNA ($p<0.001$). In the absence of PRR15, 448 genes were up-regulated and 748 were down-regulated more than 1.5-fold with $p<0.05$. These changes included significant up-regulation of GDF15, a cytokine increased in pregnancies with preeclampsia, and significant down-regulation of OVOL2, a transcription factor associated with placental labyrinth development in mice. To assess the phenotype of PRR15 knockdown, we compared proliferation in control and shRNA-expressing cells. Proliferation decreased in the absence of PRR15 when measured by the production of formazan. We will determine the rate of cell death in the PRR15 knockdown using TUNEL. Our results suggest that PRR15 may be required for driving trophoblast proliferation and survival during early development of the placenta.

Sensitivity of protein misfolding cyclic amplification vs. immunohistochemistry in antemortem detection of chronic wasting disease.

Nicholas J. Haley, Candace K. Mathiason, Scott Carver, Glenn C. Telling, Mark D. Zabel, and Edward A. Hoover

As the only prion disease affecting free-ranging animals, the antemortem identification of affected cervids has become paramount in understanding chronic wasting disease (CWD) pathogenesis, prevalence, and control of horizontal or vertical transmission. To seek maximal sensitivity in antemortem detection of CWD infection, we used paired tonsil biopsy samples collected at various time points from 48 CWD-exposed cervids to compare blinded serial protein misfolding cyclic amplification, or sPMCA – a means to amplify low levels of prion proteins in vitro, with the assay long considered the gold standard for CWD detection, immunohistochemistry (IHC). Serial PMCA negative controls – 34% of the samples evaluated – included tissues from sham-inoculated animals and unspiked negative controls, all of which tested negative throughout the course of the study. We found that sPMCA on tonsil biopsies detected CWD infection significantly earlier (2.78 months, 95% CI 2.40-3.15) than conventional IHC. Interestingly, we observed a correlation between early detection by sPMCA and host PRNP genotype. These findings demonstrate that in vitro amplification assays provide enhanced sensitivity and advanced detection of CWD infection in the peripheral tissues of cervids, with a potential role for spike or substrate genotype in sPMCA amplification efficiency.

Nonspecific Induction of Gut Mucosal Immunity and Colonization Resistance against Salmonella by Rice Bran in Mice

Ajay Kumar, Angela Henderson, Tiffany L. Weir, E.J. Ehrhart, Genevieve M. Forster, Andrew W. Goodyear, Jan E. Leach, John E. Bauer, Steve Dow and Elizabeth P. Ryan

Purpose: Dietary modulation of the intestinal immune environment represents a novel approach for enhancing protective responses against pathogens and inflammatory diseases. We conducted a study to determine the effects of whole dietary rice bran, which contains numerous bioactive components, on mucosal immune responses. Material/methods: ICR mice were fed a 10 percent rice bran or control AIN93M diet for 28 days and 129Sv mice were fed 10 percent rice bran diet one week before and during infection for model of Salmonella infection. Results: ICR mice B lymphocytes in the Peyer's patches of rice bran fed mice displayed increased surface IgA expression compared to control mice, and a significant increase in myeloid dendritic cells residing in the lamina propria and mesenteric lymph nodes was observed. Rice bran fed 129Sv mice had significantly reduced Salmonella fecal shedding as compared to control diet fed mice. Gut microbiome analysis using 454-adaptor pyrosequencing revealed that rice bran modulated the phyla Firmicutes and Verrucomicrobia. The number of Lactobacillus spp increased in mice (170 fold) after rice bran consumption and retained after one week of Salmonella infection compared to control diet fed animals. Also, we found that rice bran across diverse rice varieties differentially inhibited Salmonella fecal shedding and that polyphenols, fatty acids and certain minerals are candidate bioactive components for correlation with Salmonella fecal shedding. Conclusion: Dietary rice bran consumption represents a novel means of reducing susceptibility to enteric infection with Salmonella and potentially other enteric pathogens as well by inducing protective gut mucosal immunity.

Cardiac Mitochondrial Phenotype of the Tafazzin shRNA Mouse Model of Human Barth Syndrome

Catherine H. Le, Anthony B. de Mooy, Adam J. Chicco

Barth syndrome (BTHS) is an X-linked mitochondrial disease that is due to a mutation in the Tafazzin (taz) gene and is characterized by dilated cardiomyopathy, exercise intolerance, chronic fatigue, delayed growth, and neutropenia. Tafazzin is a mitochondrial transacylase required for the remodeling of cardiolipin (CL), a unique tetra-acyl phospholipid that is almost exclusively located in the inner mitochondrial membrane. It is believed that CL is required for the structural integrity of the mitochondria as well as for the proper function of the electron transport chain (ETC). Purpose: In this study, the cardiac mitochondrial phenotype of a new mouse model of BTHS (taz shRNA; TAZ) was characterized. Methods: Cardiac mitochondria were isolated from male TAZ and wild type (WT) mice at 3-4 months of age for assessment of respiratory parameters, H2O2 production and Ca2+ tolerance. Results: Mitochondria from TAZ mice had 50-60% lower rates of state 3 and state 4 respiration, with little change in respiratory efficiency compared to WT. Absolute levels of H2O2 production were similar in TAZ and WT but were higher in TAZ when normalized for electron flow rate. Ca2+-induced swelling was impaired in TAZ, as was sensitivity to cyclosporin A, indicating resistance to mitochondrial permeability transition. Conclusions: These findings demonstrate that taz deficiency impairs several aspects of mitochondrial function and provide the basis for further inquiry into the precise roles of taz and cardiolipin metabolism in mitochondrial (patho)physiology.

Oral Presentations

Session III ~ Salon II
1:00-5:00PM

BASIC SCIENCE

Host Factors Influence Prion Strain Adaptation

Crystal Meyerett Reid, Mark Zabel

Purpose: Chronic Wasting disease (CWD) is one of many prion-mediated diseases known as transmissible spongiform encephalopathies (TSEs). There is ever-increasing biological and biochemical evidence that prion pathogenesis is caused by the conversion of the normal host protein (PrPC) into an abnormal disease causing conformation (PrPRES). How prions encipher heritable strain properties without nucleic acids remains unclear. Previously, we have shown that host factors have contributed to the adaptation of an original deer CWD prion strain to transgenic cervidized mice. In this study we show that prion strain adaptation and mutation is highly dependent upon host factors and host-encoded PrPC primary sequence. Materials and Methods: We assessed cervid prion strain differences using biological and biochemical assays and found that amplified cervid prions and serial-passaged cervid prions were significantly different than that of the original cervid strain. Results: Previous reports indicate that there is a strict species barrier preventing CWD infection in wildtype mice. However, we show it is possible that prion mutation and adaptation can broaden the host range. We generated a mouse-adapted strain of CWD (crossing the species barrier) upon serial passage into transgenic cervidized mice and then subsequent passage into wildtype mice. All wildtype mice remained non-clinical upon first passage but became completely susceptible after second passage with similar incubation times to those of mice terminally ill from a mouse adapted scrapie strain. Inoculation of our mouse adapted CWD strain back into cervidized mice delayed progression to terminal disease. Conclusion: We conclude that prion strain adaptation and mutation is highly dependent upon host factors and host-encoded PrPC primary sequence. Upon serial passage the adapted prion strain shares more characteristics with prion strains from the new host rather than the original species.

Global analysis reveals pathways of unique regulation of mRNA decay in human induced pluripotent stem cells

Ashley Neff, Ju Youn Lee, Bin Tian, Jeffrey Wilusz, and Carol Wilusz

Reprogramming of differentiated cells into pluripotent stem cells requires global changes in gene expression. Although the rate of transcription is an important modulator of steady-state transcript levels, mRNA decay also plays a significant role in regulating the expression of cell type-specific genes. We sought to determine global mRNA decay rates in human induced pluripotent stem (iPS) cells and the fully differentiated human foreskin fibroblasts (HFFs) they were derived from. Using a microarray-based approach, we have determined half-lives for 5,481 mRNAs in both cell lines. Functional and sequence analyses have identified three independent classes of mRNAs whose decay is differentially regulated. First, we find that replication-dependent histone mRNAs are stabilized and more abundant in iPS cells. Second, a large set of KRAB-C2H2 zinc finger mRNAs are significantly stabilized in iPS cells, likely through reduced abundance of miRNAs that target their ORFs. Finally, a set of mRNAs containing C-rich sequence elements in their 3'UTR, many of which encode transcription factors, are significantly less stable in iPS cells. Interestingly, two poly(C) binding proteins, PCBP3 and PCBP4, are differentially expressed in iPS cells and HFFs and could play a role in this regulation. PCBP4 is a tumor suppressor and inhibits cell cycle progression, consistent with our finding that it is expressed more highly in HFFs than iPS cells. Thus, several intriguing classes of mRNAs are differentially regulated at the level of decay in iPS cells compared to fibroblasts. We are currently working to identify the mechanisms by which iPS cell-specific mRNA decay regulation is achieved and to determine how differential mRNA stability may contribute to the achievement and maintenance of pluripotency. This work was funded by an NIH R01 award (GM072481) and ARRA supplement, as well as a Colorado State University CVMBS College Research Council Award to JW.

Equine Mesenchymal Stem Cells in vitro Differentiation Capacity: Differences Between Sternum and Ilium

Karla G. Penman, Laurie R. Goodrich, Jennifer N. Phillips, John D. Kisiday, C. Wayne McIlwraith

Osteoarthritis is a major cause for loss of athleticism, chronic pain and death in horses. Equine mesenchymal stem cells (MSCs) have been a primary focus for tissue regeneration, especially in joint injuries. The two sites of bone marrow aspiration in the horse are the sternum and ilium. Each site has been shown to offer a rich supply of MSCs that have similar growth rate characteristics in vitro. The next step in guiding practitioners in their aspirate location is investigating the capacity of MSCs to differentiate into chondrocytes, osteocytes and adipocytes. We hypothesized that MSCs acquired from sternum and ilium in horses will have a similar capacity to undergo chondrogenic, osteogenic and adipogenic differentiation regardless of their aspiration location. Five horses had sternum and ilium marrow aspirates performed. Cells were cultured and passaged three times and treated with appropriate differentiation media for tri-lineage evaluation. Flow cytometry was conducted looking at CD11/18a, CD34, CD44, CD90 and MHC1 cell surface markers. These markers identify cells capable of multi-lineage differentiation or multipotency. Differentiation assays yielded MSCs with desired morphologic changes, confirming tri-lineage differentiation. Chondrogenic and osteogenic propensity were not found to be significantly different between the two aspirate locations. Flow cytometry results yielded no statistically significant differences in cell surface markers from the two aspiration locations. We found no difference between the aspiration location on MSCs differentiation capacity in vitro nor on expression of cell surface markers testing multipotency. These findings support previous work that demonstrated that there is no significant difference between the nucleated cell counts between aspiration locations. Thus, clinicians can draw from either sternum or ilium when attempts at regenerative therapy are pursued without concern that one site would be superior to the other.

Alterations in the immunopathogenesis of tuberculosis associated with dietary-induced formation of advanced glycation end products

Brendan K. Podell, David F. Ackart, Natalie M. Kirk, Sarah Eck, Marcela Henao-Tamayo, Elizabeth Creissen, Diane Ordway, Randall J. Basaraba

Purpose: Diet-induced obesity often leads to a state of pre-diabetes where without appropriate intervention full type 2-diabetes mellitus (DM) may develop. The formation of non-enzymatic modifications to host macromolecules known as advanced glycation end products (AGEs) is central to the pathogenesis of DM and DM-associated complications. DM is a known risk factor for development of tuberculosis however the pathogenesis of this comorbidity is poorly understood. A diet-induced model of pre-DM in guinea pigs was utilized to investigate the role of AGEs on the disease progression following infection with Mycobacterium tuberculosis (Mtb). Materials/Methods: Guinea pigs were randomly assigned to a high-fat, high-carbohydrate diet (fat-fed) or normal guinea pig chow for 6 weeks then infected with Mtb. At 60 days post-infection, histopathology, tissue bacterial counts, and flow cytometry for immune cell phenotypes were performed on tissue homogenates. Fasting serum glucose and total antioxidant capacity (TAC) were assessed by microtiter enzyme assay and serum AGEs by ELISA. Tissue AGEs were assessed by IHC. Results: Fat-fed guinea pigs had elevated AGEs in serum as well as tissue, particularly associated with Mtb lesions and necrosis. Consistent with this, there was also a decrease in total antioxidant capacity. Splenic pathology was significantly more severe in the fat-fed group corresponding with higher bacterial burden in the spleen. Also, pulmonary lesions of hematogenous origin were greater in the fat-fed group. This was supported by higher numbers of infiltrating inflammatory cells. Conclusions: Feeding of a high fat, high carbohydrate diet promoted the formation of AGEs in both the tissues and serum. The presence of more severe disease and early extrapulmonary dissemination in the face of elevated AGEs is consistent with a pro-inflammatory immune response that may be driven by interactions of AGEs with AGE receptors.

Characterization of Semliki Forest fluorescent reporter viruses

Krystle L. Reagan, Rennos Fragkoudis, Margit Ool, Alain Kohl, John K. Faszakerley

Semliki Forest virus (SFV) infection in mice provides an experimental model for the study of viral encephalitis. The use of engineered reporter alphavirus systems expressing fluorescent proteins allows for rapid determination of viral replication and in vivo imaging to monitor progression of viral infection. Three reporter viruses expressing fluorescent markers, either fused with nsP3 or under a double subgenomic promoter, were examined and compared to wild type SFV infection to validate their use in future pathogenesis experiments. Wild type SFV does not induce mortality or morbidity in adult balb/c mice, even while replicating to high titers in neural tissue. Likewise, the tested reporter viruses did not induce illness and all led to establishment of high-titer infections in the brain, when delivered intra-cranially. Intra-peritoneal delivery resulted in viremia and high-titer brain infections comparable to wild type SFV with two of the three reporter viruses tested. Tissue sections were collected to analyze histopathology. Attenuation in viremia and brain titers of the SFV A7(74) T37/17 eGFP when delivered peripherally may preclude it from use in future studies on pathogenesis. However, both SFV A7(74) nsH-eGFP and SFV A7(74) Xho-zsGreen show similarity to wild type SFV in levels of viral replication and tropism and are valid models of wild type SFV infection. Future pathogenesis studies will be carried out using the later two viral constructs that approximate wild type SFV.

Differential gene expression in corpora lutea from non pregnant and pregnant sheep

Jared J Romero, AQ Antoniazzi, JS Davis and TR Hansen

Ovine interferon-tau (IFNT) is released from the conceptus by Day 12 of pregnancy, silences up-regulation of estrogen receptor and consequently the oxytocin receptor. IFNT protects the CL during pregnancy by acting on the endometrium and disrupting the pulsatile release of prostaglandin F2 alpha (PGF). IFNT also has endocrine action inducing interferon stimulated genes such as ISG15 in the CL. We hypothesized that gene expression in the CL was similar on day 12, but differed by day 14 due to the presence (pregnancy: P) or absence (estrous: E) of a conceptus. RNA isolated from CL on day 12 and 14 of the estrous cycle or pregnancy (n = 3/group) was used to screen the Affymetrix bovine microarray (23,000 targets; n = 1 chip per ewe). Signal transduction pathways were analyzed using Metacore. Differential gene expression (1.5 fold, $P < 0.05$) was confirmed using RTPCR. Serum progesterone decreased ($P < 0.05$) from 1.7 ng/ml on Day 12 to 1.3 ng/ml by day 14 in EC ewes suggesting initiation of luteolysis. Serum progesterone remained > 1.7 ng/ml in Days 12 and 14 P ewes. The initial stage of luteolysis from Day 12 to 14 in EC ewes was associated with differential expression of 683 genes. Pathways implicated were: cell adhesion, chemokines and cytoskeletal remodeling. Day 12 CL had 55 genes that were differentially expressed in P vs EC ewes. Affected pathways were cell cycle, cytoskeleton-cell adhesion and GTPase. Pregnancy induced 21 differentially expressed genes on Day 14 vs 12. Steroid biosynthesis, immune response and interferon signaling were pathways induced by pregnancy. On Day 14 there were 734 differentially expressed genes in CL from EC compared to P ewes. The pathways implicated were cell adhesion, chemokines and cytoskeletal remodeling. Microarray analysis confirms endocrine action of pregnancy on the corpus luteum, which entails differential expression of ISGs, cytoskeletal, angiogenic and epithelial to mesynchmal transition genes. USDA AFRI 2011-67015-20067

Post-Exposure Immunization Against Francisella tularensis Membrane Proteins Augments Protective Efficacy of Gentamicin in a Mouse Model of Pneumonic Tularemia

Marjorie D. Sutherland, Andrew W. Goodyear, Ryan M. Troyer, Jeffrey C. Chandler, Steven W. Dow, John T. Belisle

Francisella tularensis, the causative agent of tularemia, is a highly virulent pathogen that has recently resurfaced as a potential bioterrorism agent. Tularemia is treatable by several different antibiotics but patient relapse still occurs. Thus, there is a need to augment current therapeutics against tularemia to reduce patient relapse and decrease mortality. Previous reports show that the membrane protein fraction (MPF) from F. tularensis LVS, given along with cationic liposomal DNA complexes (CLDC), provides protection when administered just prior to a lethal F. tularensis Schu S4 infection in mice. Therefore, we wanted to examine the ability of MPF to augment a sub-therapeutic antibiotic regime in a post-exposure immunotherapeutic model. To address this, we established a sub-therapeutic gentamicin regime and administered MPF, 24 h post-exposure and boosted again at 4 days post-exposure while simultaneously giving gentamicin for 10 days. We found that MPF was able to augment the sub-therapeutic gentamicin regime and restore complete protection against a lethal F. tularensis Schu S4 infection in Balb/c mice. Further, MPF administration reduced bacterial burden in the lungs, liver, and spleen of infected animals. The protective effects of MPF immunization were found to be mediated primarily by antibodies against MPF antigens but also required NK cells for complete protection. These studies demonstrate that post-exposure administration of MPF antigens successfully augments a sub-therapeutic gentamicin regime and that MPF could potentially be used in a clinical setting to reduce patient relapse.

Liposomes Combined with TLR9 Agonist Produce Effective Mucosal Vaccine against Mycobacterium tuberculosis

Amber R Troy, S Dow, AA Izzo

The battle against Mycobacterium tuberculosis (M. tb) has been prevalent since a time when the pharaohs ruled Egypt, but the disease remains the dominant cause of death from infectious disease to this very day. Despite decades of vaccine development, humans are still tragically vulnerable to the disease. Current vaccine strategies now extend beyond vaccine components and equal focus is now placed on the route of administration as well. Due to the particular virulence of M. tb when aerosolized and inhaled, we have developed a vaccine strategy which addresses not only the stimulation of the immune system but also the priming of cells in the local pulmonary environment. We have determined that CpG Oligodeoxynucleotides (ODN), in particular the sub-type CpG-C ODN, stimulates a robust Th1 cellular immune response capable of providing significant protection against pulmonary bacterial burden following aerosol challenge. This CpG-C ODN was used in combination with a strongly stimulating M. tb antigen, ESAT-6 to elicit the induction of antigen-specific T-cells. Lastly, when the antigen vehicle consisting of cationic liposomes was added to the CpG ODN adjuvant and the ESAT-6 antigen the cellular immune response was significant. When this vaccine formulation was administered intra-nasally the mice developed a robust Th1 type cellular immune response within the local mucosa and developed a significant reduction in bacterial burden when compared to saline controls. This vaccine containing CpG ODN, ESAT-6, and cationic liposomes may prove to be a promising vaccine capable of limiting the infection and dissemination of disease following exposure to aerosolized M. tb.

Hyaluronan cisplatin conjugate in five dogs with soft tissue sarcomas

Rachel O. Venable, Deanna R. Worley, Daniel Gustafson, Ryan J. Hansen, E.J. Ehrhart, Laird Forrest, Mark Cohen

Purpose: This study investigates intratumoral delivery of a novel hyaluronan-cisplatin nanocarrier with goals of reducing systemic toxicity and enhancing tumor and lymphatic exposure of cisplatin.
Material and Methods: 5 dogs were used having spontaneously-occurring soft tissue sarcomas (STSs). Approximately 1.5 mL of nanocarrier (20 mg cisplatin) was injected into one external STS per animal. Blood was collected at ½, 1, 2, 3, 4, 24, and 96 hr, for pharmacokinetics . Urinalysis was performed at initiation and 96 hr. Each STS and the draining lymph node(s) were removed at 96 hr utilizing sentinel lymph node mapping for localization. Platinum levels were measured in the blood samples, tumor, and lymph nodes via ICP-MS analysis.
Results: There were no observed tissue reactions 96 hr following injection into the tumor. The average AUC in the blood for the unbound platinum was 774.6 +/- (222.1) ng/mL and total platinum was 3562.1 +/- (2031.1) ng/mL. The Cmax of the unbound was 56.47 +/- (20.9) ng/mL and total platinum was 81.6 +/- (40.4) ng/mL. The t1/2 was 33.6 and 51.2 hr for unbound and total platinum. Average platinum concentrations within STSs ranged from 3324.5 ng/g to 8228.8 ng/g and from 129.5 ng/g to 6066.0 ng/g in the lymph nodes.
Conclusions: Hyaluronan-cisplatin nanocarrier is well tolerated following intratumoral injection and systemic platinum exposure is reduced compared to traditional intravenous delivery. Hyaluronan-cisplatin nanocarrier demonstrated up to 1000-fold higher levels in tumors versus systemic circulation, and was concentrated by local lymphatics at levels up to 100 fold greater than hemovascular circulation. These characteristics make it a promising new chemotherapy modality.

Estimating Prion Binding Capacity of Soil

Christy Wyckoff, Kurt VerCauteren, and Mark Zabel

Prions, misfolded isoforms of normal mammalian prion protein, are considered the putative infectious agents of transmissible spongiform encephalopathies. Studies indicate that prion aggregates form close associations with soil components, enhancing the persistence and surprisingly, the transmissibility of the infectious agent. Currently few detection, and no quantification methods exist for prions in soil, hindering an understanding of prion persistence and infectivity in the environment. Our objective is to quantify the binding capacity of whole soil, and purified montmorillonite clay (mtl), by measuring the unbound prion remaining in solution after the binding treatment. Treatments consisted of either 1% RML (mouse adapted scrapie strain), or 1% TgA20-RML brain homogenate treated with 20% whole soil or mtl. Samples were mixed for 24 hours at 23ºC to allow prions to adsorb to soil and be removed from solution. Low speed centrifugation pelleted soil components and 30µl of soil-free supernatant was intracerebrally inoculated into susceptible mice. Control samples included non-soil treated 1% RML or TgA20-RML, and soil treated negative brain homogenate. The number of days post inoculation to clinical disease was used to calculate the infectious titer of each treatment. Our results indicate a 1.45 log10 (96%) lower titer in the whole soil treated 1% RML inoculum compared to the control, and a 1.17 (93%) log10 lower titer in the whole soil treated 1% TgA20-RML inoculum compared to the respective control. Surprisingly, the mtl treatment of both RML and TgA20-RML rescued mice from disease, demonstrating that the mtl removed lethal doses of prions from inoculum. These data reveal significant prion-binding capacity of soil and the utility of this assay to further investigate persistence and decomposition of prions in the environment. Additionally, since mtl successfully rescued the mice from prion disease, mtl might be used for reclamation, remediation and decontamination.

The PARN deadenylase regulates decay of a discrete set of transcripts in mouse myoblasts

Jerome E. Lee, Ju Youn Lee, Jarrett Trembly, Jeffrey Wilusz, Bin Tian, and Carol J. Wilusz

Global control of gene expression is a tightly regulated process which involves coordination of events (transcription, splicing, export, translation, et cetera) at many levels. One way gene expression is controlled is through regulated mRNA decay. Transcript decay is primarily initiated by removal of the poly(A) tail through a process called deadenylation. One of the many enzymes responsible for this is Poly(A) RiboNuclease (PARN). Despite the fact that deadenylation is often the primary and rate-limiting step in message decay, very few mRNA targets for deadenylation by PARN have been identified in mammals. Moreover, very little is known about the role of PARN in regulating message stability. Herein we have taken a global approach using microarrays to assess mRNA decay rates in proliferating myoblasts and have identified decay rates for 16,976 transcripts. In addition, decay rates were determined for 16,939 transcripts in a myoblast cell line where PARN was stably knocked down. The median half life in PARN KD cells was increased from 4.2 to 4.5 hours, while comparison of these two datasets identified 1,478 transcripts which are stabilized in the absence of PARN and potential targets for deadenylation by this factor. One such transcript that was stabilized in the PARN KD cells encodes the AU rich element binding protein ZFP36L2. Subsequent qPCR assays found this transcript to be stabilized in a PARN-dependent fashion and, the poly(A) status of the Zfp36l2 message was also impacted by PARN levels. Finally, transcripts encoding other RNA binding proteins were found to be impacted by PARN levels. Taken together our data indicates that deadenylase complexes interact with distinct groups of RNA binding proteins and these interactions determine which mRNAs will be acted substrates for specific components of the RNA decay machinery.

Poster Presentations

Endocrine Delivery of Interferon-tau into the Uterine or Jugular Veins at Different Concentrations Protects the Corpus Luteum from Prostaglandin F2 Alpha Induced Luteolysis

Alfredo Q. Antoniazzi, Brett T. Webb, Jared J. Romero, Natalia P. Smirnova, Gordon D. Niswender, Fuller W. Bazer, and Thomas R. Hansen

The ovine conceptus secretes interferon-tau (IFNT) during early pregnancy (Days 10 to 21). It acts in a paracrine manner on the endometrium to silence transcription of endometrial estrogen receptor alpha and, consequently, preventing luteolytic pulses of prostaglandin F2 alpha (PGF). Endocrine action of IFNT also occurs by Days 14-15 of pregnancy inducing IFN-stimulated genes (ISGs). We have demonstrated extension of estrous cycles beyond 32 days after 7 days infusion of recombinant ovine (ro)IFNT (200 mcg/day) into the uterine vein (UV). Also, 12h infusion of roIFNT (200 mcg/day) into the UV partially protected the CL against PGF challenge. Because IFNT release from the conceptus is lower prior to Day 15, two concentrations of roIFNT (200 or 20 mcg/day) were tested herein for the ability to induce ISGs in CL and protect the CL against luteolysis. It was hypothesized that infusion of 20 mcg roIFNT/day into the UV and infusion of 200 mcg/day into the jugular vein (JV) would protect against PGF-induced decline in serum progesterone concentration. Osmotic pumps loaded to deliver 200 or 20 mcg/day of roIFNT or BSA into the UV and JV for 3 days (n = 10/group) were installed on Day 10 of the estrous cycle. One half received an injection of 4 mg/58 kg of body weight of PGF on Day 11 (n = 5/group) of the estrous cycle. Serum progesterone concentrations were determined using RIA. CLs were collected on Day 13. Differences were considered at $P < 0.05$. All concentrations (20 and 200 mcg/day) and modes of delivery (UV and JV) of roIFNT induced ISG15 mRNA (qRTPCR) in CL. In roIFNT-infused ewes, serum progesterone concentrations were maintained to 24h and 48h following injection of PGF and were not different from BSA and roIFNT controls, but were greater when compared to BSA/PGF group. It is concluded that endocrine delivery of roIFNT, whether through UV or JV, protects the CL from luteolytic actions of PGF. USDA NIFA AFRI Competitive Grant 2011-67015- 20067.

Role of Autophagy in Tumor Development and Metastasis

Rebecca A. Barnard, Ryan J. Hansen, Paola Maycotte, and Daniel L. Gustafson

Purpose: Autophagy is an intracellular process that degrades proteins and damaged organelles. This process may act as a survival mechanism during cellular stress to maintain homeostasis or remove irreparable cells. Autophagy has a conflicting role in cancer, either promoting or suppressing tumor progression. Thus, it is imperative to have a more complete understanding of the role autophagy plays in tumorigenesis and metastasis. Methods: In order to observe the effect of autophagy inhibition on tumor growth and metastasis development, an inducible knock down system of Atg12, a protein necessary for autophagy, was constructed in highly metastatic 4T1 and rarely metastatic 67NR murine mammary cancer cells. In this system, the presence of doxycycline knocks down Atg12 expression, thus preventing autophagy. A proliferation assay was used to measure growth rate in vitro. To determine growth rate in vivo, cells were orthotopically implanted into nude mice and tumor volume was measured over time. Results: In vitro, knock down appeared to have no effect on the proliferation of both 4T1 cells and 67NR cells. In mice, knock down of Atg12 appeared to significantly increase tumor growth rate in 4T1 cells, but decreased the rate in 67NR cells. Knock down of Atg12 decreased the number of lung metastases in mice challenged with 4T1 cells, but had no effect on the number of lung metastases in mice challenged with 67NR cells. Conclusions: Further investigation is needed to ensure that Atg12 knock down was achieved in vivo and these results can be reproduced. If these results are confirmed, then continued research will investigate the differences seen between in vitro and in vivo models. Additional studies will be conducted in syngeneic mice challenged with 4T1 cells to determine the role of an intact immune system. Lastly, pharmacologic modification of autophagy will be used to determine the applicability to the clinical setting and therapeutic intervention.

Evaluation of Point of Care Glucose Meters in Alpacas

Oriana Beemer, Stacey Byers, Andrea Bohn

Purpose: Hospitalized alpacas are often hyperglycemic requiring repeated blood sampling to monitor glucose levels and response to insulin therapy. Point-of-care (POC) glucometers are commonly used to measure whole blood glucose concentrations due to the rapid results and low expense. There is one POC glucometer currently marketed for use in animals; however this meter has not been validated for use in alpacas. Therefore human meters are used but also have not been validated or compared to clinical pathology laboratory glucose values for alpacas. Potential sources of variation include meter accuracy, hematological variations, and drug interactions. The purpose of this study was to compare 3 commercial glucometers to laboratory glucose readings. Materials and methods: Four alpacas were used in this randomized cross-over study. The alpacas were given either 0.4 U/kg of regular insulin intravenously or 500 mg/kg of dextrose intravenously with a 1 week washout between crossover periods. Blood samples were obtained from a jugular catheter at specific time periods starting 10 minutes before drug administration until 6 hours post administration. Whole blood and plasma samples were measured on 3 glucometers, and serum glucose was measured on a laboratory chemistry analyzer. Results: The POC meters had variable results compared to the serum glucose and results also varied based on testing plasma or whole blood. All 3 meters had either proportional or constant bias but the variations were not considered clinically relevant. Conclusion: The POC meters can be used for alpacas but if more accurate blood glucose values are desired, reference intervals for each meter should be established.

Behavioral and Cognitive Differences of Mice Inoculated with Mouse Adapted Prion Strains

Heather Bender, Crystal Meyerett, Mark Zabel

It is hypothesized that prion diseases are caused by the recruitment of the cellular prion protein, PrPC, into an infectious protease resistant isomer, PrPRes. PrPC knockout mice (PrP-/-) are known to be resistant to disease compared to wildtype mice; however, the function of PrPC and its relationship to behavior and cognition remain relatively unknown. In a pilot study comparing the behavior and cognition skills of PrP-/- mice to wildtype mice, we found that PrP-/- mice have a significant cognitive deficiency in odor-guided tasks ($P < 0.05$), but not spatial guided tasks as compared to the wildtype. We are now conducting a longitudinal study using behavioral and cognitive tests with PrP-/-, wildtype, and PrPC overexpressing mice to determine the effects of mouse adapted prion strains on behavior and cognition. We hypothesize that mice that overexpress PrPC should show more behavioral and cognitive deficits as the mouse adapted prion strains deplete or sequester PrPC as compared to wildtype mice. It appears that control PrPC overexpressing mice resemble a wildtype phenotype more than the PrP-/- phenotype in cognitive tasks; whereas, the phenotype of the inoculated PrPC overexpressing mice does not resemble the cognitive phenotype of either the wildtype or PrP-/- mice three months post inoculation. So far we have only seen behavioral differences in the PrPC overexpressing mice at the onset of clinical signs; whereas, the inoculated wildtype mice show behavioral differences before the onset of clinical signs of prion disease. Therefore, the overexpression of PrPC seems to impair the ability of PrPRes to affect behavior functions in mice inoculated with a mouse adapted prion strain, but does not rescue the animal from clinical disease. This pilot study of the behavioral and cognitive differences between control and inoculated PrP-/-, wildtype, and PrPC overexpressing mice could potentially lead to a pre-clinical behavioral test of prion diseases in mice.

Increasing Consumption of Rice Bran and Navy Beans for Colon Cancer Control and Prevention: A Randomized-Control Pilot Investigation

Erica Borresen, Kerry Doyle Gundlach, Adam Heuberger, Tiffany Weir, Melissa Wdowik, Regina Brown, Elizabeth Ryan.

Purpose: Navy beans and rice bran have demonstrated compelling chronic disease fighting properties in animal and human studies, yet a small fraction of rice bran produced is used for human consumption and dietary bean consumption remains low in the United States. BENEFIT (Beans/Bran Enriching Nutritional Eating For Intestinal health Trial) is a growing community-academic partnership to advance staple food-nutrition research on navy beans and rice bran in northern Colorado. The major goal is to address a significant gap in our knowledge regarding the feasibility of increasing bean and rice bran intake in adults and colon cancer survivors for colon cancer control and prevention. Methods: 14 healthy adults (11 non-cancer, 3 colon cancer survivors) participated in a 4-week dietary intervention trial. Participants were randomized into either placebo, navy bean powder (35 g/day) or rice bran (30 g/day) groups. Seven meals and six snacks were developed and provided one-third of the participants' total dietary intake. Three-day dietary food logs were completed weekly and were used to calculate total percent of navy bean or rice bran intake as well as macronutrient distribution and total calories. Results: A decrease in total caloric intake at 4 weeks in navy bean consuming individuals was detected compared to the control group. Navy bean and rice bran groups showed an overall increase in dietary fiber intake compared to control. Pilot analysis of blood and stool samples revealed novel dietary metabolite biomarkers of rice bran and bean consumption that elicit changes to the intestinal microflora. Conclusion: Adding navy bean powder and rice bran into prepared meals and snacks is a feasible and safe approach to achieve levels that inhibit colon cancer formation seen in animal models. This approach can inform public health nutrition programs to enhance awareness and promote consumption of chronic disease fighting foods in an evidence-based manner.

Mycobacterium bovis model of infection in goats

Angela Bosco-Lauth, Richard Bowen, Brendan Podell, Torsten Eckstein, Elizabeth Brooks, Mercedes Gonzalez-Juarrero

Purpose: Tuberculosis causes sanitary problems in livestock and wildlife populations and results in serious limitations to trade and economic losses to the food industry. Bovine tuberculosis is a contagious and infectious disease caused by Mycobacterium bovis (M.bovis). The use of animal models has been crucial in the characterization of host and bacterial factors that determine the immune response against tuberculous bacteria, and is also important in the testing of novel vaccines against bovine tuberculosis. For vaccine testing purposes, cattle are difficult to house under high containment conditions for long periods of time. Goats are very susceptible to M. bovis infection and the prevalence of infection within-herd can be high. Therefore, goats may represent a cheaper alternative for testing prototype vaccines in large ruminants and humans. Materials/Methods: Using a low and high dose of infection, we developed a M. bovis infection model in goats. Using a nebulizer, two groups of goats received an aerosol inoculum of 1,000 or 10,000 cfu M. bovis cells respectively. After 8 and 16 weeks of the infection, goats were euthanized and detailed post-mortem analysis of lungs, lymph nodes, spleen and liver lesions were performed. Selected lung lesions and respiratory lymph nodes were evaluated and cultured for bacteriological and histological analysis. Results: All infected animals presented severe tuberculosis lesions in lung and associated lymph nodes. M. bovis was recovered from pulmonary lesions and lymph nodes in all inoculated goats. Conclusions: The present work shows a reliable new aerosol model of animal model of infection to be used in the understanding of tuberculosis disease and future development of vaccine trial in this and other species.

Intranasal Administration of a Modified Live Feline Herpesvirus 1 and Feline Calicivirus Vaccine Induces Cross Protection Against Bordetella bronchiseptica

Allison Bradley, Joann Kinyon, Timothy Frana, Denise Bolte, Doreene R. Hyatt, Michael R. Lappin

Purpose: Vaccination against all feline upper respiratory pathogens is not possible. Results of previous studies suggest intranasal vaccination may stimulate nonspecific immunity against agents not contained within the vaccine, but no study has directly examined this in cats. We hypothesized that cats administered a modified live feline herpesvirus-1 (FHV-1) and feline calicivirus (FCV) intranasal vaccine would have fewer clinical signs after challenge inoculation with Bordetella bronchiseptica than unvaccinated controls.
Materials/Methods: Twenty specific pathogen free 12 week-old kittens were randomized into 2 groups of 10 cats each. The vaccinated group was administered a single intranasal dose of a commercially available vaccine containing modified live strains of FHV-1 and FCV, and the control group remained unvaccinated. All 20 cats were administered B. bronchiseptica by nasal inoculation seven days later and were observed daily for clinical signs of illness for 20 days.
Results: In the first 10 days after B. bronchiseptica challenge, vaccinated cats were less likely to be clinically ill (indicated by lower cumulative clinical scores) than control cats ($p = 0.01$). The most commonly observed clinical sign was sneezing. Overall, 9 of 10 control cats and 2 of 10 vaccinated cats were noted to sneeze at least once during days 1-10 after inoculation with B. bronchiseptica ($p = 0.006$). These differences were no longer apparent during days 11-20. Finally, the percentage of observation points with sneezing recorded was significantly greater in control cats than in vaccinated cats over days 1-10 ($p < 0.0001$) and days 1-20 ($p = 0.02$).
Conclusions: Intranasal vaccination against FHV-1 and FCV decreased signs of illness due to an infectious agent not contained in the vaccine. This nonspecific immunity could be beneficial for protection against organisms for which vaccines are not available and as early protection while specific adaptive immunity is developing.

Optimizing immunostaining of fibrillin-1 in canine tissue: Implications for quantifying changes in canine mitral valve disease

Cord M Brundage, C Lu, M Davis, K Summers, BM Corcoran

Myxomatous mitral valve changes in canine heart disease are characterized in part by proliferation of glycoaminoglycans within the heart valve connective tissue matrix weakening the valve. Similar connective tissue changes are seen in human Marfan syndrome, a genetic connective tissue disorder resulting from altered expression of the fibrillin-1 encoding gene. Fibrillin-1(FBN1) is a highly conserved glycoprotein essential for the formation of elastic fibers. Using immunostaining we sought to identify FBN1 expression in normal canine tissue, with the intent of quantifying expression in animals with mitral valve disease. Multiple staining and imaging techniques were employed to optimize the protocol for this study. These included both biotin-labeled and fluorescence immunohistochemistry with a variety of antigen retrieval methods. Preliminary results are presented here. This project was conducted at the Roslin Institute as part of a summer research exchange with the Royal (Dick) School of Veterinary Studies in Edinburgh Scotland.

Normal canine brain glucose uptake and distribution using FDG-PET-CT

Cord Brundage, Lawrence Whalen, Billie Arceneaux, Jeffrey Stewart, Susan Kraft

The radioactive tracer 18-fluorodeoxyglucose (FDG) can be used in positron emission tomography – computed tomography (PET-CT) imaging as a measure of glucose uptake and thus brain metabolic activity. The distribution of brain metabolic activity may be altered in seizure patients, those with ischemic injury, inflammatory disease, brain tumors and patients with advanced stages of dementia. PET imaging is gaining recognition for assessing human patients with these conditions. However the normal distribution of FDG in the brain has not been well characterized in dogs or cats. Six neurologically normal adult dogs obtained from a commercial supplier underwent FDG-PET-CT (Philips Gemini TF) and magnetic resonance (MR) imaging (GE Signa LX). All procedures were approved by CSU Animal Care and Use Committee. PET-CT scans were coupled with MR images for neuroanatomic detail. Brain PET-CT scans were evaluated qualitatively to determine areas of greatest glucose uptake. FDG uptake was also quantified on PET images using a Philips commercial workstation as the standard uptake value (SUV) in multiple bilateral prosencephalic and rhombencephalic brain regions. Additionally, in 3/6 dogs, blood uptake curves and dynamic PET acquisitions were performed over 1 hr to generate time-activity curves for the dynamic glucose uptake rate and intensity in various brain regions. Frontal and occipital lobe grey matter prosencephalic regions demonstrated increased glucose uptake relative to rhombencephalic brainstem regions. The results of this study provide a framework to begin studying altered FDG metabolism related to veterinary neurological diseases. The ultimate goal is to explore the application of PET imaging in veterinary neurology. This study was funded by a CSU Companion Animal research grant, Department of ERHS and the Animal Cancer Center.

Rapid Salmonella detection in experimentally-inoculated equine feces and environmental samples using two commercially available lateral flow antigen detection systems

Brandy A. Burgess, Noelle R. Noyes, Denise S. Bolte, Doreene R. Hyatt, Dave C. Van Metre, Paul S. Morley

Salmonella enterica is a zoonotic pathogen that has tremendous impact on many animal production and management systems. Rapid detection of S. enterica in fecal or environmental samples may facilitate effective infection control practices. Current detection methods require approximately 48-hours to obtain results. Alternatives have been developed, lateral flow antigen detection systems (LFADS), which are presently marketed for use in food safety microbiology. The objective of this study was to evaluate two commercially available rapid Salmonella detection systems in fecal and environmental samples. Fecal samples collected from repeatedly culture-negative horses were inoculated with known concentrations of Salmonella enterica serotype Typhimurium. All fecal samples were cultured using two enrichment techniques, 1) 1 gm of feces in TET and 2) 10 gm feces in BPW-RV. Environmental samples collected using electrostatic dust wipes from a veterinary teaching hospital were inoculated with known concentrations of S. enterica serotype Typhimurium. All environmental samples were cultured using four enrichment techniques, 1) BPW-RV, 2) BPW-TET-RV, 3) BPW-TET, and 4) BPW. In a blinded fashion, samples were tested using two different LFADS as well as plated on agar media for confirmatory testing. Of inoculated fecal samples, Test 1 correctly identified 100% using the TET method and 30% using the BPW-RV method; Test 2 correctly identified 100% using the TET method and 33% using the BPW-RV method. Of inoculated environmental samples, Test 1 correctly identified 100% using BPW-RV and BPW-TET-RV methods, 95% using PBW-TET method, and 65% using BPW. Test 2 correctly identified all inoculated environmental samples. This study demonstrates that both commercially available rapid detection systems evaluated may be valuable diagnostic tools when managing veterinary hospitals and other animal facilities. However, further evaluation of naturally-infected samples is warranted.

Evaluating Hypertension in the General Canine Population

Melody C. Cerra, Jessica Malberg, Rebecca Ruch-Gallie

Purpose: Pulmonary hypertension is recognized as a clinical syndrome in canines, but blood pressure is not taken during routine appointments in many veterinary clinics. Recognizing whether a patient is hypertensive can serve as an indicator of underlying disease and can provide valuable medical information. The objective of this study was to determine if hypertension is present in the general population of dogs seen for routine appointments at Community Practice at the Veterinary Teaching Hospital and what patient factors influence blood pressure measurements. Materials/Methods: Blood pressures (indirect, Cardell 9402) were evaluated in 223 dogs presenting for annual examination from November 2010 to August 2011. Breed, size (small, medium, large), body condition score (underweight, normal, overweight), current and prior medical conditions, sex and age were recorded for each patient. Patient position and cuff position were recorded for each reading. Results: ANOVA with a significance set at p= 0.05 was utilized to determine patient and test factors. Of patient factors, size had the only influence on measurements. Of test factors, dogs in a sitting position had higher readings than sternal or recumbent. Conclusion: Small breed dogs have a higher mean arterial pressure than large breed dogs. Blood pressures read higher in the sitting position than in the sternal or recumbent position. Although 100 dogs (including small and large breeds) had systolic pressures greater than 150, none exhibited clinical signs.

Pilot study evaluating the risk factors associated with zoonotic disease transmission in a goat contact area

Tina Chen

Purpose: Venues which allow animal contact give the public opportunities for entertainment and education, but public health measures must be taken to decrease the risk for disease transmission. Even apparently healthy animals can shed zoonotic pathogens to humans. The purpose of this study was to investigate two hypotheses. First, we wanted to know if there is a difference in risk factor behaviors between age groups. Second, we sought to determine if hand washing compliance at our goat contact exhibit is similar to that of other animal contact venues.
Methods: During the study, 154 visitors to a goat contact area were observed to see if they demonstrated behaviors which have been documented to be known risk factors for zoonotic disease transmission. A range of risk factors were observed, including touching an animal, touching the fence, and failure to wash hands. Chi-squared statistics were used to determine the relationship between risk factor behaviors among age groups and to determine if hand washing compliance at this goat contact area was similar to that of other facilities.
Results: Hand washing compliance among adults was significantly less than that of children ($p=0.036$). Among children under 13 years of age, 87 percent washed their hands, a significant difference from the expected proportion of 60 percent ($p<0.05$). Among individuals 13 years old and older, 73 percent washed their hands. This was also greater than expected, but the difference was not statistically significant ($p=0.076$).
Conclusions: Overall, hand hygiene compliance at this goat contact area was higher than expected, especially among children. Other animal contact facilities should conduct risk assessments to determine the likelihood of zoonotic disease transmission and to safeguard the health of both animals and people.

Effects of platelet-rich plasma on chondrogenesis of bone marrow derived mesenchymal stem cells in dilute fibrin gels

Jaclyn Christakos, Laurie Goodrich, Jennifer Phillips, John Kisiday, C. Wayne McIlwraith

Purpose: Platelet-rich plasma (PRP) is considered a promising method for delivery of growth factors to musculoskeletal defects. Mesenchymal stem cells (MSCs) possess the ability to differentiate into cells of connective tissue origin. Previous studies have shown that PRP enhances proliferation and mitogenic potential of MSCs. In this study, the effect of PRP on the chondrogenic potential and differentiation of MSCs seeded in fibrin gel scaffolds was examined. It was hypothesized that MSCs treated with PRP would demonstrate greater chondrogenic potential and differentiation than control groups, providing evidence for use as a regenerative therapeutic in poorly healing cartilage defects.
Materials and Methods: Fibrin gels and PRP were prepared from equine plasma. Equine MSCs were harvested, expanded, and seeded in fibrin gels. Four treatment groups were utilized: chondrogenic media only, chondrogenic media and PRP, control media only, and control media and PRP. Evaluation of type II collagen production by polymerase chain reaction (PCR), colorimetric assay quantification of total glycosaminoglycans (GAGs), and fluorescent DNA quantitation was performed.
Results: It was hypothesized MSCs treated with PRP would show greater chondrogenic potential and differentiation than control groups as determined by measurement of increased proliferation and greater production of type II collagen and GAGs. Initial data was supportive of this hypothesis. Complete evaluation of replicate samples will provide a greater sample set and quantitative analysis of chondrogenic markers.
Conclusion: While currently utilized clinically based on anecdotal evidence, additional objective data is required to validate use of PRP in combination with MSCs. Increased proliferation of MSCs treated with PRP and greater production of markers of chondrogenesis identified in this study would provide evidence to support evaluation of this multi-modal regenerative therapy in clinical studies.

Prevalence of select vector borne agents in owned dogs of Ghana

Lorelei L. Clarke, Lora R. Ballweber, Kelly Allen, Susan Little, Michael R. Lappin

While many dogs in West Africa are seen in clinics for evaluation of clinical syndromes related to vector-borne diseases, such as anemia, weakness, and lethargy, the resources for definitive diagnosis are not available. The purpose of this study was to evaluate for evidence of vector borne infections in pet dogs in Ghana. Ticks, sera, and blood samples in EDTA were collected from dogs evaluated in the Amakom Veterinary Clinic in Kumasi, Ghana between Dec 2010 and Jan 2011. The ticks were identified to the genus level by microscopy. Sera were evaluated for Dirofilaria immitis antigen and antibodies against Borrelia burgdorferi, Anaplasma phagocytophilum, and Ehrlichia canis (SNAP®4DX). Conventional polymerase chain reaction assays designed to amplify the DNA of Ehrlichia spp./Anaplasma spp./Neorickettsia spp./Wolbachia spp., Babesia spp., Rickettsia spp., Hepatozoon spp., Bartonella spp. and the hemoplasmas were performed. Positive amplicons were sequenced. All of the ticks were Rhipicephalus spp. There were 8 positive dog samples: 4 Hepatozoon canis; 2 E. canis; 1 A platys; 1 Wolbachia). In addition, 3 samples were positive for A. phagocytophilum antibodies, 2 samples were positive for E. canis antibodies and 1 sample was positive for E. canis antibodies and D. immitis antigen. Of the 3 dogs positive for E. canis antibodies, 2 were PCR positive and confirmed by sequencing to be E. canis and Wolbachia. The Wolbachia PCR positive dog was also positive for D. immitis antigen in serum. Bartonella henselae DNA was amplified from 2 ticks and Hepatozoon canis DNA was amplified from 1 tick. Vector borne diseases are common in this part of Ghana. Bartonella henselae is generally considered to be vectored by Ctenocephalides felis but has been amplified from both Ixodes and Rhipicephalus ticks. Hepatozoon canis is known to be carried by Rhipicephalus ticks and were the likely vectors in these dogs.

Understanding Trends in Dog Ownership, Health, and Veterinary Care to Save Dog Lives

Christina Collins, Melody Cerra, Rebecca Ruch-Gallie

This study is the first of a series in identifying the trends of dog ownership and relinquishment and dog health, allowing scientists and veterinarians to conduct research and develop interventions based on sound evidence. This is a retrospective demographic study. Targeted demographic data are: Breed-primary and secondary, sex and reproductive status. Additional datum from medical databases is disease diagnoses over the animal lifetime. From shelter databases, source of animal, reason for relinquishment (if appropriate) and outcome were determined. Although data is currently undergoing collection and analysis, several key elements in determining breed-specific causes of relinquishment or disease have been identified. There is an apparent trend toward small breed dogs, either purebred or mixed-bred. Whether this is a reflection of societal preference or demographics/lifestyle is beyond the scope of this study and warrants further attention. There are challenges in breed-specific data collection with the most concerning occurring in accurate breed identification. This is true for both medical and shelter databases. Caution should be utilized when interpreting breed-specific data since current breed populations are not well defined. For this reason, the CDC no longer reports dog bite data by breed. Finally, existing shelter statistical recommendations, such as the Asilomar Accords, American Society for Prevention of Cruelty to Animals and Maddie's Fund, do not include breed-specific data. Although most shelters maintain this information, it is not routinely reported and difficult to obtain on a national basis.

Evaluation of the soft tissue attachments of the equine stifle using radiographic analysis

James D Conway, LR Goodrich, A Valdes-Martinez

Purpose- Radiography of the equine stifle is an important diagnostic tool for visualization of bony changes. Unfortunately there is little research describing the soft tissue attachments (entheses) within the stifle when using radiography. We describe the anatomical location of the soft tissue attachments of the equine stifle using 4 standard radiographic views: caudocranial, lateromedial, caudolateral-craniomedial, and caudomedial-craniolateral views.
Materials and Methods- Phase 1 consisted of locating entheses of the various tendons and ligaments in the equine stifle during dissection. Once the precise limits of each enthesis were determined, they were mapped onto one stifle specimen, dissected of soft tissues, using barium paste. The standard radiographic views were obtained to determine the location of the different entheses. Phase 2 consisted of the assessment of all radiographs for determination of the best view(s) for evaluation of each individual enthesis.
Results- In phase 1, the entheses were precisely identified radiographically using the method described above. These structures include the collateral, cruciate, and meniscal ligaments; and the popliteal tendon. Localizing the precise boundaries of all the entheses in all views helped determine the potential superimposition of structures, especially menisco-tibial ligaments, when these structures were evaluated in only one plane. This demonstrated the advantages of multiple views for a complete radiographic evaluation of the stifle. Phase 2 is still in progress.
Conclusions- The results of this study will assist the practitioner to make a more informed diagnosis when evaluating the equine stifle. The radiographs from this study can and should serve as a reference for soft tissue structural evaluation of the equine stifle.

MiRNA regulation of aromatase (CYP19) in equine granulosa cells

Juliano da Silveira, Julhiano Rossini, Jacobo Rodriguez, Jennifer Halleran, Stephanie Krick, Elaine Carnevale, and Gerrit Bouma

Purpose: Recently, small RNA molecules (miRNAs) were identified in cell-secreted vesicles (exosomes) that are present in equine ovarian follicular fluid (FF). Furthermore, these exosomes containing miRNAs appear to originate from within the follicle and can be taken up by granulosa cells. MiRNAs bind to target mRNAs regulating gene expression and function. Aromatase (CYP19) enzyme activity (necessary for estradiol biosynthesis) is critical for follicle development and selection in mammalian species. We postulated that exosomal miRNAs in FF can bind CYP19 mRNA and modulate its translation. Material and Methods: Ovarian activity of 10 mares was monitored daily to identify dominant follicles. Immature dominant follicles (30-35 mm) and mature dominant follicles (30-35 mm and 24 hr after deslorelin) were aspirated to collect granulosa cells (GC) and FF. Experiment 1: Treatment of cultured immature and mature GC with exosomes isolated from mature and immature follicles, respectively. Experiment 2: Treatment of cultured mature GC with a let7a miRNA mimic, (predicted to target CYP19). Data was analyzed using T-test to compare treatment vs control. Results: Treatment of mature GC with exosomes from mature or immature FF, did not affect CYP19 levels. Treatment of immature GC with exosomes from mature FF revealed a decrease in CYP19. Finally, GC transfection with let7a miRNA mimic ($p<0.001$) revealed a decrease in CYP19 mRNA and protein levels ($p<0.07$). Conclusion: Our data indicates that exosomes in mature FF can regulate CYP19 activity in GC. In addition, we demonstrate that let7a miRNA directly targets CYP19 mRNA. FUNDED BY HYLTON FOUNDATION

Cancer stem cells in canine malignant melanoma and osteosarcoma have stable phenotypes and enhanced survival in the tumor microenvironment

Michael P. Deogracias, Amanda M. Guth, Leah A. Mitchell, Steven W. Dow

Purpose: Cancer stem cells (CSCs) are small populations of tumor cells that self-renew and maintain cellular heterogeneity in tumors. CSCs generate much of the tumor mass and can be resistant to many chemotherapeutic agents. Markers of CSCs have not been well characterized or compared among different tumor types, particularly in canine cancer. We compared phenotypes of CSCs in canine malignant melanoma (MM) and osteosarcoma (OSA), which are cancers that also affect humans, often metastasize, and may not respond well to traditional chemotherapeutics. We also observed the effect of stromal cells in the tumor microenvironment on expression of CSC markers. We hypothesized that MM and OSA would have stable populations of CSCs, with similar markers in both types and CSC proliferation enhanced by stromal cells. Methods: Flow cytometry was utilized to screen the samples for stem cell markers. Cultured cell lines (7 MM and 7 OSA), fine needle aspirates (FNAs) from 8 canine MM cases, and biopsies from 18 OSA tumors were tested. Two-tailed t-tests compared data from the different groups. The panel of stem cell markers included the surface proteins, CD34, CD24, CD44, and CD133, and the transcription factor Oct3/4. Results: In MM and OSA cell lines, small populations of cells were positive for CD24 and Oct3/4, while low percentages of MM cells also expressed CD34. In both cancers, a majority of cells expressed CD44, and thus, this marker could not reliably identify CSCs. In MM FNAs versus cell lines, proportions of CD133 and Oct3/4 positive cells were significantly increased, while there were fewer CD44 positive cells. Compared to OSA cultured cells, biopsies had significantly more CD34, CD24, and CD133-expressing cells, while fewer CD44 positive cells were detected. Conclusions: We observed stable profiles of CSCs in canine MM and OSA cell lines and FNAs/biopsies. There were shared markers between these two cancers, and the tumor microenvironment enhanced CSC proliferation.

Regulation of GABA and Glutamate Release from Proopiomelanocortin Neuron Terminals in Intact Hypothalamic Networks

Matthew S. Dicken, Ryan E. Tooker, Shane T. Hentges

Purpose: Hypothalamic proopiomelanocortin (POMC) neurons and their peptide products mediate important aspects of energy balance, analgesia, and reward. In addition to peptide products, there is evidence that POMC neurons can also express the amino acid transmitters GABA and glutamate, suggesting these neurons may acutely inhibit or activate downstream neurons. However, the release of amino acid (AA) transmitters from POMC neurons has not been thoroughly investigated in an intact system. In the present study, the light-activated cation channel channelrhodopsin-2 (ChR2) was used to selectively evoke transmitter release from POMC neurons. Materials/methods: Whole-cell electrophysiologic recordings were made in brain slices taken from POMC-Cre transgenic mice that had been injected with a viral vector containing a floxed ChR2 sequence. Pharmacology was used to identify current phenotype and test for current modulation by opioid and GABA-B receptors. Results: Brief pulses of blue light depolarized POMC-ChR2 neurons and induced the release of GABA and glutamate onto unidentified neurons within the arcuate nucleus (ARC), as well as onto other POMC neurons. In testing the ability of the observed AA transmitter release to be modulated, opioid and GABA-B receptor agonists readily inhibited GABA and glutamate currents from POMC neuron terminals. This regulation indicates that opioids and GABA released from POMC neurons may act at presynaptic receptors on POMC terminals in an autoregulatory manner to limit continued transmission. Conclusions: The results show that in addition to the relatively slow and long-lasting actions of peptides, POMC neurons can rapidly affect the activity of downstream neurons via GABA and glutamate release.

Retrospective review of Northern fur seal (Callorhinus ursinus) placentas for Coxiella burnetii on St. Paul Island

Katherine Dirsmith, Terry Spraker, Kristy Pabilonia, Christina Weller, Tom Gelatt, Colleen Duncan

Purpose: In 2010, Northern fur seal placentas were examined, and a high prevalence of Coxiella burnetii was detected by PCR. Because C. burnetii is an intracellular organism and does not grow in routine culture, it was unclear if the organism has been present in this population prior to 2010 and simply was not identified, or if it is an emerging disease. The purpose of this study was to determine whether C. burnetii is an endemic organism and has been present within the population for an extended amount of time or if it is an emerging pathogen.
Materials/methods: Diagnostic pathology reports from this Northern fur seal population, dating back to 1986, were reviewed for reference to placental tissue. The corresponding slides and paraffin embedded blocks of placenta were located. These slides of placenta were examined microscopically for evidence of inflammation or intra-trophoblastic bacteria. Paraffin curls were made from 40 of the oldest samples in which blocks were identified. On these tissues, real-time polymerase chain reaction (qPCR) to test for the presence of the marine mammal strain of C. burnetii was run.
Results: To date, 40 slides of placenta have been microscopically examined. Only rare inflammation has been observed, and no C. burnetii or other organisms have been detected. qPCR is pending.
Conclusions: Because C. burnetii is not uniformly distributed throughout the placenta, multiple diagnostic methods are required to identify the bacterium in tissues. This retrospective tissue review will contribute significantly to the understanding of C. burnetii in the Northern fur seal population of St. Paul Island.

The effects of intensive forest management on the prevalence of Hantavirus and gastrointestinal parasites in wild deer mice

Katie Doepker, Rhea Hanselmann, Anna Jolles

Purpose: Anthropogenic environmental disturbances may compromise the immune system of wildlife inhabiting disrupted ecosystems, making them more susceptible to disease. The prevalence of gastrointestinal (GI) parasites and Sin Nombre Virus (SNV), a strain of Hantavirus, was determined in wild deer mice (Peromyscus maniculatus) inhabiting intensively managed forests in Oregon. We hypothesized that changes in environment would impair immunity of deer mice and cause higher prevalence of SNV and GI parasites (helminths and coccidia). Materials/methods: Management levels of forest plots were: undisturbed for 40-50 years (older stand control), clear cut within one year, but not additionally managed (clear cut control), and recently clear cut and intensively managed with herbicides and manual vegetation control (treatment). An enzyme-linked immunosorbent assay (ELISA) was used to test for SNV and standard fecal flotation techniques were used to assess presence of GI parasites.
Results: Statistical analysis was not performed due to small sample sizes (older stand control, n=4, clear cut control, n=8, treatment, n=4). However, prevalence of SNV was highest in mice on treatment plots. Of the mice trapped, 2/4 tested positive on treatment, 2/8 on clear cut control, and 1/4 on older stand control plots. Gastrointestinal parasite prevalence on older stands was 3/4, 4/8 on clear cut control, and 2/4 on treatment plots.
Conclusion: Our results suggest that forest management intensity may affect prevalence of SNV and GI parasites in wild deer mice. The higher prevalence of GI parasites in older stands may be due to lesser ability of parasites to survive under the environmental conditions created by clear cutting. Prevalence of SNV was highest in treatment plots, possibly related to the effects of intensive management strategies on immune function. These results could have important implications for disease risk in wildlife populations inhabiting disturbed habitats.

Site-directed metabolic biotinylation of AMPA receptors may perturb protein-protein interactions with TARPs

Amanda M. Dudek, Leslie M. Stone, Kathryn M Partin

AMPA receptors are glutamate-gated ion channels that mediate fast excitatory synaptic responses. AMPA receptor expression is regulated by stargazin, a transmembrane AMPA receptor regulatory protein (TARP). The goal of the present study is to probe the quarternary structure of AMPA receptor-TARP complexes at the plasma membrane. We hypothesized that biotin acceptor domains (BADs), expressed as cassettes of 32 amino acids that include the 17 amino acid BAD motif and a flexible glycine/alanine linker, could be inserted at putative sites of interaction in rat GluA2, and that subsequent biotinylation of these sites might sterically block functional interactions between GluA2 and stargazin protein. The sites at which BADs were inserted (GluA2-BAD mutants) include parts of the receptor that lie directly above, below and within the transmembrane helices. GluA2-BAD mutants were transiently transfected into HEK 293 cells with and without stargazin and viewed using confocal fluorescence microscopy. We found that GluA2-BAD mutants were efficiently expressed in HEK 293 cells, suggesting that the insertions alone did not significantly impair receptor trafficking or function. Specifically, expression of GluA2-BAD MKV led to predominantly intracellular localization of the protein, similar to the wild type receptor, and co-expression of GluA2-BAD MKV with stargazin resulted in efficient trafficking of the mutant receptor to the plasma membrane, similar to wild type receptor. Our results demonstrate that insertion of the biotin acceptor domain itself at this site is not enough to prevent association with stargazin. Ongoing experiments will include a functional analysis of GluA2-BAD MKV using patch clamp electrophysiology, as well as the testing of other GluA2-BAD mutants. These experiments will lead to a better understanding of how the activity-dependent association of GluA2 with auxiliary proteins impact AMPA receptor synaptic physiology.

Novel vaccination strategy for feline immunodeficiency virus

Mauren Emanuelli, Ryan Troyer, Martha MacMillan, Wendy Sprague, Chris Grant, John Elder, Sue VandeWoude

Feline immunodeficiency virus (FIV) is a lentivirus that causes an AIDS-like disease in domestic cats. FIV utilizes cat CD134 as a primary binding receptor for cellular entry. FIV infected cats that produce anti-CD134 antibodies have lower viral loads and better health status than cats that lack anti-CD134 antibodies. Furthermore, ex vivo studies demonstrated that anti-CD134 antibodies only bind to CD134 after attachment to the viral surface (SU) protein, displacing SU and blocking infection. Based on these findings we proposed to immunize cats with FIV SU-CD134 complexes to investigate if the immunization will generate anti-receptor-SU humoral antibodies and whether or not these antibodies would provide protection against FIV infection. In a pilot study we compared the antibody response in cats vaccinated with this complex in different adjuvants and subsequent challenge with FIV. Four groups of cats (2 cats per group) were immunized by subcutaneous route with a preparation of FIV SU-CD134 antigen plus aluminum, Freund's, antigen only, and a control group with saline only. Two cats from Freund's group, one cat from aluminum group and another one from antigen only developed an antibody response to the vaccine complex. Cats were challenged orally with FIV 16 weeks post final boost and re-inoculated via subcutaneous route 56 days after the first challenge. All cats became infected after FIV challenge. However, one of the strongest antibody responders had delayed infection. In conclusion, there was no clear difference in immune response or protection between the different adjuvant groups. Because aluminum is the only adjuvant approved for use in humans, we will use this in future studies. We will next perform a larger scale vaccine trial to further evaluate humoral immune response and protective efficacy.

Evaluation of novel treatments for shelter cats with suspected viral causes of upper respiratory disease

Audra Fenimore, Kasey Carter, Madeline Fujishiro, Ryan Peters, Michael Lappin

Purpose: Feline upper respiratory tract disease (URTD) is a leading cause for euthanasia in shelter cats. The purpose of this study was to gather controlled data on novel treatment strategies for shelter cats with suspected persistent viral URTD that have failed conventional therapy. Materials/Methods: Cats from shelters that failed lysine and antibiotics for 2-3 weeks were transported to CSU. Prior to treatment, each cat underwent a 3 day equilibration period. The cats were then randomly assigned to one of two treatment groups. Group A received human interferon alpha (10,000 unit/kg, SQ) once daily for 14 days. Group B received an intranasal FHV-1/FCV vaccine on day 1 as immunotherapy followed by 1 mL of sterile saline SQ once daily for 14 days. A clinical score for each cat was determined daily by blinded individuals. Cats who improved (clinical score of < 3) by day 14 were eligible for adoption. Cats with a clinical score of = 3 were entered into the crossover treatment group. Cats that failed both therapies or had severe ocular clinical signs were administered famciclovir as a rescue drug for FHV-1. Results: A total of 44 cats have been entered to date. Clinical signs resolved without anti-viral treatment in 16 cats during the equilibration period. Famciclovir therapy was needed for 3 cats and 1 cat was euthanized prior to treatment due to severe glossitis. Based on Fisher's exact test, a greater percentage (P = 0.01) of Group B cats (12 of 12 cats; 100%) responded to primary therapy by Day 14 than Group A cats (6 of 12 cats; 50%). However, the mean time to resolution for Group A (8.6 days) and Group B (8.4 days) was not significantly different (P = 0.8). Correlation of treatment responses to FHV-1/FCV molecular diagnostic results and lymphocyte subset distributions is ongoing. Conclusions: Administration of human interferon alpha or topical FHV-1/FCV vaccine as immunotherapy may be beneficial in alleviating clinical signs of feline viral URTD in some cats.

Metabolomics as a novel tool to assess dietary modulation of the canine metabolome in response to navy bean consumption

Genevieve M. Forster , Adam L. Heuberger, Corey D. Broeckling , & Elizabeth P. Ryan

Metabolomics is a novel research tool for assessing local and systemic metabolic changes in response to diet. The purpose of this study was to evaluate the changes in canine fecal and plasma metabolite profiles in response to consumption of unique dietary components. Twenty-one male and female adult dogs were enrolled in a 4-week dietary intervention study at CSU Veterinary Teaching Hospital to investigate effects of 25% navy bean intake. Diet formulations, safety, and apparent total tract digestibility of the navy bean and control diets were previously demonstrated (Forster et. al. under review). Clinical serum biochemistry and experimental metabolic analyses were performed on plasma and fecal samples at baseline and 4 weeks. Diet, fecal, and plasma samples were extracted using an aqueous-methanol solvent and metabolites were detected by GC-MS. Metabolites were identified by screening mass spectra in the NIST and GOLM databases. Dogs that consumed the navy bean diet showed a 22.7% reduction in serum cholesterol compared to controls ($P < 0.01$). In the navy bean diet, 262 molecular features were found to be at least 1.5 times higher than the control diet ($P < 0.05$). Pipercolic acid was identified as a unique metabolite in the navy bean diet. Fecal metabolite profiles of dogs consuming the navy bean diet revealed a subset of dogs with altered glucose, galactose, and ribose metabolism. This study has first demonstrated the utility of metabolomics to assess a) metabolite differences in diet components after controlling for macro and micronutrients and b) metabolic response to a dietary intervention in dogs as evidenced by changes in the plasma and fecal metabolome. Changes in plasma cholesterol and fecal excretion of carbohydrates by the navy bean diet in dogs may implicate a role for navy bean intake to alter lipid metabolism and sugar utilization for chronic disease prevention.

Developing cross-species predictions of drug sensitivity for canine cancer

Jared S. Fowles, Ann M. Hess, Dawn L. Duval, Daniel L. Gustafson

Purpose: Gene expression signatures for determining drug sensitivity in cancer are becoming more utilized in cancer research. The recently developed COXEN (co-expression extrapolation) method has proven successful in predicting drug sensitivity in vitro and in vivo in human cancer. Canine tumors show great biological and genetic similarities with humans. Our purpose is to explore the COXEN method's utility in a cross-species extrapolation of gene expression signatures to predict drug sensitivity in canine cancer cells from a human training set. Methods: Microarray gene expression and drug sensitivity data for doxorubicin was publicly available for the human NCI60 panel. Microarray gene expression analysis was performed for 16 canine cancer lines (ACC16). Sensitivity of the ACC16 to doxorubicin (DOX) and vinblastine (VBL) was calculated via Alamar Blue assays. SAM analysis with FDR cutoff of 0.1 was performed on the 12 most sensitive and resistant lines from the NCI60 to obtain a gene expression signature. Corresponding data from the ACC16 was compared to the NCI60 signature via correlation matrices for co-expression to identify genes for prediction model development. Results: GI50 values for DOX and VBL against the NCI60 panel ranged from 6 -13,000 and 0.25-2500nM, respectively. Drug sensitivity in the ACC16 was comparable to the NCI60. 34 human probeset IDs were identified after SAM analysis mapping to 27 genes. 55 canine probeset IDs mapping to 27 genes were found on the canine 2.0 Affy chip. COXEN analysis generated a 2 gene model for DOX which accurately predicted 75% of ACC16 sensitivity ($R=0.49$, p.val=0.0517, Spearman). A 6 gene model for VBL was 75% accurate in prediction ($R=0.64$, p.val=0.0082, Spearman). Conclusions: Initial results are encouraging for the use of COXEN in cross-species prediction models, but we are still in the exploratory phase. Further study may show this method beneficial to predict canine patient response to cancer drugs.

Serological responses against antigenically distinct contemporary equine influenza virus strains (H3N8) induced by commercially available vaccines

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Equine influenza is a contagious respiratory disease caused by H3N8 equine influenza virus (EIV). Despite the availability of efficacious vaccines, equine influenza has remained an economically important disease. Vaccine failure can be explained by the continuous genetic evolution of the virus. Genetic drift has resulted in the recent emergence of two antigenically distinct H3N8 Florida sublineage virus clades (1 and 2). As evidence suggests vaccines containing earlier American lineage viruses do not provide adequate protection against contemporary EIV isolates, the 2011 OIE vaccine recommendations state that vaccines should contain isolates from clades 1 and 2. We hypothesized that a vaccine recently updated with a clade 1 virus (Calvenza-03, Boehringer-Ingelheim) induces higher titered serological responses to contemporary clade 1 viruses than a vaccine containing an earlier American lineage virus (Fluvac Innovator, Pfizer Animal Health). Sera collected on days 113, 126, and 296 post vaccination from ponies receiving either Calvenza-03, Fluvac Innovator, or a placebo control were tested by hemagglutination inhibition assay against H3N8 isolates from the predivergent, American (Kentucky), and clades 1 and 2 phylogenetic groups. Geometric mean titers were calculated and differences between groups were analyzed by Repeated Measures ANOVA. Ponies vaccinated with Calvenza-03 demonstrated no significant difference in antibody titers against viruses from clade 1 and clade 2 of the Florida sublineage. In contrast, ponies receiving Fluvac Innovator had significantly higher antibody titers to clade 2 than to clade 1 isolates ($P=0.0018$ after vaccinations). As the risk of vaccine breakdown increases with a lengthening of the antigenic distance between the vaccine strains and the circulating viruses, our results support the recommendation of the OIE that vaccines should include contemporary viruses from both clades of the Florida sublineage to confer clade-specific immunity.

Genetic polymorphisms in fabI in Burkholderia species and resistance to fabI inhibitors

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The studies of this project ultimately compliment a larger series of studies with the long-term goal of developing novel broad-spectrum chemotherapeutics against bacterial infections. These studies are based on the central hypothesis that enoyl-ACP reductases in the FAS-II pathway are clinically valid targets for the development of novel chemotherapeutics. To date, this group has developed a series of lead fabI inhibitors with potency against the pathogen causing melioidosis, Burkholderia pseudomallei. This study seeks to confirm the molecular target and further define the mode of action of lead inhibitors by developing drug resistant mutants in B. psuedomallei (Bp82) and the plant pathogen B. thailandensis (Bt38), and then identifying the genetic polymorphism. Mutants were positively selected for each fabI inhibitor investigational compound and the classic fabI inhibitor triclosan at multiple concentrations of MIC, and evaluated for cross-resistance. Bp82 colonies grown in the presence of the compounds showed altered morphology and delayed growth time but did not show an increased MIC when evaluated in mircrobroth assay, while Bt38 colonies showed increased resistance and cross-resistance among the compounds. While a fabI-1 mutation was investigated first with attempted PCR amplification and sequencing of the fab-1 gene, there are many other possibilities that could potentially be investigated, such as the use of alternate enzymes or overexpression of an efflux pump. The results of this study will ultimately add to a larger hypothesis investigating inhibitors of the enoyl-ACP reductase enzymes using mechanism-based structural optimization for establishing drug target essentiality and drug specificity in the discovery of one or more compounds that can undergo pre-commercialization development for treating a variety of priority pathogens.

Ocular toxicity following stereotactic radiotherpay for canine nasal tumors

Matthew Goldrick, Michael Nolan, Susan LaRue, Cynthia Powell

Canine sinonasal tumors are most effectively treated with radiation therapy. Traditional treatment protocols deliver 42-54 Gray (Gy) over a 2-3.5 week period, achieve average survival of about one year and are associated with significant radiation side effects. A stereotactic radiotherapy (SRT) protocol, which delivers 20-30 Gy over a 1-3 day period, has been developed at the Colorado State University Animal Cancer Center. Initial review of survival data suggests this technique provides tumor control similar to that achieved with more traditional radiotherapy protocols; anecdotally, the majority of acute side effects appear to be mild if at all present. Late radiation-associated toxicity has not been rigorously evaluated. Therefore, the aim of this study was to describe late ocular toxicity associated with nasal SRT. This retrospective study identified potential ocular complications of stereotactic radiotherapy by comparing results of ophthalmologic examinations performed 6-12 months following completion of treatment with pre-treatment findings in those patients that were evaluated by a board-certified veterinary ophthalmologist prior to receiving nasal SRT. The majority of dogs in this study suffered late ocular complications following irradiation. Cataracts were the most frequently noted ophthalmologic lesions; retinopathies and neuropathies, as well as keratitis and conjunctivitis/blepharitis was documented less commonly. The majority of lesions were subclinical and all were mild at the time of diagnosis. In conclusion, though late ocular toxicity is common following nasal SRT, lesions are less severe and more amenable to successful medical and/or surgical management than those reported following more traditional forms of curative-intent nasal irradiation.

Urinary Cytokine Concentrations in Normal Cats and Cats with Chronic Kidney Disease

Lauren Habenicht, Tracy L. Webb, Laurie Clauss, Stephen W. Dow, Jessica M. Quimby

Chronic kidney disease (CKD) is a common cause of illness and death in cats. The hallmark of CKD in cats is chronic tubulointerstitial nephritis, and inflammation contributes to the progression of renal fibrosis. However, at present it is difficult to directly assess the degree of intra-renal inflammation without renal biopsy. Measurement of urine pro-inflammatory cytokine concentrations may provide a non-invasive means of assessing intra-renal inflammation. Therefore, we quantitated urine cytokine concentrations in 34 healthy cats and 34 cats with CKD. When urine cytokine concentrations in healthy and CKD cats were compared, we found significantly higher concentrations of IL-8, MCP-1, and TGF-B1 in urine of CKD cats, along with significantly lower VEGF concentrations. A significant positive correlation between serum creatinine and urinary IL-8 and TGF-B1 concentrations were found, as well as a negative correlation between serum creatinine and urine VEGF concentrations. We concluded therefore that urinary cytokine measurement may be a potentially useful means of assessing intra-renal inflammation in cats with CKD.

Sheep placenta and developmental programming

Jennifer Halleran, Juliano Da Silveira, Quinton Winger, Gerrit Bouma

Environmental factors can alter fetal development and tissue differentiation. This phenomenon is known as fetal programming. An example would include reproductive disorders, such as intrauterine growth restriction potentially leading to abnormal fetal tissue development. Unfortunately, little is known regarding the molecular control of normal and abnormal fetal programming. We postulate micro RNA (miRNA) molecules and epigenetic factors are involved in abnormal fetal tissue programming. miRNAs are small RNA molecules about 22 nucleotides in length. These molecules play an important role in gene regulation of many tissues, can cause gene silencing, and can be used for diagnostic tests. Epigenetics refers to chemical modifications of DNA that can lead to changes in gene expression and function. The hypothesis of the current study is that prenatal testosterone exposure in the ewe alters placental function, leading to impaired fetal reproductive tissue differentiation. First, we determined if prenatal testosterone treatment leads to hypomethylation in the placenta. This was accomplished by assaying global methylation in control and treated cotyledon and caruncle placentomes. Second, we examined if prenatal testosterone treatment alters placental expression of miRNA and mRNAs, specifically estrogen receptor (ESR1) mRNA. Results reveal a decrease in global methylation of prenatal testosterone treated caruncles and an increase in global methylation of prenatal testosterone treated cotyledons. Levels of miRNAs targeting different signaling pathways were either decreased or increased. Finally, it was shown there was an increase in relative ESR1 levels in the testosterone treated caruncles and decreased ESR1 levels in the testosterone treated cotyledons, possibly through altered methylation of receptor promoter regions. In conclusion, prenatal testosterone treatment alters placental development by affecting levels of miRNA expression and changing epigenetic states.

The use of novel lymphatic endothelial cell-specific immunohistochemical markers to differentiate angiosarcomas in dogs

Charles H.C. Halsey, E.J. Ehrhart, Brad Charles, Deanna Worley, Daniel L. Gustafson

Lymphangiosarcomas are uncommon vascular neoplasms that arise from lymphatic endothelial cells. They efface and replace normal subcutaneous tissue and are characterized by arborizing, vascular channels lined by a single layer of pleomorphic endothelial cells and a paucity of erythrocytes. Lymphangiosarcomas are architecturally similar to hemangiosarcomas, a common malignancy of vascular origin arising from blood vascular endothelial cells. Common immunohistochemical markers for vascular endothelium, such as Factor VIII-related antigen and CD31, have traditionally been used to confirm the diagnosis of tumors of vascular origin. However, these markers fail to differentiate between lymphangiosarcoma and hemangiosarcoma, which often demonstrate diverse clinical behavior and require different treatment modalities. Here we describe for the first time the use of two novel lymphatic endothelial cell-specific markers, LYVE-1 and PROX-1, to further differentiate between vascular tumors of lymphatic (lymphangiosarcoma) and blood (hemangiosarcoma) endothelial cell origin in the dog.

Design and profiling of a series of AMPA receptor modulators

Jonathan E. Harms, Kathryn M. Partin, Craig Jamieson

Glutamate is the primary excitatory neurotransmitter in the mammalian CNS. One of its targets, the AMPA family of ion channels, is responsible for mediating neural processes involved in learning and memory. Further, it has been shown that these processes are enhanced by modulatory drugs acting directly on AMPA receptors (AMPARs). Due to these positive effects, AMPARs and their allosteric modulators present key targets of study in the treatment for cognitive disorders. To expedite development of positive AMPAR modulators, a unique structure-based approach to drug design (SBDD) is being used to rapidly screen for modulatory compounds and optimize them for use as drug based treatments. SBDD has been successful at creating and testing drugs with positive in vivo results, but the method of action for these drugs is not well understood. In the present study, we focused our efforts on using SBDD to better understand AMPAR mechanisms of gating. We compared the effects of two well documented allosteric modulators (cyclothiazide and CX614) to one of Merck's new chemotypes, JAMI 1001A ((2-(2-4-(hydroxymethyl)-3-(trifluoromethyl)-1H-pyrazol-1- yl)acetamindo)-4,5,6,7 tetrahydrobenzo[b]thiophene-3-carboxamide). Outside-out patches excised from HEK 293 cells transiently transfected with the AMPAR GluA2 were exposed to glutamate agonist with or without modulator. The JAMI 1001A compound was found to be more efficacious on GluA2 than its predecessors, slowing the onset of receptor deactivation by more than 2-fold. In addition, JAMI 1001A significantly modulated desensitization, resulting in a robust steady-state current in the continued presence of glutamate. Computational modeling suggests that JAMI 1001A may provide new insight into AMPAR channel gating. The present study demonstrates a two-fold benefit of SBDD: it confirms SBDD as an effective means for drug development, and further demonstrates SBDD as a useful tool in understanding drug and receptor mechanisms of action.

Disruption of advanced glycation end products by the antimicrobial drug, isoniazid

Jessica D. Haugen, David Ackart, Brendan K. Podell, Randall J. Basaraba

Purpose: Recent studies have linked the formation of advanced glycation end products (AGEs) with diabetes, Alzheimer's disease, cardiovascular disease, stroke, and the development of cataracts. These compounds are natural products of the body, formed through a non-enzymatic process in which the aldehyde of a reducing sugar binds to free amino groups of proteins. Particularly high levels of AGEs are seen in diabetes patients, whom in turn have an established risk factor with the development of tuberculosis. Currently there are few drugs tested against the development and treatment of AGEs. In this study isoniazid (INH), a compound used as a front line tuberculosis drug, is tested for anti-AGE properties. Materials/Methods: AGEs were formed in an in-vitro setting by combining various reducing sugars or reactive aldehyde intermediates with bovine serum albumin. Samples were then incubated at 37°C for various durations of time at which point they were read by fluorescent analysis for AGE formation. Inhibition of AGE formation through the addition of INH was performed by combining stocks of glycoaldehyde and bovine serum albumin with various concentrations of INH. Samples were incubated at 37°C and fluorescent measurements were taken at EX:370/EM:440 and EX:335/EM:385 to derive RFUs. Results: An exponential trend in AGE accumulation is seen as the time of incubation is increased. Variations in AGE formation also appeared to be compound dependent, as results show that reactive aldehyde intermediates are more reactive than reducing sugars. The addition of various concentrations of isoniazid (INH) to AGE causes an observed decrease in AGE levels. Conclusion: When compared to a known AGE inhibitor, aminoguanidine, and a known breaker, ALT-711, INH displays both inhibition and breaking affects. With fluorescent analysis, INH has also proven to be more effective at inhibiting the formation of AGEs at various concentrations compared to aminoguanidine.

Detection of Salmonella spp. in the environment at agricultural fairs in association with poultry and waterfowl exhibitions

Talor Hodge, Kyran Cadmus, David Van Metre, Denise Bolte, Michael M. Russell, Kristy L. Pabilonia

Purpose: This study was conducted to determine if Salmonella is a common environmental contaminant at poultry and waterfowl exhibitions. Poultry and waterfowl can be asymptomatically infected with Salmonella and may shed the organism into the environment, particularly during periods of stress. Animal to human transmission of Salmonella has been well documented and potential risks for human exposure should be evaluated. Materials/methods: We sampled ten Colorado 4-H fairs and the Colorado State Fair during July through August 2011. We collected five environmental samples from the poultry and waterfowl exhibits at each fair: floors, tables, poultry feed, poultry litter and waterfowl litter. Floors and tables were sampled using sterile drag swabs moistened with double strength skim milk. Feed and litter samples were collected in Whirl Pak bags. Samples were cultured for Salmonella at the CSU Veterinary Diagnostic Laboratory. All Salmonella isolates were sent to the National Veterinary Services Laboratory in Ames, Iowa for serotyping. Results: Salmonella was isolated from 10 out of 11 fairs. Isolated serotypes included Enteritidis, Kentucky, Bredeney, Derby, Braenderup, Infantis, Montevideo, Thompson, Meleagridis and Cubana. Based on our findings, Salmonella is commonly present in poultry and waterfowl exhibits. Conclusions: Some of the Salmonella serotypes detected in this study are associated with poultry, while others are not, suggesting that Salmonella may have been transferred from other animal exhibits at the fair through animal or human movement. These findings suggest that poultry exhibits are a possible venue for animal to human Salmonella transmission via handling of poultry and waterfowl or via environmental exposure. Visitors to fairs should maintain good hand hygiene practices while attending poultry exhibits and exercise caution when eating or drinking at the fair to avoid contamination with Salmonella.

DNA strand break induced bystander effect (DBIBE) linked to gene mutations and telomere double strand break fusions in naïve cells

Jalal Nasir, Idate Rupa, Warner Christy, Liber Howard.

Purpose: The purpose of this experiment was to investigate if non-radiation dependent DNA damage can induce the production of bystander signal; a cell signal that can induce genomic instability in non-targeted E18 cells. Materials and methods: Modified TK6 cells (E18) with an ISce1 insert in intron 2 of the TK1 gene were allowed to generate a bystander signal following the electroporation of rare cutting restriction enzyme ISce1 carried by a plasmid to induce DNA damage at the ISce1 site in intron 2 of the thymidine kinase locus. Mutation assays using medium transfer were carried out to measure an increase in direct or bystander mutation fractions (MF) as an indirect measure of bystander signal production. Alkaline comet assay was used to confirm the DNA damage caused by electroporation of ISce1. Results: We demonstrated that the transfection of plasmid carrying ISce1 gene produced a single double strand break and released sufficient bystander signal into the medium that it would increase the bystander MF in naïve cells. The relative direct and bystander MF increase due to the electroporation of three rare cutting restriction enzymes (Not1, an 8 base cutter, Sfi1 a 13 base cutter and ISce1 an 18 base cutter) showed that DBIBE does not show a dose-response. The DBIBE did not exhibit any temporal kinetics over 10 hour duration. Tail lengths and single DNA strand breaks measured from comet assays were used to confirm the DBIBE mutation data. The mutants were isolated and shown to be viable for up to 20 days when grown in TFT supplemented RPMI 1640 medium. The bystander signal inhibition using superoxide dismutase and PD 98059 indicated that the signal involved the MAPK pathway. Conclusion: Sufficient bystander signal can be produced by inducing as little as one DNA break. This bystander signal does not have dose-response or temporal kinetics in naïve cells and involves the MAPK signaling pathway.

Proopiomelanocortin neurons in the arcuate nucleus have inhibitory and excitatory subpopulations

Brooke C Jarvie, Shane T Hentges

Purpose: Hypothalamic proopiomelanocortin (POMC) neurons have traditionally been defined by their ability to make multiple peptides implicated in feeding behavior and reward. However, there is also evidence that subpopulations of these neurons release the inhibitory and excitatory amino acid (AA) transmitters, GABA and glutamate. Materials/Methods: In the present study fluorescent in situ hybridization and immunohistochemistry was used to describe POMC neurons with respect to their AA transmitter phenotype by labeling mRNA for a variety of markers which are indicative of GABA release, GAD67, GAD65 and vGAT, or glutamate release, vGLUT2. This method was compared to the use of transgenic GAD67-GFP and vGAT-GFP animals. Results: Only about 7% of POMC neurons expressed vGLUT2, with a higher probability of occurrence in the retrochiasmatic and rostral-most arcuate nucleus. Roughly half of the vGLUT2 expressing POMC cells also expressed GAD65, although GAD65 and GAD67 were found in ~40% of POMC neurons. Interestingly, despite multiple reports of GABA release from POMC neurons, the presence of the vesicular transporter responsible for packaging GABA, vGAT, was not detected. Conclusions: Subpopulations of POMC neurons can release inhibitory and/or excitatory AA onto downstream target sites which may mediate their actions in food intake and reward, allowing for a fast-acting mechanism of action rather than the slower release of POMC peptides.

Transcriptome Analysis of Murine Osteosarcoma

Brian T. Kalet, Liza E. O'Donoghue, Richard M. Casey, Richard A. Slayden, Douglas H. Thamm, Debra A. Kamstock, Dawn L. Duval

Purpose: Next-generation sequencing (NGS) for whole transcriptome analysis enables unbiased detection of all RNA. This technology was employed to evaluate differential expression between a non-metastatic murine osteosarcoma (OSA) cell line and a metastatic derivative. Transcriptomes from each cell line and from cell line-induced orthotopic tumors were analyzed. Materials/Methods: mRNA was isolated by TRIzol extraction and dual purification rounds using a Poly(A) Purist kit (Ambion). Purified mRNA was analyzed on an Agilent Bioanalyzer for integrity and ribosomal RNA contamination. Samples were processed and sequenced using the SOLiD4 system (Applied Biosystems). Resulting sequences were aligned to the mouse genome and gene expression counts determined using NextGENe Software. Expression counts were normalized and differential expression between groups was determined using R/Bioconductor edgeR (R/BeR) analysis. Expression analysis of 4 housekeeping genes by qRT-PCR from the purified RNA samples was used to validate sample consistency, sequencing and analysis procedures. Results: NGS was performed on triplicate cell and tumor samples. Average reads/sample were 2,655,122 (2,251 - 7,773,426). Analysis by qRT-PCR revealed consistent levels of housekeeping gene expression across samples indicating the samples with the lowest read counts may have encountered a technical error during sequencing. R/BeR analysis of expression in successful samples also confirmed levels of housekeeping genes were unchanged between groups. Pathway analysis identified changes in genes associated with cell motility and immune function. Conclusions: Analysis of sequence and gene expression changes in this OSA mouse model will provide new insights into the genetic and microenvironmental changes that contribute to metastasis in osteosarcoma. Appropriate validation and normalization procedures are important considerations in assessing differential gene expression using NGS technology.

Studying the role of ADHFe1v3 and CCDC3 in canine osteosarcoma cell resistance to chemotherapy drugs

Dorna Khamsi, Dawn L. Duval

Osteosarcoma (OSA) is the most common primary bone cancer in canine patients, most often occurring in giant and large breeds. Nearly all primary tumors will eventually metastasize. Studies in our lab have found certain genes to be either up- or down-regulated in tumor tissue from patients with a disease free interval (DFI) of fewer than 100 days compared to those with a DFI of greater than 300 days. We are now studying how two of these underexpressed genes (ADHFe1 and CCDC3) affect resistance to chemotherapy and are using for our study three different OSA cell lines (Abrams, Gracie and D17) obtained from patients at the ACC. Canine ADHFe1 variant 3 was obtained from cells to make a mammalian expression construct and subsequently transfected into Abrams and Gracie cell lines. A CCDC3 shRNA knockdown construct was made, and due to difficulties amplifying canine CCDC3 a mammalian expression construct was made using human CCDC3. Abrams and D17 cell lines were transfected with CCDC3 constructs, a process involving electroporation of cells to increase plasmid construct uptake. After cells have adhered to a plate and multiplied in several days, isolated cell populations with one genetic make up are selected. Western blot analysis confirmed significant overexpression of CCDC3 in at least four cell populations from one transfection of Abrams cells, while RT-PCR suggested successful underexpression of CCDC3 in several Abrams cell populations transfected with one shRNA construct. Drug susceptibility assays performed on cell populations containing shRNA constructs show variable effects on the IC50 of cells treated with either doxorubicin or carboplatin. Currently, drug susceptibility of overexpressing cell populations is also being tested. Findings from this research will lead to a better understanding of the role of these genes in the progression of canine OSA and has the potential to improve prognostic methods, as well as aid in the development of tailored treatment regimens.

Effects of Synthetic Feline Facial Pheromone Use on Reducing Incidence of Upper Respiratory Tract Disease

Heather Y. Kihara, Rebecca Ruch-Gallie

Purpose: Cats entering shelters are more susceptible to disease due to many environmental and host factors, stress being one factor. Synthetic feline facial pheromone has been developed to help reduce stress in cats. Several studies have explored the use of these synthetics as adjunct treatment for inappropriate urination, cystitis, and inappetence. There have been no studies published looking at their use in prevention of upper respiratory tract disease (URTD). This study looks at the effect of synthetic feline facial pheromone on the reduction of incidence of URTD clinical signs in cats that are entering an animal shelter. Materials/methods: A total of 100 cats were studied. One group of 50 cats was exposed to synthetic feline facial pheromone via aerosolization (Feliway™ Diffuser) and a second group of 50 cats was exposed to the aerosolized base without pheromone present. Only cats entering the shelter's holding ward without prior clinical signs of URTD (i.e. sneezing, oculonasal discharge) were enrolled and observed. Study cats were housed in the holding ward with URTD cats that were not enrolled. Study cats were then scored daily for the development of these signs using a previously validated method. Data was also collected on the outcome (live or fatal) of each cat's stay. Shelter staff and scorer were blinded to the diffuser contents. Results: Using Chi-square analysis there is no statistical significance between treatment and clinical signs of URTD ($p=0.57$), outcome of stay ($p=0.21$) or severity of signs ($p=0.13$). Conclusions: Use of synthetic facial pheromone has no statistical impact on incidence or severity of clinical signs of URTD in this animal shelter. However, the observed severity of clinical signs was decreased. Therefore, this may warrant further investigation with a larger study.

Association of PECAM-1 and idiopathic pulmonary fibrosis

Lauren B. Kinner, Maggie Escobar, Stephanie Moon, Alan R. Schenkel

Purpose: Platelet endothelial cell adhesion molecule (PECAM-1/CD31) plays critical roles in the function of blood vessel cells known as endothelial cells and platelets, the removal of aged white blood cells, and is essential for white blood cell movement into tissues. There are two human PECAM-1 alleles which are most common: Leucine98Serine536Arginine643 (LSR) and Valinie98Asparagine536Glycine643 (VNG). The VNG allele has been shown to be associated with several inflammatory diseases, like coronary artery disease and atherosclerosis. This study is currently investigating the hypothesis that distinct human PECAM-1 alleles can be used as susceptibility markers for idiopathic pulmonary fibrosis (IPF).
Materials/Methods: Solid human lung tissue samples from anonymous donors were used for RNA extraction, followed by cDNA PCR amplification and sequencing. PECAM-1 alleles were then determined and analyzed. Of the 19 lung tissue samples that have been analyzed, 10 were IPF positive and 9 were non-IPF cases.
Results: Thus far, there has been a high correlation between those patients who had died from IPF and the occurrence of the LSR PECAM isoform. Of the 10 IPF samples that were sequenced, 9 were either LSR homozygous or heterozygous for the LSR/VSR allele. This is contrasted by the controls, in which 7 out of the 9 were homozygous VNG genotypes. Control group (non-IPF) samples consisted of a variety of patients, including some who had succumbed to COPD. These findings are consistent with previous studies that showed a correlation between the VNG isoform and COPD.
Conclusion: Our current data shows that the LSR PECAM isoform may be used as a susceptibility marker for IPF, due to its occurrence in IPF positive lung tissue samples.

Immunohistochemical detection of CWD prions in the CNS of Muntjac Deer

Shana F. Kitchen, Davis M. Seelig, Candace K. Mathiason, and Edward A. Hoover

As many studies of Chronic Wasting Disease (CWD) are hindered by the large size of the disease's native hosts, the identification of a smaller and more manageable model system which accurately reproduces the neuropathologic features of the disease as seen in the native host is desired. As such, the chief objective of this work was to document the neuropathologic features and pattern of accumulation of the pathologic, misfolded prion protein (denoted PrPRES) in CWD-infected Muntjac deer and compare results with native species. Materials and methods: Tissues were collected at terminal disease from experimentally CWD- infected Muntjac deer and from negative control animals. Following tissue fixation, the left hemisphere of the brain was coronally sectioned and, following routine paraffinization, six micron thick tissue sections were mounted onto glass slides, deparaffinized, rehydrated, and subjected to Heat Induced Epitope Retrieval. The IHC protocol used for the specific detection of PrPRES employed a combination of a mouse monoclonal antibody, an HRP-conjugated polymer secondary antibody, and the red AEC chromagen. Results: Initial optimization of the PrPRES detection was achieved by increasing primary antibody dilution, decreasing primary antibody incubation time , and decreasing AEC incubation time. In contrast to negative control animals, CWD-infected Muntjac demonstrated marked neuronal and neuropil spongiosis. Lastly, PrPRES deposits in CWD-infected Muntjac were seen throughout the CNS and in a pattern similar to CWD-infected mule deer. Conclusions: In this work, we present an optimized protocol for the specific detection of PrPRES in the CNS tissues of CWD-infected Muntjac deer. Moreover, through the demonstration of prion-associated neuropathology and the widespread CNS accumulation of PrPRES in infected Muntjac, we confirm the utility of this species as a model system for the neuropathologic features of CWD in native species.

Characterization of FIV sequences in Bobcats (Lynx rufus)

Danielle Lagana, Justin Lee, Jesse Lewis, Sarah Bevins, Alex Griffith, Linda Sweanor, Kevin Crooks, Sue VandeWoude

Purpose: Feline immunodeficiency virus (FIV) is an enveloped RNA retrovirus of the family Lentiviridae. Previous research shows that each felid species harboring an FIV-like virus appears to have a species-specific strain that is highly divergent from other FIVs. Contrary to this trend, FIV sequences from pumas (Puma concolor) cluster into two distinct monophyletic clades: FIVPcoA and FIVPcoB. FIVPcoB is the most widely described lentiviral type infecting pumas. The rarer and highly divergent clade, FIVPcoA, was previously only detected in Florida panthers and California pumas. Recently, FIV isolates sequenced from infected bobcats (Lynx rufus) in CA clustered with FIVPcoA, as did a FL bobcat FIV sequence. This was the first documented report of a strain of FIV that infects more than one species in the wild, providing possible evidence of cross species transmission between bobcats and pumas. The aims of this study were to determine the prevalence of FIV in bobcats in Southern FL and Colorado and to describe the genetic characteristics of these isolates. Materials/Methods: DNA was isolated from peripheral blood mononuclear cells from live-captured (n=43) or hunter-killed (n=47) bobcats in CO and live captured (n=25) bobcats from FL. Nested PCR was performed using degenerate primers designed to amplify a region of FIV pol-RT gene. Results: No FIV was detected by PCR in any of the CO bobcats we tested. Five samples from the FL bobcats were FIV-positive by PCR. Sequence analysis of these isolates demonstrates that FL bobcats are infected with FIVPcoA. The FIVPcoA sequences show greater similarity to samples from both bobcats and pumas identified in the same geographic area than to samples from bobcats in other geographic areas. Conclusion: Our data supports the previous finding that bobcats in FL and CA are infected with FIVPcoA, and supports the hypothesis that contact with pumas in these two geographic areas has resulted in cross-species transmission of FIV.

The effects of maropitant (Cerenia) on the clinical recovery of dogs with parvoviral gastroenteritis

Jamie Lenberg, Lauren Sullivan, Pedro Boscan, Timothy Hackett, David Twedt

Objective: To determine if hospitalized dogs affected with parvoviral gastroenteritis, when treated with maropitant compared to ondansetron, demonstrate an improved clinical recovery and shorter duration of hospitalization.
Procedures: This prospective randomized study involved 22 dogs that were naturally infected with parvovirus. Dogs were included following a positive result on a snap ELISA parvovirus test and if they were not previously treated for their disease. All dogs were treated with IV fluids, cefoxitin, and enteral nutrition. They were randomized to receive either maropitant (1 mg/kg IV q24h, n=11) or ondansetron (0.5 mg/kg IV q8h, n=11) as a primary antiemetic. Frequency of vomiting, nausea, and visceral pain scoring were evaluated twice daily. Rescue analgesics and antiemetics were administered as dictated by specific pain and nausea criteria. Clinical severity scoring, body weight, and caloric intake were monitored daily.
Results: When comparing maropitant and ondansetron groups, there was no statistical difference in: duration of hospitalization (3.36 +/- 0.56 vs. 2.73 +/- 0.38 days, p = 0.36); frequency of rescue antiemetic use (3/11 vs 5/11 dogs, p= 0.66); duration of vomiting (5 vs 4 days, p= 0.65); days to voluntary appetite (2 vs 1.5 days, p= 1.0); mean nausea score (27.9 +/- 6.3 vs 22.7 +/- 5.9, p= 0.56); or mean CSU pain score (1.25 +/- 0.18 vs 1.10 +/- 0.16, p= 0.87). Dogs treated with maropitant gained significantly (p=0.01) more weight during hospitalization (0.10 +/- 0.10 kg), whereas dogs treated with ondansetron lost weight (-0.43 +/- 0.16 kg). Maropitant was administered IV in dogs > 9 weeks of age, consecutively for up to 6 days, without any noted adverse effects.
Conclusions: In dogs with parvoviral gastroenteritis, maropitant appears to be equally effective in controlling clinical signs when compared to ondansetron but dogs treated with maropitant demonstrated better ability to maintain body weight during hospitalization.

Comparative Analysis of Bleomycin In Pulmonary Disease Susceptible PECAM Deficient Mice

Marta Lishnevsky, Steven J. Woods, William A. Muller, D.W.H. Riches, Alan R. Schenkel

Platelet Endothelial Cell Adhesion Molecule (PECAM/CD31) deficient mice in the FVB/n background spontaneously develop a chronic interstitial pneumonia. It has similarities to human interstitial pneumonia, including early alveolar collapse, subsequent development of fibrosis, and proliferation of myofibroblasts. This disease affects mice of all ages but is not fully penetrant, developing in about 40% of the mice. We show here that there appears to be leakage of blood into the lungs of affected mice. This implies that these mice would be susceptible to pulmonary insults. Surprisingly, these mice are resistant to infection. They also do not show any disease after intratracheal histamine instillation to induce vascular leakage. Bleomycin was used to induce lung injury and subsequent disease in PECAM deficient and wild type mice in order to compare the disease process in this well-characterized model of pulmonary fibrosis. As shown with other pulmonary insults, the FVB/n strain is highly resistant to bleomycin, paradoxically suggesting that rather than being more susceptible to damage, these mice may have highly active but improper repair mechanisms that may contribute to subsequent fibrosis.

Alternative Methods for Cryopreservation of Stallion Spermatozoa

Luisa M. Manzanares, J.K. Graham, K. Tarvis

Effective cryopreservation techniques have been developed for freezing bull sperm using glycerol as the cryoprotectant. However, results have not been so promising for stallion sperm, since glycerol crosses the cell-membrane slowly, creating osmotic stresses during the cooling, freezing and thawing process. Four experiments were designed to develop an alternative method of cryopreserving stallion sperm. We examined alternative low molecular weight cryoprotectants Methyl Formamide (MF), DiMethyl Formamide (DMF) and Methyl Acetamide (MA) compared to Glycerol (G); at various concentrations in two diluents (Lactose and FR4). Stallion ejaculates were split, and portions diluted into different treatments, packaged into 0.5ml straws, cooled and frozen in liquid nitrogen vapor. After storage in liquid nitrogen, straws were thawed in 39°C water and samples evaluated using a computer assisted sperm analysis (CASA) system for Motility, Progressive Motility, Velocity, and Straightness prior to freezing and post-thaw. Flow cytometry was used to evaluate cell viability. The first experiment evaluated the ability of MF, DMF, MA and G (each at 5%) to cryopreserve sperm. Experiments 2, 3 and 4 compared MF, DMF and G at different concentrations. In Expt 1, DMF protected sperm (Mot & Viability) as well as G (37-41%; $p< 0.05$), while MA was inferior (32%; $p<0.05$). Expts 2 and 3 indicated that 10% CPA was too high ($p<0.05$). When DMF was used at 5-12.5%, 5% DMF resulted in the highest percentage of motile sperm (44%) which was higher than that for G (31%; $p<0.05$). In conclusion, MF and DMF in the FR4 diluent are potential alternatives to glycerol for freezing stallion sperm. Diluents containing 5% DMF improved stallion sperm survival after freezing and thawing compared to Glycerol. Therefore, diluents using DMF as the cryoprotectant improve stallion sperm cryosurvival, however the fertilizing ability of these sperm must be determined.

The Analgesic Effect of Maropitant as a Pre-anesthetic Agent During and After Canine Ovariohysterectomy

Megan Marquez, Pedro Boscan, Heather Weir, Pamela Vogal, David Twedt

The objective of our study was to determine the analgesic effects of Maropitant used as a pre-analgesic agent for canine ovariohysterectomies. Healthy female dogs (n=40) between 6 months and 6 years of age presented for routine ovariohysterectomy were divided into three groups to receive either morphine, maropitant or 0.9% physiological saline (control) as a pre-anesthetic agent. Each dog was assessed for pain before and after the pre-anesthetic agent was given, and for 3 hours after the ovariohysterectomy. Pain level was recorded using the visual analogue scale (VAS) and the CSU pain score by 4 blinded investigators. The investigators made a unilateral decision if pain was present at each evaluation and administered rescue doses of analgesia as needed, which were compared between groups. During surgery parameters were also measured to assess: heart rate, blood pressure, respiratory rate and the percentage of isoflurane required. Data was compared with one way-ANOVA and posthoc Bonferroni test. The maropitant group required less isoflurane during ovarian ligation compared to control (1.41±0.25% vs 1.65±0.39%; $p<0.01$). The maropitant group was similar to morphine (1.53±0.37%). Similarly, the maropitant group required less isoflurane during skin closure compared to control (1.26±0.19% vs 1.52±0.26%; $p<0.05$). The maropitant group was more comfortable when compared to control 2 and 3 hours after extubation ($p<0.05$). The morphine group was more comfortable to the control only 2 hours after waking up ($p<0.01$).Of clinical interest, the maropitant group required less morphine rescue doses over three hours, when compared to control and morphine groups. Similarly, only 18% of dogs in the maropitant group required carprofen rescue analgesia, when compared to 30% in both the morphine and control groups. In this study maropitant appears to confer similar or better analgesia compared to this specific morphine dose for canine ovariohysterectomy.

Vaccine-associated Leptospira antibody responses in client-owned dogs

Laura E.R. Martin, K. Tomo Wiggans, Michael R. Lappin

Purpose: The purpose of this study was to determine the antibody response of client-owned dogs vaccinated with 1 of 4 commercially-available 4-serovar Leptospira vaccines.
Materials/methods: Thirty-two client-owned dogs that had not been vaccinated against Leptospira in at least 1 year were enrolled. Dogs were randomly assigned to receive 1 of 4 vaccines (with booster 3 weeks later) against the Canicola, Grippotyphosa, Icterohemorrhagiae and Pomona serovars, including Leptovax 4 (Boehringer-Ingelheim), Nobivac Lepto4 (Intervet Schering-Plough), Recombitek 4 (Merial) and Vanguard L4 (Pfizer Animal Health). Sera were collected prior to vaccination (week 0), at booster (week 3) and 1 and 4 weeks post-booster (weeks 4 and 7). Titers for the vaccine serovars were measured using the microscopic agglutination test (MAT). Titers for serovars Bratislava and Hardjo were also measured to assess cross-reactivity.
Results: For all dogs, the highest titers occurred during week 4 for the Canicola, Grippotyphosa and Icterohemorrhagiae serovars with 100%, 72% and 94% of dogs having titers >=1:100 and 66%, 38% and 44% of dogs having titers >=1:1,600, respectively. Titers of >=1:100 against Bratislava, Hardjo and Pomona were observed in 44%, 3% and 81% of dogs, respectively, during both weeks 3 and 4. Variations in titers were found between and within vaccine groups. For example, 100% of dogs administered Nobivac Lepto4 remained seropositive against Canicola at week 7 compared to 38% of the Recombitek 4 group. Week 4 titers for Grippotyphosa in dogs administered Vanguard L4 ranged from negative to 1:6,400.
Conclusions: The differences in antibody response within and between vaccine groups and cross-reactivity to serovars not included in the vaccines indicate that vaccines are not equivalent and MAT titers are not serovar specific. MAT titers were commonly >1:800, suggesting that titer magnitude cannot be used reliably to differentiate vaccinated from naturally-infected dogs.

A Rationale for Evaluating Livestock Contaminated with Radioactive Materials

Dayton D. McMillan, Jessica Gillis, Pete J. Sprenger, Thomas E. Johnson

Purpose: Recent events at Fukushima nuclear power plant have demonstrated the importance of having in place a sequential methodology of evaluating and handling radiologically contaminated animals. The methodology developed will serve to mitigate economic, livestock health, and consumer concerns. Materials/Methods: This assessment was formulated using literature regarding cost, livestock health, and risk perception. The cost of decontamination and projected market value of livestock were examined in relation to consumer perception. Disposal of radioactive carcasses and waste from decontamination were important economic considerations. The maximum acceptable dose where livestock health was not impacted was determined based upon an extensive literature search and international recommendations. Previous research on consumer risk perception of radioactively contaminated livestock was utilized to ascertain the likelihood of consumer consumption of decontaminated livestock.
Results: The most important aspect of this project was found to be consumer risk perception. Based on risk perception, it is unlikely consumers will purchase or accept food products that had been contaminated, even if verified to be decontaminated to acceptable levels. Economically, decontamination of animals would be the best course of action, even if consumption by humans would not be accomplished due to perceived risks, as contaminated carcass radioactive material disposal would prove expensive. Using decontaminated animal materials for such uses as pet food, leather products, or rendering should be considered. Livestock exposed to radiation doses less than their LD10 and not suffering deterministic effects were considered suitable for human consumption. Conclusions: Overall, livestock will most likely be suitable for alternative market use if successfully decontaminated, as it is unlikely consumers will find decontaminated products acceptable for food use.

The characterization of glutamate-gated chloride channels from Anopheles gambiae as insecticidal drug and vaccine targets

Jacob I. Meyers, Leslie M. Stone, Meg Gray, Ines Marques da Silva, Kathryn M. Partin, Brian D. Foy

Background: Malaria sickens and kills millions of people across the tropics annually. Current malaria control methods are reliant on established insecticides used to kill mosquito vectors. However, mosquitoes have adapted and become resistant to current insecticides creating a need for the development of new insecticides with new modes of action to control the spread of malaria parasites. We have recently demonstrated the potential of mass drug administration of the anthelmintic drug ivermectin to control mosquito populations. Ivermectin agonizes glutamate-gated chloride channels (GluCl) on neuronal and muscle tissue causing paralysis and death. Purpose: To assess GluCl as a target of mosquitocidal drugs and vaccines in Anopheles gambiae mosquitoes, the primary vector of malaria in Africa. Methods: Immunohistochemical staining with a polyclonal antibody directed against the extracellular domain of GluCl was used to reveal GluCl expression. Transient transfection of GluCl into the C6/36 mosquito cell line and conventional outside-out patch clamp technique was used to measure GluCl activity. Blood meals spiked with anti-GluCl antibodies were fed to mosquitoes to assess mosquitocidal properties of anti-GluCl antibodies. Results: GluCl is expressed in the head, thoracic ganglia and proximal antennal segments of A. gambiae mosquitoes. Electrophysiological recordings show glutamate-induced activity in C6/36 cells transfected with GluCl. Anti-GluCl antibodies caused a significant decrease in mosquito survival with an LD50 of 2.82g/mL. Serially feeding mosquitoes blood containing 282 mcg/mL of anti-GluCl antibodies significantly reduced survival over a 20-day period. Conclusion: A. gambiae GluCl are expressed in vital mosquito tissues that can affect fitness and survivorship. Targeting these channels with antibodies administered through a blood meal reduces the survivorship of laboratory reared A. gambiae mosquitoes.

Viral characterization of feline immunodeficiency virus in saliva and salivary tissues

Craig Miller, Karen Boegler, Susan VandeWoude

Feline immunodeficiency virus (FIV), a naturally-occurring lentivirus of wild and domestic felids, is shed in saliva and thought to be transmitted primarily via bite wounds. Viral pathogenesis and cellular mechanisms associated with salivary transmission of FIV have not been well studied. Human immunodeficiency virus (HIV) is also known to be present in the saliva of infected individuals and has been shown to be genetically, structurally, and biochemically similar to FIV. Studies involving HIV salivary pathogenesis have increased the prospect of alternative antiviral therapies and diagnostic methodologies in endemic areas. Further elucidation of lentiviral mechanisms of salivary excretion and transmission may therefore have significant implications in both medical and veterinary research. To evaluate viral kinetics and salivary shedding, 20 cats were exposed to a well-characterized, highly pathogenic strain of FIV. We demonstrated that saliva contains significant amounts of both viral RNA and proviral DNA. Quantification revealed that viral RNA present in saliva is on average 4 times higher than in plasma, while proviral DNA in saliva is within one order of magnitude of peripheral blood mononuclear cell (PBMC) proviral DNA. RT-PCR analysis revealed that viral and proviral loads in oral lymphoid tissues (tonsil, retropharyngeal lymph node) were significantly higher than oral mucosal tissues (tongue, buccal mucosa, salivary gland). Histologic evaluation of oral tissues revealed mild to moderate reactivity of the retropharyngeal lymph node and tonsil, as well as mild to moderate, subepithelial, lymphoplasmacytic, histiocytic, and mastocytic infiltration of the tongue and buccal mucosa. Results to date suggest multi-organ involvement in viral shedding and infectivity that appears to predominate in oral lymphoid tissues. Future studies will further evaluate viral pathogenesis in salivary tissues via immunohistochemistry, in-situ hybridization, and in vitro experiments.

The taste receptor T1R3 is expressed in at least 2 different cell populations in the mammalian hypothalamus

Sara H. Monahan, Leslie M. Stone

Dysregulation of hypothalamic circuits is linked to obesity, type II diabetes and heart disease. Circulating factors such as insulin and leptin are released in response to feeding states and influence these circuits. In addition, direct sensing of nutrients by hypothalamic and other brain regions likely plays a role in monitoring levels of available nutrients. Glucose-sensing cells are present in the hypothalamus and brainstem and appear to use a variety of transduction mechanisms. Recent evidence indicates that the sweet taste receptor heterodimer T1R2/T1R3 is involved in glucose sensing in the CNS and is expressed in neurons located in the arcuate nucleus of the hypothalamus (Ren et al., 2009). Cells in this region are well situated to detect circulating levels of nutrients due to a local disruption of the blood brain barrier. The goal of the current study is to test the hypothesis that T1R3 is expressed in a subpopulation of neurons in the hypothalamus that is important for nutrient sensing, and that these cells use an intracellular transduction pathway similar to that used by peripheral taste receptor cells to detect nutrient changes in the brain. Immunocytochemical analysis of mouse brain tissue was done to better characterize both the distribution of T1R3 positive cells and to begin to identify the specific cell types that express this taste receptor. We found that T1R3 antibodies label at least two populations of cells in the hypothalamus: one that is prevalent in the arcuate nucleus, and another, less common subset of cells that extend processes both toward the ventricle and toward neurons of the hypothalamus. Some of the latter cells are double-labeled for vimentin, an intermediate filament protein associated with tanycytes, glial like cells that line the 3rd and 4th ventricle. Our initial studies support the idea that T1R3 cells are present in the hypothalamus and are ideally situated to detect glucose changes in the brain.

A non-coding RNA produced by all arthropod-borne flaviviruses inhibits the cellular exonuclease XRN1 and modulates messenger RNA stability

Stephanie L. Moon, John R. Anderson, Carol J. Wilusz, Jeffrey Wilusz

All arthropod-borne flaviviruses produce a highly structured non-coding RNA that contains the 3' untranslated region (UTR) of the viral genome. These short flavivirus RNAs (sfRNAs) are implicated in viral pathogenicity, but the function and mechanism of action of sfRNAs remain unknown. We have determined that Dengue virus sfRNA is formed by the major cellular exoribonuclease XRN1 in human and mosquito systems. This stalling of XRN1 at structural elements in the 3' UTR is very unique and is not known to occur in host transcripts. The XRN1 enzyme degrades cellular transcripts in the 5' to 3' direction, and is important for normal cellular mRNA turnover, transcript quality control, and small RNA-induced gene silencing in human and mosquito cells. Purpose: We hypothesized that sfRNA formation, in addition to stalling the exonuclease XRN1, might also inactivate it. Materials/Methods: Human and mosquito cell extracts and recombinant XRN1 protein were used to show the effect of sfRNA formation on RNA decay in vitro. RNA was co-immunoprecipitated with XRN1 using a specific antibody against the protein. Cellular mRNA decay rates and the accumulation of uncapped mRNA decay intermediates were determined in West Nile virus and Dengue virus infected cells in culture using RT-qPCR. Results: sfRNA formation was associated with RNA stabilization in vitro and in infected cells in culture. Uncapped decay intermediates were increased in flavivirus infected cells. XRN1 was shown to be physically associated with viral RNAs encoding the 3' UTR. Conclusions: We propose that XRN1 stalls on the highly structured viral 3' UTR and remains associated with the RNA, inhibiting XRN1 activity. We further hypothesize that sfRNA formation may induce cytopathology by inhibiting 5' to 3' exonucleolytic mRNA decay in cells. Future studies will determine the impact of XRN1 inactivation on stability of immunomodulators in flavivirus infections.

Identification of Methicillin-Resistant S. aureus (MRSA) of Animal Origin Using Bacteriophage Amplification and a Lateral-Flow Immunoassay

Paul S. Morley, Denise Bolte, Joyce D. Rousseau, Dipankar Manna, Breanna Dreiling, J. Scott Weese

Background: Rapid laboratory confirmation is essential for appropriate clinical management of (MRSA) and for appropriate control of nosocomial and zoonotic transmission risks. Recently, bacteriophage amplification has been used as the basis for a commercial test designed to rapidly identify MRSA in human clinical specimens. The purpose of this study was to evaluate a similar prototype test to identify animal-source MRSA and methicillin-susceptible (MS) Staphylococcus aureus (SA) and to evaluate the test's reactivity with relevant non-SA staphylococcal isolates. Methods: Bacteriophage specific for SA were included in 2 broth media solutions, 1 that also contained cefoxitin to select for methicillin-resistant (MR) bacterial strains. A variety of genetically characterized Staph. isolates of animal origin were selected from an archival bank, and standardized concentrations were inoculated into the broth solutions. A lateral-flow immunoassay was used to detect phage that were amplified in broth cultures. Results: 36 MRSA isolates belonging to 7 major genetic types (USA 100, USA 200, USA 300, USA 500, USA 600, USA 700, and CC398) were evaluated. 32 MRSA were correctly classified; 4/15 CC398 isolates were misclassified. 11 of 15 MSSA isolates were correctly classified, as were 25 of 25 other non-SA staphylococcal isolates (including MR S. pseudintermedius, MS S. pseudintermedius, MR S. hyicus, MR S. schleiferi, and coagulase-negative Staphylococcus spp). Conclusion: This prototype rapid assay correctly classified a high proportion of widely diverse MRSA, MSSA, and other non-SA staphylococcal strains of animal-origin. Failure to detect some CC398 isolates might be problematic for some regions and animal species. Modification of the bacteriophage mixture may help overcome this misidentification. This inexpensive, rapid method has promise for practical applications requiring identification of MRSA colonization and infection in animals.

Venous Lactate Measurement in Post Operative Colic Horses

Darla K. Moser, Kris L. Perry, Eileen S. Hackett

Purpose: Colic is the leading cause of mortality in horses aside from old age. Following colic surgery, serious complications can arise, contributing to increased morbidity and mortality. Pre-operative peritoneal and venous lactate concentrations have been used in equine medicine as an indicator of intestinal ischemia and prognosis. Predictive value of venous lactate and serial lactate concentration of horses in the post-operative period is unknown.
Materials/Methods: Horses undergoing surgery for gastrointestinal disease were enrolled with owner permission. A portable lactate meter (Accutrend, Roche Diagnostics, Indianapolis, IN) was used to analyze venous samples immediately following anesthetic recovery and daily throughout hospitalization. Complications arising during hospitalization and survival to hospital discharge were recorded.
Results: 51 horses were enrolled, ranging in age from 2 to 29 years. Lactate concentration immediately following anesthetic recovery was significantly higher in horses that developed complications during hospitalization ($p=0.046$). The odds of developing complications post-operatively was doubled for horses with a venous lactate concentration >5mmol/L. Lactate concentration tended to be higher in non-survivors 24 hours following gastrointestinal surgery ($p=0.063$).
Conclusions: Elevations in lactate in the post-operative colic period were associated with increased risk of complications and death. Association of post-operative venous lactate and outcomes in horses undergoing surgery for gastrointestinal disease warrants further investigation.

Evaluation of maxillary blockade via the infraorbital foramen approach – A magnetic resonance imaging (MRI) study in equine cadavers

Darla Moser, Marlis L Rezende, Alex Valdes-Martinez, Khursheed Mama

Purpose: Maxillary nerve blockade is used in horses to provide anesthesia for surgical procedures in the maxilla. Anesthesia of entire maxilla requires blockade of the nerve before entering the infraorbital canal – a technique relatively difficult to perform and associated with complications. The aim of this study is to determine if complete maxillary block can be achieved via the infraorbital foramen, by evaluating the distribution of local anesthetic using MRI.
Material/methods: 20G 1.88-inch catheters were introduced into both infraorbital foramens of 4 equine cadaver heads. After MRI control scans were performed, a 50-50% mixture of lidocaine and contrast was injected: 7.5ml in one side and 15ml on the alternate side. Immediately following the injections, new images were obtained using spoiled gradient echo 3D sequences.
Results: The control scans demonstrated that the entire infraorbital canal could be identified and the maxillary nerve path could be traced. Images obtained after the injections showed the occurrence of artifact along the nerve path, which seems to have been created by the presence of contrast and the fast MRI sequence used. The anatomical localization and distribution of the artifact suggest that the spread of both volumes is compatible with complete maxillary block.
Conclusions: Complete maxillary blockade via infraorbital foramen seems to be achieved with the volumes used, suggesting that this approach can be an alternative to traditional maxillary blocks. However, different MRI scans need to be performed to eliminate artifact and enhance visualization of fluid distribution. This is the focus of the second part of this study.

Effects of Interactions Between Antimicrobial Peptides and Antibiotics on Bacterial Killing

Kara Mosovsky, Ediane Silva, Steven Dow

Purpose: Antimicrobial peptides (AMPs) are cationic peptides produced by myeloid and epithelial cells that serve as a first line of innate immune defense against pathogens. AMPs kill bacteria by creating pores in the bacterial cell membrane. Beta-lactam antibiotics, kill bacteria by inhibiting cell wall synthesis. Thus, we hypothesized that combined treatment of bacteria with AMPs and antibiotics would elicit synergistic bactericidal activity. Therefore, we examined the interaction of the cathelicidin AMP LL-37 with the beta-lactam antibiotic ceftazidime for killing of the gram-negative pathogen Escherichia coli (E. coli). Materials/Methods: Bacteria were incubated in vitro with LL-37 and low-doses of ceftazidime, alone or in combination, and the effects on bacterial killing were assessed by plating bacteria and determining colony forming units. In particular, we examined the effects that the sequence of adding the AMP and the antibiotic had on bacterial killing. Results: We found that simultaneous treatment of bacteria with low-dose ceftazidime and LL-37 produced synergistic killing of E. coli. However, we were surprised to observe that treatment of bacteria first with ceftazidime, followed later by addition of LL-37, produced resistance to bacterial killing. Likewise, pre-treatment of E. coli with subinhibitory concentrations of LL-37 also produced bacterial resistance to killing by ceftazidime. Conclusions: Our findings indicate that bacterial exposure to low doses of either compound, AMPs or antibiotics, induces resistance to killing by the other compound. Only when bacteria are exposed to both compounds simultaneously is synergistic killing observed. Thus, the bacterial response to low-doses of either AMPs or antibiotics appears to induce protective responses involving changes in the cell wall or cell membrane. These findings have implications for the deleterious effects of low dose exposure to antimicrobials on innate resistance to bacteria.

Apoptosis in Normal and Coxiella burnetii Infected Placentas from Alaskan Northern Fur Seals (Callorhinus ursinus)

Elizabeth Myers, EJ Ehrhart, Brad Charles, Terry Spraker, Colleen Duncan

Purpose: Northern Fur Seal (NFS) pup decline on the Pribilof Islands has incited investigations of reproductive health in this population. In 2010, Coxiella burnetii was identified in 3% of NFS placentas from a single rookery on St. Paul Island, Alaska by immunohistochemistry (IHC) and 75% were positive for C. burnetii via PCR. The significance of this finding for the reproductive health of this population was unclear. While many placental infections result in increased cell death, C. burnetii has been shown to suppress apoptosis of the host macrophages as a survival mechanism. Methods & Materials: To compare the amount of placental apoptosis in infected and non-infected placentas, IHC for antibodies to cleaved caspase-3 and the (TDT)-mediated dUTP-digoxigenin nick end labeling (TUNEL) technique were used. Placentas evaluated represented three groups; those infected with histologically identifiable organism (n=5), PCR positive without organism seen on H&E (n=5), and tissues negative on IHC and by H&E (n=5). Results: There was a statistically significant difference in the frequency of apoptotic cells between all three infection groups (TUNEL p=0.012, Caspase-3 p=0.023), with significantly more apoptosis identified in the uninfected placentas. Conclusion: This finding suggests that the survival mechanism of C. burnetii in host macrophages to reduce apoptosis may also be utilized in trophoblasts. The significance of decreased trophoblastic apoptosis for the fetus requires further investigation.

Chemically Induced Retinal Degeneration Model in Goldfish

Shyla Myrick, Jozsef Vigh

Retinal degenerative diseases ultimately lead to permanent vision loss in both animals and people. The loss of photoreceptors triggers reorganization of the remaining retinal circuitry. This substantial, chronic morphological change ("remodeling") challenges the success of treatment strategies. We sought to establish a natural model to examine stem cell-based photoreceptor replacement schemes using the Goldfish (Carassius auratus auratus) retina, which retains stem cells throughout life, and thus capable to regenerate retinal damage. In this study we tested various strategies to induce complete and selective photoreceptor loss, prerequisite to study consequent remodeling and regeneration processes. Enrofloxacin, and NMU (N-Nitroso-N-methylurea) were selected for their documented retinal degenerative potential, along with Vincristine (mitotic blocker). To assess the effectiveness of these drugs unilateral intraocular injections were performed under anesthesia, then fish were exposed to various lighting intensities. Retinal tissue was harvested from treated and control contralateral eyes at various time points and subsequently evaluated using standard immunohistochemical techniques.
Enrofloxacin did not cause retinal degeneration in the fish retina, regardless of the light conditions post-injection (normal room light vs. bright light) treated group showed no significant reduction in cell count in the photoreceptor / outer nuclear layer (ONL) or the inner nuclear layer (INL). The MNU + Vincristine treated group showed a 61% reduction in the ONL with no significant cell loss in the INL. This data demonstrates the potential of MNU as the chemical agent for the characterization of the remodeling process following photoreceptor degeneration although the mechanism through which to achieve full degeneration will require further investigation.

Incidence of upper respiratory disease in cats at an emergency shelter

Sarah E. Iverson, Rebecca Ruch-Gallie

In response to lessons learned from Hurricane Katrina, the US Congress passed the Pets Evacuation and Transportation Standards Act (PETS Act) in 2006 requiring that state and local emergency preparedness plans address the needs of individuals with household pets and service animals following a major disaster or emergency. To date, the incidence of disease has not been evaluated in companion animal in emergency shelters. Cats housed in US animal shelters face many difficulties, not least of which is dealing with highly contagious upper respiratory tract disease (URTD). Factors contributing to URTD prevalence in shelters include stress and high concentration of cats and agents. The purpose of this study was to evaluate the incidence of URTD in an emergency shelter and to determine what housing variables were associated with disease. Approximately 175 cats at an auxiliary emergency shelter in Minot, ND were observed during a three week confinement in a grain warehouse. Cats were observed and received a clinical score for signs of URTD such as nasal and ocular discharge, sneezing, and coughing. Ninety-three percent of the cats housed showed clinical signs of URTD during confinement, with 14% exhibiting significant disease (mean clinical score >1). Incidence rates are compared to multiple variables, including size of crate, number of cats in crate, presence of an object from home, and availability of a place to hide.

Effect of Maropitant, an Antiemetic Neurokinin-1 Receptor Antagonist for Dogs and Cats, on the Sevoflurane Minimum Alveolar Concentration During Ovarian Stimulation in Cats

Sirirat Niyom, Pedro L. Boscan, David C. Twedt, Eric Monnet

Purpose: Maropitant is a neurokinin (NK-1) receptor antagonist, approved for use in dogs and cats to treat emesis. The hypothesis is that Maropitant will decrease the sevoflurane minimum alveolar concentration (MAC) during visceral stimulation. Materials/methods: Ten, female cats weighting 2.55 ± 0.07 kg (mean ± SE) were used. General anesthesia was induced and maintained with sevoflurane. MAC evaluations were performed prior to and after 1 and 5 mg/kg Maropitant intravenous administration. The right ovarian ligament was accessed using a laparoscopic approach. MAC was determined when stimulating the right ovary and ovarian ligament with 4.9 Newton's of tension force. MAC was defined as the average of the cross-over concentrations and performed in triplicate. Reported MAC was adjusted to sea-level and depicted as mean ± SE. Repeated measure ANOVA was used for comparison. Results: Sevoflurane MAC was 2.96 ± 0.3%. Maropitant 1 mg/kg decreased the MAC to 2.51 ± 0.3% (15%, $p < 0.01$). A higher Maropitant dose of 5 mg/ kg did not change MAC further when compared to the low dose (2.46 ± 0.4%). Conclusions: Maropitant decreased the anesthetic requirements during visceral ovarian stimulation in cats. However, the MAC sparing effect was not dose dependent.

Detection and isolation of pH1N1 influenza A virus from a privately owned small swine herd in Colorado

Kristy L. Pabilonia, Kyran J. Cadmus, Christina B. Weller, EJ Ehrhart, Barbara E. Powers

Purpose: This case report documents the first known report of transmission of 2009 pandemic H1N1 influenza virus in the United States from a human to a swine herd. Lungs and intestines from two piglets were submitted to the Colorado State University Veterinary Diagnostic Laboratory in November 2010. The piglets were from a small, privately owned herd and had a history of weight loss and pneumonia. The referring veterinarian reported that other piglets on the farm had died. Materials/Methods: Necropsy (on-farm) and histopathology were performed. Real-time reverse transcription polymerase chain reaction (rRT-PCR) assays to detect the 2009 pH1N1 influenza A virus were conducted on RNA isolated from lung homogenates from both piglets. The assays included detection of the matrix gene of influenza A virus and the neuraminidase gene specific for 2009 pH1N1 virus. The virus was isolated in Madin-Darby canine kidney (MDCK) cells and specific pathogen free embryonated chicken eggs. Results: Gross lesions detected on necropsy included cranioventral pneumonia in both piglets, a section of thickened small intestine in Piglet 1, and in Piglet 2 peritonitis, abnormally colored small intestine and an enlarged mesenteric lymph node. Histopathological lung lesions included segmental airway epithelial necrosis and effacement with consolidation by filling of alveolar spaces with neutrophils and histiocytes, loss of alveolar walls in the absence of distinct necrosis, prominent perivascular lymphocytic cuffs, and alveolar wall congestion. Both samples were positive on both PCR assays. Sequence analysis showed 98% homology with 2009 pH1N1 human isolates from across the U.S. and 98% homology with two recent U.S. swine isolates from Nebraska and Minnesota. Conclusions: The owner was employed as a pharmacist and participated in pH1N1 vaccination programs, making occupational exposure to the 2009 pandemic H1N1 virus a possibility and the suspected route of transmission from human to swine.

Presynaptic Gi/o-coupled receptors resist acute desensitization

Reagan L. Pennock, Matthew S. Dicken, Shane T. Hentges

Purpose: Gi/o coupled receptors located in the somato-dendritic region of neurons modulate neuronal activity through a direct inhibition of firing, while the same receptor located on presynaptic terminals modulates activity by inhibiting neurotransmitter release. Previous studies suggest that the location of a Gi/o coupled receptor on a neuron may determine that receptor's resistance to acute desensitization, however these studies generally focus on a single type of Gi/o coupled receptor and are spread across many regions of the brain. The present study examines the desensitization of multiple Gi/o-coupled receptors in a single population neurons. Materials/Methods: Whole cell voltage clamp experiments were performed in proopiomelanocortin (POMC) neurons of the hypothalamus. Results: Agonists for the mu opioid receptor (MOR), GABAB receptor (GABABR) and nociceptin receptor (ORL-1) inhibited POMC neurons directly through the induction of a postsynaptic potassium conductance as well as inhibited the release of GABA onto POMC neurons presynaptically, which was measured as a reduction in the amplitude of evoked inhibitory postsynaptic currents (eIPSCs). The potassium current induced by all three agonists desensitized rapidly in the prolonged presence of agonist, reaching a steady state within minutes of the peak effect. No desensitization of the inhibition of neurotransmitter release by MOR or ORL-1 was detected under a similar exposure to agonist, however GABABR mediated inhibition did desensitize in ~25% of recordings. Conclusions: Resistance to desensitization is a property shared by Gi/o coupled receptors found on presynaptic terminals. Further, the fraction of presynaptic GABABRs that do desensitize suggest that resistance to desensitization may be conferred by a property of the receptors and not an intrinsic property of the terminals on which they are located.

Encephalitic alphavirus infection of outbred mice visualized using in vivo and ex vivo imaging

Aaron T. Phillips, Tawfik Aboellail, Ken Olson

These studies use an infectious clone of Western equine encephalitis virus McMillan strain, a highly neurovirulent strain in mice, that was engineered to express a transgene under the control of a 3' double subgenomic promoter. Constructs include firefly luciferase (LUC), DsRed, and GAL4-expressing variants. Outbred CD-1 mice were used with LUC and DsRed–expressing variants and Tg UAS-LUC mice were used with the GAL4 –expressing variant. This system was used to show that virus dissemination may occur through neuronal connectivity in the case of intranasal challenge and descriptions of the resulting pathogenesis are presented through histological examination. DsRed-expressing virus was used to demonstrate viral replication at the cellular level using microscopy. Focal concentrations of fluorophore along neuronal processes were found. To validate this system as a convenient proxy of antiviral efficacy, mice were treated with liposome-associated-nucleic acid-antigen-complex based on the WEEV-E1 ectodomain and infected with LUC-expressing virus. The results demonstrate excellent antiviral assessment as treated animals survived and showed minimal luminescence while untreated animals did not survive and showed very high levels of luciferase expression.

Body condition score does not predict myocardial triglyceride content in canids

Cara Porsche, E Mehlman, M Frye

Purpose: The prevalence of overweight and obese canine patients approaches 40%, and is likely to parallel the increasing prevalence of obesity in the human population. Increased body weight in humans predicts greater morbidity and mortality attributed to cardiomyopathy and heart failure. A predictor of disease progression is left ventricular (LV) hypertrophy. Using echocardiography, we identified that LV wall thickness is increased in obese dogs. The cellular mechanisms underlying the structural change in this species are not fully understood. Accumulation of excess myocardial triglycerides (TG) is variably present in obese humans. The purpose of this study was to quantify myocardial TG content in obese dogs as a potential contributor to gross LV hypertrophy in this species.
Materials/methods: Subjects included 4 obese dogs and 12 age-matched lean controls, without a history of cardiac disease. Dogs were submitted for necropsy at the CSU Veterinary Medical Center after euthanasia for reasons unrelated to this study. Body condition scores (BCS) were determined using a 9-point system, with a score = 7 defining obesity. LV tissue was snap frozen in liquid nitrogen for quantitation of TG content using the Biovision Triglyceride Assay Kit (Biovision Research Products, Mountain View, CA).
Results: Myocardial TG concentrations were similar between lean and obese dogs (37.78 ± 7.57 nmol/mg wet weight and 81.99 ± 39.46 nmol/mg wet weight, respectively; $p = 0.215$).
Conclusion: Body condition is not likely to be the primary determinant of myocardial TG content. Dietary macronutrient composition and adiposity may partly determine myocardial TG accretion; however, this study was limited by a lack of information regarding these factors. Further studies would ideally include dietary history, % body fat determination, and additional BCS groupings (underweight, normal, overweight, obese). Due to high variability within groups, a larger sample size is also needed.

Computed tomography mapping of distal limb synovial structures studied in twelve horses

Amanda E. Rauhauser, Christopher Kawcak

Diagnostic analgesia of the distal limb is a commonly used tool for localizing lower limb lameness. Because the specificity of distal limb analgesic procedures is unknown, the proximity of joints and tendon sheaths to neurovascular structures may influence clinical diagnostic outcome. However, little research has successfully provided accurate three-dimensional mapping of the communication between joint spaces and associated synovial structures. The specific goal of this research study was to improve the specificity of local injections in order to allow veterinarians to more effectively diagnose lameness and better alleviate pain for the horse. Based on the hypothesis that three dimensional contrast-enhanced computed tomography (CECT) will provide a specific map and increase effectiveness of regional analgesia of the distal limb, limbs were recovered post-mortem, injected with contrast in several joints, and scanned using computed tomography. This method allowed more thorough characterization of the differences between distal limb structures. In particular, this study found that contrast infiltrated the tarsal sheath in addition to the tarsal metatarsal joint in six out of ten tarsal metatarsal joints injected. Also, five out of twelve intracarpal joints injected also had evidence of contrast entering the fenestrated origin of the proximal suspensory ligament. It can be concluded from these results that synovial structures may overlap in some cases, leading to confounding evidence and inaccurate interpretation of lameness diagnostics. These preliminary conclusions demonstrate the need for continued CECT mapping of joint injection infiltration. If performed on live tissues with minimal injector error and concurrent lameness exams, CECT could improve the accuracy of diagnostic analgesia.

Plasma concentrations, behavioral and physiological effects following IV administration of dexmedetomidine in horses

Marlis L Rezende, Khursheed Mama, Kristin Grismund, Eugene Steffey, Scott Stanley

Purpose: Alpha2 agonists are the most commonly used drugs to provide sedation and analgesia in the horse. Dexmedetomidine is the newest, most potent and most selective of the a2 – agonist agents available for clinical use. The characteristics of dexmedetomidine indicates that the drug has great potential for use in equine anesthesia; however the knowledge of its effects in the horse is extremely limited and warrants further studies. The aim of this study is to evaluate the use of IV dexmedetomidine in the horse by assessing plasma concentrations of the drug as well as its behavioral and physiological effects.
Material/methods: 5 mcg/kg of dexmedetomidine was administered IV to 8 horses. Blood was sampled at predetermined time points from 1min to 10h after drug administration for plasma dexmedetomidine concentration measurements using liquid chromatography-mass spectrometry. Behavioral (e.g. head position, response to noise) and physiological responses (e.g. heart and respiratory rates) were also recorded at fixed time points from 4min to 6h post dexmedetomidine administration. Data were summarized as mean ± s.d.
Results: Dexmedetomidine plasma concentration peaked at 1 minute (3.4 ± 4.3ng/ml) and quickly decreased over time to 0.2 ± 0.1ng/ml at 30 minutes, until no longer detected at 1h post injection. Behavioral and physiological responses were also of greater magnitude at the earlier time points, returning to baseline after 30 minutes.
Conclusions: Dexmedetomidine plasma concentrations correlated well to the behavioral and physiological responses observed, which were of short duration. Dexmedetomidine may be used to provide sedation for short procedures in the horse.

Transferrin receptor expression in serum exosomes as a marker of regenerative anemia in the horse

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Purpose: Anemias are often identified as either regenerative (indicating that the bone marrow is producing new red blood cells), or non-regenerative. Across many species, the presence of immature red blood cells, known as reticulocytes, in the circulation helps to identify an anemia as regenerative. However, in horses, reticulocytes are not found in the circulation. Currently, regeneration in the horse can only be confirmed with a bone marrow aspirate, which is a painful and complicated procedure. We investigated the expression of the transferrin receptor (a membrane protein involved in iron regulation) in anemic horses as a non-invasive way to identify a regenerative anemia. Transferrin receptor expression is high in early erythroid precursors and decreases via exosome secretion as erythrocytes mature. Exosomes are 30 – 100 nanometer vesicles containing proteins and RNA, which are actively secreted from the plasma membrane of cells. We hypothesized that transferrin receptor expression would increase in serum exosomes during a regenerative anemia. Materials/methods: Six horses were phlebotomized to induce anemia. Serum was collected on Days 0, 4, 7, 10, 14 and 21 and exosomes were isolated. The level of transferrin receptor on the exosomes was determined by Western blot. Results: Anemia was confirmed by decreased hematocrits and regeneration was confirmed by decreased myeloid:erythroid ratios in the bone marrow. In all six horses, transferrin receptor expression increased significantly over Days 7-10 ($p < 0.05$). Transferrin receptor levels peaked on Day 10 and were a mean 4-fold higher than levels on Day 0. Conclusions: These data suggest that transferrin receptor expression in serum exosomes may provide a marker for a regenerative response in an anemic horse.

Isolation of Salmonella enterica from the environment in a large animal hospital

Audrey Ruple-Czerniak, Brandy Burgess, Paul Morley

Purpose: The objective of this study was to compare two methods of detecting environmental contamination with Salmonella enterica in a large animal veterinary teaching hospital.
Methods: Environmental samples were collected using both electrostatic wipes and sponge collection devices from stalls used to house either horses, cows, or camelids that were confirmed to be shedding Salmonella enterica by fecal culture. Samples were obtained from stall sites after cleaning and disinfection had been completed. Areas within the stall that were sampled included floors, walls, drains, cracks, and feed and water containers, with a similar amount of surface area covered with both sampling devices. Multiple enrichment protocols were used for bacteriologic culture of samples obtained using each of the sampling devices.
Results: A total of 100 paired environmental samples were collected and bacteriologic culture and 14% of samples collected using the electrostatic wipes resulted in detection of Salmonella enterica while only 4% of samples collected using the sponge collection device resulted in detection of S. enterica. Results were concordant in 88% of the samples. Of the paired samples with discordant bacteriologic culture results, Salmonella organisms were detected in the sample collected with the electrostatic collection device in 11 out of 12 (91.7%) of the samples.
Conclusions: These results suggest that the sampling and culture method used with the electrostatic collection device is more sensitive than is the sampling and culture method used with the sterile sponge collection device.

Potential effects of volcanic emissions (VOG) on respiratory health of free-ranging Mouflon Sheep

Bridget A. Schuler, Jon Faford, Jenny G. Powers, Terry R. Spraker, and Colleen G. Duncan

Though volcanic eruptions have occurred throughout history, effects on humans, animals and the environment have only recently been studied. In March 2008, the Kilauea volcano on the main island of Hawai`i increased SO2 emissions from 200 tons per day to 2,000 tons per day, making it the largest point source of SO2 in the United States. Sulfur dioxide is the primary gas emitted; however, other gases, trace minerals, and heavy metals are also released. Collectively emissions are referred to as volcanic smog or VOG. Since 2008, areas downwind of the plume have experienced extreme vegetation loss associated with VOG exposure. There is anecdotal evidence of negative effects on lung function, reproduction, and bone health in domestic livestock. Adverse human respiratory effects have also been documented. Effects on free-ranging animals are not well studied. The objective of this study compared gross and histologic changes in the respiratory tract of Mouflon Sheep (Ovis gmelini musimon) directly downwind from Kilauea to a population of Mouflon/domestic hybrid sheep rarely in contact with volcanic emissions. In conjunction with a management cull, sections of respiratory tract were collected and examined. In the downwind (exposed) sheep, gross and/or histologic evidence of pulmonary nematodiasis (Muellerius capillaris) infection was observed in 44/49 animals. Histologically, nodules were eosinophilic granulomas with numerous adult parasites, larvae and eggs. In contrast, 7/50 non-exposed sheep had gross and/or histologic lesions consistent with lung worm. Exposed sheep had significantly more upper respiratory tract inflammation and hyperplasia consistent with chronic antigenic stimulation, likely due to recent drought conditions. Although pulmonary disease was more severe in exposed sheep, there are numerous potential confounding factors between the two populations.

Equine endometrial concentrations of fluconazole following oral administration

David B. Scofield, Ryan A. Ferris, Luke A. Whittenburg, Daniel L. Gustafson, and Patrick M. McCue

Purpose: The objective of this study was to determine the plasma and endometrial concentrations of fluconazole after oral administration to mares. Our hypothesis was that endometrial levels of fluconazole would be maintained above the minimum inhibitory concentration (MIC) of Candida albicans.
Materials and Methods: Group one mares (n=6) in early estrus were administered a single loading dose of 14 mg/kg fluconazole (Glenmark Generics Inc., Mahwah, NJ) via naso-gastric tube. Group two mares (n=3) were administered the loading dose, followed by maintenance doses of 5 mg/kg every 24 hours for six days. Plasma and endometrial biopsy samples were collected at predetermined times and analyzed at the Pharmacology Core Laboratory, CSU using HPLC-MS-MS.
Results: Oral fluconazole was well absorbed and distributed to the plasma and uterine tissues in the mare. Mean plasma and endometrial fluconazole levels at 24 hours following loading dose were 9.53 +/- 0.82 mcg/ml (mean +/- SD) and 11.3 +/- 2.38 mcg/g respectively. Fluconazole levels in plasma and endometrium 24 hours following the last maintenance dose were 7.82 mcg/ml +/- 1.81 and 7.23 +/- 3.85 mcg/g, respectively. The published MIC for Candida spp. is 0.5 to 8.0 mcg/ml with 94% of Candida albicans isolates having an MIC value less than 0.5 mcg/ml.
Conclusions: Oral fluconazole administered as a 14 mg/kg loading dose and 5 mg/kg maintenance dose every 24 hours will result in endometrial tissue levels near the accepted minimum inhibitory concentration (MIC) values for most Candida spp. and surpass the MIC for C. albicans in the reproductive tract of the mare. Systemic administration of an antifungal agent may be an important therapeutic option in addition to local intrauterine therapy for the treatment of equine fungal endometritis. Consequently, this dosage regimen could be considered for the treatment of infectious endometritis in the mare caused by susceptible fungal organisms.

Effect of equine metabolic syndrome on the intrafollicular environment and fertility

Dawn R. Sessions, Elaine M. Carnevale

Purpose: Equine metabolic syndrome (EMS) is associated with obesity and is characterized by insulin resistance (IR), decreased adiponectin (ADIPO) and elevated insulin (INS), and leptin (LEP). We tested the hypothesis that mares with EMS have altered follicular environments and decreased pregnancy rates compared to mares with normal metabolic function. Materials and Methods: Light-horse mares (11-27 yrs) in a clinical assisted reproductive program were used with owner permission. At 24h after GnRH analog (Sucromate, 0.9mg, IM) administration, preovulatory follicles were aspirated in both normal (n=12) and mares with EMS (n=8) to collect follicular fluid (FF), granulosa cells (GC) and oocytes. Blood was collected on the morning of aspiration following an overnight fast to determine IR, and evaluate serum concentrations of INS, LEP, and ADIPO. Protein concentrations in serum and FF were determined using RIA. Gene expression for receptors of INS, LEP, and ADIPO were assessed in GC. Oocytes were used for the clinical program. Results: Age and body condition scores were not different between groups. Concentrations of INS (r^2=0.75; P<0.001), LEP (r^2=0.85; P<0.001) and ADIPO (r^2=0.82; P<0.001) were highly correlated in serum and FF. Insulin was elevated (P<0.001) in serum and FF of mares with EMS vs. normal mares; while LEP only tended to be higher (P=0.07) in the FF of mares with EMS. Conversely, ADIPO was decreased (P<0.05) in serum and FF in the EMS group vs. normal mares. Granulosa cells expressed receptors for INS, LEP, and ADIPO; however, there was no difference in receptor expression between groups. Pregnancy rates between groups were not different, however, pregnancy was associated with elevated serum LEP concentrations (r^2=0.47; P<0.05). Conclusions: The intrafollicular environment is influenced by metabolic diseases. Granulosa cells express genes for metabolic hormone receptors indicating they can affect follicular function and oocyte viability.

A Novel Detection Method for Aerosol Reactivity

Jeff Shapiro, Yupaporn Sameenoi, Kirsten Koehler, Chuck Henry, Jeff Collett, John Volckens

Purpose: Human exposure to particulate matter (PM) air pollution is a top-10 contributor to the global burden of disease on planet earth. Yet, the mechanisms by which PM exerts its ill effects are unknown. Some evidence suggests that PM exposure encourages cellular oxidative stress through the generation of reactive oxygen species in human tissue. Thus, a need exists for reliable measurement of PM's oxidative potential. The objective of this work was to develop an improved, real-time assay for the oxidative potential of PM air pollution. We coupled a particle-into-liquid sampler with a microfluidic-electrochemical sensor capable of reacting dithiothreitol (DTT; an indicator of oxidative load) with freshly-collected PM so that aerosol reactivity could be estimated in near real-time. Methods: This study assessed the novel electrochemical sensor's capacity to measure laboratory aerosol reactivity against the traditional DTT assay. Two types of laboratory PM (Urban dust NIST 1649b and fly ash) were generated at 3 atmospherically-relevant concentrations (10, 30, 90 ug/m3). PM collection and reactivity analysis were performed in parallel using both the traditional (offline) and electrochemical (online) methods. DTT consumption rates were compared using multiple regression analysis. Results: The traditional and electrochemical methods detected similar reactivity for each PM type and linear responses to various aerosol concentrations. However, the electrochemical system detected reactivity values with higher precision than the traditional method indicating lower limit of detection. Conclusions: The novel electrochemical detection system collected and assayed laboratory aerosol in real-time allowing for both high resolution measurements (~15 minutes) with equally accurate yet more precise measurements. This system will eventually permit more comprehensive and biologically relevant PM reactivity measurements.

Vaccination with attenuated Burkholderia for protection from acute and chronic melioidosis

Ediane B. Silva, Andrew Goodyear, Margie Sutherland, Steven Dow

Burkholderia pseudomallei is a soil bacterium that can also cause serious infection in humans following exposure to contaminated soil or water. Infection with this pathogen in humans causes high mortality and there is currently no vaccine available. In addition, many apparently recovered individuals go on to develop chronic meliodosis with abscesses in multiple organs. The aim of the present study was to determine whether immunization with a live but highly attenuated strain of B. pseudomallei (strain Bp82), a purM deletional auxotroph deficient in adenine biosynthesis, could produce effective protective immunity against B. pseudomallei infection. We wished to determine: (1) whether parenteral subcutaneous (s.c.) immunization with Bp82 could produce protection from acute pulmonary challenge, (2) whether mucosal immunization could also produce protection against the development of chronic meliodosis, and (3) what is the mechanism of protection following acute challenge. To address these questions, BALB/c mice were immunized twice either s.c. (10^7) and/or orally (10^8) with Bp82 or saline phosphate (control), then subjected to B. pseudomallei challenge. Two weeks following immunization, the mice were challenged intranasally with 5x103 CFU/mouse of virulent B. pseudomallei (strain Bp1026b). Immunization by s.c. generated significant protection (P = 0.0062) from acutely lethal challenge with B. pseudomallei, with 100% survival for 60 days following pulmonary challenge. The Bp82 immunizations reduced the bacterial burden but did not completely eliminate bacterial persistence. The s.c. Bp82 immunization induces Bp82 specific IgG and T cell memory immune responses. Although further studies are necessary to elucidate the immunological mechanisms of protection and to improve protection against chronic disease, these results make the Bp82 a valuable component for further evaluation in the development of a meloidosis vaccine.

LIN28A regulates human trophoblast syncytialization and preeclampsia diagnostic markers

Erin E Soisson, JL Seabrook, JD Cantlon, CM Clay, RV Anthony & QA Winger

Preeclampsia (PE) and intrauterine growth restriction (IUGR) arise from abnormal placenta development and function. A vital component of development is the formation of the syncytiotrophoblast (ST) layer, which functions as the feto-maternal interface. The ST is maintained throughout pregnancy via continual cytotrophoblast (CT) differentiation and fusion. In addition to modulating metabolic exchange, the ST layer produces human chorionic gonadotropin (hCG), which is required for pregnancy recognition, trophoblast invasion and vascular remodeling. Placental explants cultured from PE and IUGR pregnancies have lower CT fusion indices, fewer nuclei per ST, and lower hCG secretion. Serum concentrations of free-beta hCG (ß-hCG) and placental protein 13 (PP13) have been shown to be predictive markers for the development of PE and IUGR; with decreased levels of PP13 and elevated levels of ß-hCG indicative of imminent PE. Therefore, understanding the mechanisms controlling CT-ST differentiation is an important approach for understanding placental disease. LIN28A, an RNA binding protein, is high in pluripotent cells and decreases with differentiation. In the current study we investigated how LIN28A regulates CT-ST differentiation. LIN28A is highly expressed in ACH-3P cells, a first trimester trophoblast cell line. We constructed LIN28A shRNA-knockdown (KD) ACH-3P cells. KD cells were treated with forskolin, and the syncytialization response was assessed. Relative levels of ß-hCG (CGB) and PP13 mRNA were both higher (3-, 7-fold, respectively; $P < 0.01$) in KD cells vs control. Forskolin treatment resulted in higher levels of relative CGB and PP13 mRNA (3-, 11-fold, respectively; $P < 0.01$) in the KD cells vs control. These data suggest that LIN28A has an inhibitory effect on mechanisms regulating ST differentiation, as well as preeclampsia markers ß-hCG and PP13, and that deregulation of LIN28A may contribute to the development of placental pathology.

Genes relevant to cardiomyopathy are differentially expressed in response to Western diet feeding alone and with DHA supplementation

Katharine Spencer, Jeckel K, Bouma GJ, Hess A, Petrilli E, Frye M

Obesity increases the risk for cardiomyopathy in the absence of hypertension, diabetes or myocardial ischemia. The fatty acid milieu, modulated by diet, may alter myocardial gene expression relevant to structure and function, lending partial explanation for the array of cardiomyopathic phenotypy in obese individuals. Our aim was to define myocardial gene expression profiles with consumption of a Western diet, and subsequent modification with DHA supplementation. Adult male Wistar rats (n=10) were assigned to 1 of 3 dietary treatments for 3 months: control (CON), Western diet (WES) and Western diet supplemented with DHA (WES+DHA). Left ventricular myocardial tissue was subjected to RNA isolation. Biotinylated cRNA targets were hybridized to the GeneChip Rat Genome 2.0 probe array, and signal intensity was determined using the GeneChip 3000 scanning system. Fourteen genes with a log2FC > 0.8 were chosen for real time qRT-PCR validation based on relevance to hypertrophy, extracellular matrix remodeling and contractile proteins. Microarrays revealed a total of 66 genes that were differentially present in 1 or more group comparisons (ANOVA $p < 0.001$). There was agreement between relative transcript levels as determined by microarray and qRT-PCR. Transcript expression patterns and pathway analysis revealed hypertrophy in WES rats compared to CON, and antihypertrophic proceses in WES+DHA rats compared to WES. Compared to CON, WES+DHA rats had variable changes in gene expression relevant to hypertrophy, and changes favoring modification of contractile proteins. It was concluded dietary fatty acid composition modulates myocardial gene expression in dietary obese rats. Specifically, genes favoring myocyte hypertrophy appear to be upregulated in response to WES feeding, and may be attenuated with concomitant DHA supplementation. Future studies will focus on identifying changes in protein levels that are consistent with observed changes in gene expression.

Developing an Animal Decontamination Protocol

Pete Sprenger, Dayton McMillan, Dave Dolan, Jessica Gillis, Tom Johnson

Purpose: The purpose of this study was to evaluate the effectiveness of decontaminating livestock using multiple methods. Because several factors were expected to influence the efficacy of animal decontamination, a protocol was developed to compare decontamination procedures for a variety of animal hides.
Materials & Methods: Decontamination effectiveness was evaluated by contaminating animal hides before using various decontaminating methods to cleanse the hides. Tissue samples including horse, pig, goat, and sheep hides were contaminated in a glove box with a liquid solution comprised of uranium fission fragments. The radioactive liquid solution was chosen to simulate environmental radioactive contamination from fallout. Water rinsing, soap followed by water rinsing, vacuum systems, and commercially available decontamination gel were evaluated as potential decontamination methods. Samples were evaluated in the glove box using Geiger-Mueller and sodium iodide radiation detectors. Additionally, effluent material was evaluated to determine the success of the methods in removing specific isotopes. Each sample was decontaminated three times with each method to determine effectiveness per decontamination attempt.
Results: Of the procedures evaluated, commercial decontamination gel proved completely ineffective as a decontamination technique. The consistency of the gel was such that it could not be effectively removed from hide samples after application. All other techniques were more effective in removing contamination from the hides.
Conclusions: The use of water, soap and water, or a vacuum methods for external radionuclide removal from livestock is more effective than the use of decontamination gel.

Study of Spontaneous and Environmentally Induced Copy Number Variation and Possible Mechanisms

Jackie Stanton, Lucas Argueso, Ane Zeidler, Chris Puccia, Keerthi Vemulapalli

Chromosomal rearrangements resulting in Copy Number Variation(CNV) have long been recognized as contributing factors in carcinogenesis, and more recently in Autism Spectrum Disorders. The molecular mechanisms underlying the formation of CNVs are not completely understood. The goal of our research is to investigate the environmental factors and molecular mechanisms associated with spontaneous CNVs. We have developed, and are now using, a CNV detection assay using the budding yeast as a model system. Our CNV reporter contains two yeast genes, SFA1 and CUP1 that confer gene dosage-dependent tolerance to formaldehyde and copper, respectively. This system enables the detection of rare clones containing an amplification of the chromosomal segment containing the reporter by selection in media containing high levels of formaldehyde and copper. Results obtained under basal growth conditions indicated that most spontaneous CNV events are mediated through non-allelic homologous recombination(NAHR) between dispersed repetitive DNA sequences. The most abundant classes of CNVs observed involved segmental duplications and non-reciprocal translocations. However, we also detected insertional translocations(ITs) in about 2% of all the haploid CNV clones. Cases of ITs have been found at roughly the same frequency in patients referred for full cytogenetic evaluation, but their mechanism of formation is not yet understood. We are currently redesigning our yeast assay to be able to directly select for IT events and investigate their formation mechanism. Finally, in order to study the effect of environmental factors on CNV, cells were exposed to a low dose of three different known mutagens: Hydroxyurea(HU), Methyl Methanesulfonate(MMS), and Camptothecin(CPT); initial data has shown an increase in the CNV mutation rate due to these exposures. We are currently analyzing the spectra of chromosomal rearrangements induced by these exposures, and comparing them to the un-exposed pattern.

The effects of omeprazole therapy on bacterial colonization of the pharynx in healthy dogs

Lauren Sullivan, Justin Wakayama, Pedro Boscan, Doreene Hyatt, David Twedt, Michael Lappin

Purpose - To identify the commensal pharyngeal flora in healthy dogs and determine if flora is altered following omeprazole administration.
Materials and Methods – Prospective descriptive study. Three baseline pharyngeal swabs, collected 48 hours apart, were obtained from 8 Beagles. Omeprazole (1 mg/kg PO q24h) was administered for a total of 12 days. During omeprazole administration, 3 post-treatment pharyngeal swabs were obtained on Days 8, 10 and 12. All swabs were submitted for semi-quantitative aerobic and anaerobic culture. Growth of bacterial isolates, as well as genus of isolates, were compared between the pre-treatment (n=24) and post-treatment (n=24) swabs. The frequency of new pharyngeal isolates in dogs post-treatment was also evaluated.
Results- A total of 112 isolates grew in the pre-treatment samples, versus 116 isolates in the post-treatment samples. The most common pre-treatment isolates were Gram negative organisms (Pasteurella=43, E. coli=3), Gram positive organisms (Streptococcus=31, Staphylococcus=13, Bacillus=6), and anaerobes (Porphyomonas=6, Provotella=6). The most common post-treatment isolates were Gram negative organisms (Pasteurella=23, E. coli=7), Gram positive organisms (Streptococcus=36, Staphylococcus=22, Bacillus=12), and anaerobes (Peptostreptococcus=10, Provotella=4). An asymptotic equality test was performed on percentage of animals negative pre-treatment and then positive post-treatment. There was a significant (P
Conclusions and Clinical Relevance- Following omeprazole therapy, an increased frequency of Gram negative and anaerobic bacteria was observed. Further studies are warranted to determine the clinical significance of antacid therapy on pharyngeal flora in dogs.

Endothelial cell apoptosis, in vitro and in situ, as a component of the tumor control mechanism induced by stereotactic radiation therapy

Katy L. Swancutt, Susan M. LaRue

Stereotactic radiation therapy (SRT), in which large doses of ionizing radiation are administered in a precisely targeted fashion over one to five fractions, was previously thought to attain tumor control by selectively killing cells with fast replication rates, or clonogens. Recent studies have generated evidence supporting a new mechanism of tumor control, and combined with findings from our research group, we have called the previously established mechanism of clonogenic cell death into question. We suggest that the mechanism through which SRT effectively kills tumors is by radiation-induced endothelial cell apoptosis, leading to fatal changes in tumor microenvironment through damage to tumor microvasculature. The goal of this work is to obtain a basic understanding of endothelial cell apoptosis as a component of SRT. Cultures of endothelial cells were irradiated and dose-dependent survival, total cell death, and cell death specifically via apoptosis were quantified and compared to fibroblast cells. Also, apoptosis was evaluated via markers of endothelial cells in peripheral blood samples as well as in biopsy samples from a canine soft tissue sarcoma that had been treated with SRT.

Improving Rooster Sperm Cryopreservation: Effects of Alternative Cryoprotectants, Diluent and Straw Size on Cryosurvival

Kimberly M. Tarvis, Phillip H. Purdy, James K. Graham

Rooster sperm survive cryopreservation too poorly to be used in the poultry industry. Much of the damage is due to the sperm's inability to withstand osmotic stresses caused when cryoprotectants (CPA) are added and removed. When the CPA glycerol (Gly) is removed from sperm, an osmotic gradient forms since Gly permeates the plasma membrane more slowly than water, causing the cells to swell and even rupture. These experiments determined if replacing glycerol with low molecular weight CPAs that permeate the plasma membrane more quickly improves cryosurvival. Studies evaluating different diluents and straw sizes were also conducted to develop a cryopreservation protocol that could be used in the industry. In experiment 1, rooster sperm were frozen using 5 CPAs: Gly, methylformamide (MF), dimethylformamide (DMF), ethylene glycol (EG) and methylacetamide (MA), at 9% in a glutamate-based diluent (GD). Semen was packaged in 0.25mL straws, frozen in liquid nitrogen (LN) vapor and stored in LN. After thawing, motility was determined using a computer-assisted sperm analysis system and membrane integrity was determined using flow cytometry. Sperm frozen with Gly had higher motility (54%) and membrane-intact (58%) sperm than MA (47% and 52%, respectively, $p<0.05$); while DMF, EG and MF resulted in less than 45% motile and membrane-intact cells ($p>0.05$). In experiment 2, freezing sperm in two diluents, a trehalose-based diluent (TD) and GD using Gly and MA as the CPA, and in 0.25mL or 0.5mL straws was evaluated. Sperm frozen in TD + 9% MA in 0.5mL straw exhibited the higher motility (65%) than sperm frozen in TD + 9% Gly in 0.5mL straw (61%) or sperm frozen in GD (46-53%, $p<0.05$). In addition, sperm frozen in 0.5mL straws had higher motility than 0.25mL straws. In conclusion, rooster sperm can be effectively cryopreserved, when they are frozen in 0.5mL straws using TD with 9% MA. Future studies need to determine the fertilizing capacity of the frozen sperm.

Anti-microbial activity of Fuzhuan tea, a fermented preparation of Camellia sinensis (L)

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Purpose: Dark teas, such as Pu'er and Fuzhuan, are Camellia sinensis L. (Theaceae) that has undergone a microbial fermentation process. Reports suggest that the fermentation process used for production of Fuzhuan tea alters its phytochemical profile. Compounds commonly associated with the antioxidant and antimicrobial properties of tea are reduced in Fuzhuan tea compared to green teas. However, fermentation may also impart unique chemical components to the tea. The purpose of this study was to conduct a comparative metabolomic analysis of fermented Fuzhuan tea with green teas and identify anti-microbial activities and compounds that may result from fermentation. Methods: In this experiment we chemically analyzed water-soluble extracts from Fuzhuan and green tea leaves using ultra high pressure liquid chromatography-mass spectrometry (UPLC-MS).Whole crude extracts and polar and non-polar fractions were tested for their anti-microbial effects on Gram-negative and Gram-positive human pathogenic bacteria. Results: Fuzhuan and green teas had distinctly different chemical profiles. Identification of known tea polyphenols confirmed that some of these compounds were lower in Fuzhuan tea compared to green tea, but several compounds tentatively identified as flavonoids-glycosides and fatty acid amides were higher in Fuzhuan tea. Crude tea extracts had modest activity against Staphylococcus aureus and Shigella sonnei. Fractions from these extracts are being tested to pinpoint compound classes associated with antimicrobial activity. Conclusion: Initial studies support that fermentation alters the phytochemical content of Fuzhuan tea and tea extracts display antimicrobial activity. Further chemical characterization of active fractions is necessary to identify novel chemical compounds resulting from fermentation.

Osmotic Fragility and Flow Cytometric Determination of Lipid Peroxidation in Feline Erythrocytes

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Purpose: Develop assays to assess feline erythrocyte (RBC) membrane health. Oxidative stress resulting in membrane lipid peroxidation may contribute to a decrease in RBC lifespan in cats with chronic kidney disease (CKD). Antioxidant capacity in units of soluble vitamin E is significantly decreased in cats with CKD compared to controls (56 ± 21 vs. 81 ± 13, $p<0.005$) and one of the properties of vitamin E is a reduction in lipid peroxidation. Materials/Methods: RBC membrane osmotic fragility was measured in cats with a range of packed cell volumes (PCV) using a standardized protocol of exposure to NaCl dilutions. A standardized protocol for the flow cytometric determination of lipid peroxidation in feline RBCs was developed using the emission of the DHPE fluorophore, whose fluorescence attenuation inversely correlates with membrane lipid peroxidation. These assays were applied to feline RBC samples covering a variety of PCVs. Results: The varience of these assays was determined by running samples in triplicate, and the effect of sample dilution and storage time was determined. Linear regression gave an R-squared of 0.45 for the fit between PCV and the NaCl concentration producing 50% hemolysis. Linear regression gave an R-squared of 0.75 for the fit between PCV and the DHPE fluorescence. Conclusions: These assays may be measuring different aspects of feline RBC membrane health. The correlation between the assays will be determined, and the relationship between RBC membrane health, oxidative stress, and vitamin E antioxidant capacity will be investigated in anemic cats with CKD.

The evaluation of biochemical markers and an in vitro prion amplification assay for the diagnosis of CWD using cerebrospinal fluid

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Transmissible spongiform encephalopathies(TSEs), or prion diseases, are a uniformly fatal family of neurodegenerative diseases in mammals that includes chronic wasting disease (CWD) of cervids. The early and ante-mortem identification of TSE-infected individuals using conventional western blotting and immunohistochemistry has proven difficult, as the low level of detectable prions in readily obtainable samples are beyond the limits of detection of such traditional assays. This has necessitated the development of assays employing either amplification of the abnormal prion protein or detection of readily identifiable biomarkers for prion infection.
In the present study, we evaluated CSF from CWD-exposed or naïve whitetail deer: 1) using a modified Protein Misfolding Cyclic Amplification (PMCA) assay employing PTFE beads; and 2) for the presence of two biochemical markers of prion disease, ERK1/2 and 14-3-3. In PMCA, low levels of PrPCWD serve as a seed for the conversion of a substrate (transgenic Tg[CerPrP] mouse-origin PrPC) to levels detectable by conventional Western blotting (WB). Constitutively expressed 14-3-3 and ERK1/2 proteins, alternatively, have been demonstrated to be elevated in CSF samples of Creutzfeldt-Jakob patients, inflammatory CSF ischemic events, and paraneoplastic neurological disorders. Results from our study indicated that the modified PMCA assay may have the appropriate sensitivity and specificity needed to be used as a diagnostic tool for CWD. Further statistical analysis and research needs to be carried out on a greater number of samples to determine the suitability of this assay for either ante- or post-mortem CWD diagnosis. ERK1/2 and 14-3-3 proteins were detectable in the CSF of both naïve and CWD-exposed WTD and therefore they may not serve as prion disease-specific biomarkers in the CSF of WTD.

Combined Immuno-antimicrobial Therapy for the Treatment of Chronic Staphylococcal Infection

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Staphylococcus aureus is a common biofilm producing pathogen that is frequently involved in the development of nosocomial and community acquired infections. The emergence of new, highly resistant strains continues to impede efforts to reduce morbidity and mortality rates, necessitating the development of new therapeutic measures for defense against this pathogen. Biofilm formation serves as one of many important resistance mechanisms for the S. aureus organism, and is a major cause of post-operative complications in surgical patients. It is therefore of great interest to target specific components of the biofilm or biofilm bacterial proteins in the development of effective therapies. In vitro experiments performed in our laboratory have demonstrated a synergistic relationship between antimicrobials and immunotherapeutics when used in combination, resulting in increased clearance of planktonic bacteria. In the current studies, biofilm infections were induced in mice through subcutaneous implantation of S. aureus inoculated surgical mesh. Mice were treated with either pre-exposure subcutaneous administration of a vaccine composed of S. aureus biofilm antigens, oral administration of antibiotics (amoxicillin/clavulanic acid), or a combination of these treatments. In vivo bioluminescent imaging was performed to monitor the progression of the infection over time. Results indicated that mice receiving combination therapy responded significantly better in terms of bacterial clearance than those receiving antibiotics or vaccine alone. These studies strongly support the continued investigation of combined immuno-antimicrobial therapy protocols for the treatment of chronic staphylococcal infections.

Development of an indirect enzyme-linked immunosorbent assay for the detection of feline antibodies against Mycoplasma felis

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Purpose: The purpose of this study was to develop an indirect enzyme-linked immunosorbent assay (ELISA) for detection of antibodies against M. felis and to test this assay using sera collected from shelter cats with and without signs of upper respiratory tract disease (URTD).
Materials/methods: A checkerboard titration was performed to determine the optimal ELISA conditions using antigen isolated from a specific pathogen-free (SPF) cat exposed to SPF cats inoculated with M. felis. Sera from SPF cats prior to and after exposure to M. felis and shelter cats with and without signs of URTD were tested on the ELISA. Polymerase chain reaction (PCR) assay for M. felis was also performed on swabs from the shelter cats. Potential for M. felis antibody cross-reactivity was tested by creating an ELISA using Ureaplasma antigen under the same sensitization conditions as the M. felis assay.
Results: The optimal sensitization parameters for the M. felis ELISA included using a 1:1,600 dilution of antigen in carbonate buffer on an Immulon 2 plate (Thermo Scientific, Rochester, NY). Distinct differences between positive and negative control sera were most prominent using a 1:1,000 dilution of horseradish peroxidase-conjugated goat anti-feline antibody. The coefficient of variation for negative and positive absorbance values were 4.9% and 3.1%, respectively. There was a significant difference ($P = 0.0007$) between absorbance values from healthy and sick shelter cats, but the percent agreement with PCR was 36.5%. Using PCR as a comparison, the sensitivity and specificity of the ELISA was 100% and 19.5% respectively. There was no significant difference in absorbance values for paired sera run on the M. felis and Ureaplasma ELISAs.
Conclusions: This ELISA may be a useful epidemiological tool for determining seroprevalence of M. felis in cats with upper respiratory tract disease. Further work is required prior to using this assay in a clinical setting.

Identification and characterization of metastasis-related microRNAs in osteosarcoma

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Purpose: Recently, the role of microRNAs (miRNAs) as regulators of oncogenes and tumor-suppressor genes, and therefore regulators of the proteins involved in cancer progression and metastasis has emerged. The purpose of this study was to examine the miRNA expression profiles in 2 isogenic mouse osteosarcoma (OSA) cell lines with differing metastatic capacity, identify candidate miRNAs as potential novel diagnostic or therapeutic targets in OSA and characterize their functional roles in a highly metastatic mouse OSA cell line. Materials/Methods: A quantitative real-time PCR array was used to detect differentially expressed miRNAs in a highly metastatic mouse OSA cell line, DLM8 and the parental Dunn mouse OSA cell line. Total RNA was extracted from 4 biological replicates of each cell line and miRNA expression was evaluated using primers for all 709 known murine miRNAs. Relative expression levels were analyzed with a t-test of normalized values. Results: We identified a potential metastatic miRNA signature in mouse OSA in the highly metastatic DLM8 cell line. We identified 108 miRNAs that were differentially expressed in DLM8 cells compared to Dunn cells ($p<0.05$). The expression of both miR-218 and miR-322 was markedly downregulated in DLM8 cells. While some miRNAs such as miR-218 have a well-characterized association with cancer progression, others such as miR-322 appear to be unique to OSA and whose functions are unknown. Conclusions: Our results suggest that specific miRNAs including miR-218 and miR-322 may be involved in the metastasis of mouse OSA as candidate tumor-suppressive miRNAs. We are currently confirming specific roles of miR-218 in the metastatic phenotype by creating a DLM8 cell line that stably expresses miR-218 and evaluating its ability to regulate tumor cell invasion and migration. Further studies will identify the expression of miR-218-regulated target genes such as Runx2, a key transcription factor associated with osteoblast differentiation.

Development of microsphere immunoassays for the detection of domestic cat antibodies

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Microsphere immunoassays (MIAs) are capable of detecting multiple analytes simultaneously. Assay kits are commercially available for the quantification of various analytes, including cytokines, chemokines and antibodies; however, there are currently no kits for the domestic cat. Our objective is to develop MIAs for the quantitation of total IgG and IgA in domestic cat plasma samples, and determine the percentage of IgG and IgA antibodies that are feline immunodeficiency virus (FIV)-specific during experimental or naturally occurring FIV infection. Domestic cat reference serum, IgG and IgA antibodies, and recombinant FIV proteins are being used to develop these assays. The standard curve range of IgG is 1-125 ng/mL and IgA is 2-250 ng/mL. After development is complete, we will validate the assays to determine the accuracy and precision of the methods. These methods will allow precise quantitation of FIV-specific IgG and IgA kinetics in domestic cats infected with pathogenic and/or apathogenic FIV.