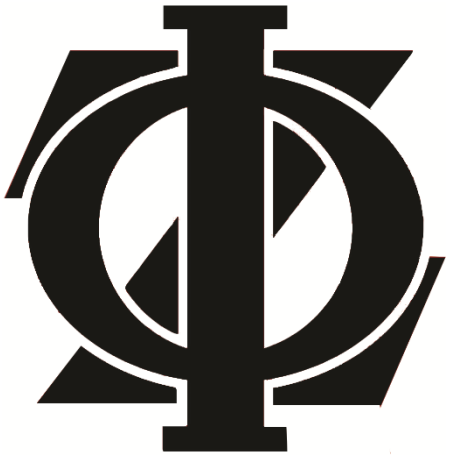
PROCEEDINGS

The 29th Annual

Phi Zeta Research Day

MICHIGAN STATE
UNIVERSITY

COLLEGE OF VETERINARY MEDICINE



Friday, October 4, 2019

8:30 a.m. to 4:30 p.m.

The College of Veterinary Medicine is pleased to welcome you and Dr. Jason Bartz to the 29th Annual Phi Zeta Research Day at Michigan State University. The College sponsors this colloquium to foster interactions in research and scholarship between students and faculty within the College, as well as with other colleges and departments on the MSU campus.

The program this year features poster and oral presentations, as well as an address from **Dr. Jason Bartz,** who is the Associate Dean for Academic and Faculty Affairs and Professor and Vice Chair of Medical Microbiology and Immunology at the *School of Medicine at Creighton University in Omaha, NE.* Dr. Bartz, is today's Jaqua Foundation Symposium keynote speaker, and is presenting "Environmental and host factors that contribute to prion strain emergence."

Dr. Bartz graduated from the University of Wisconsin with a PhD in virology. He is a pioneer in prion disease pathogenesis and biology prion strain emergence and interference. His group investigates - alternative routes of prion entry into the host to better define the possible pathways taken by prions to gain access to the central nervous system (CNS); the role of the innate immune system in processing and transport of prions to secondary lymphoreticular system tissues; and the factors that influence susceptibility of neurons to prion infection and/or replication. He is an established investigator in virology and prion biology.

The MSU College of Veterinary Medicine realizes the importance of biomedical research for the advancement of science, animal and public health, and the veterinary profession. In this spirt, the College invites you to explore some of its current affiliated research at Phi Zeta Research Day.

ACKNOWLEDGMENTS

The following individuals have contributed their time and effort to ensure the success of our forum:

Dalen Agnew
Evan Brenner
Anum Hadi
Colleen Hegg
Susan Holcombe
Emily Lenhard
Dimity Palazzola
Ashley Russell
Srinand Sreevatsan
Jaimie Strickland
Arun Varun
Colette Zulu

We are grateful to the support given by all of the College of Veterinary Medicine's departments and units. We would also like to acknowledge all of our volunteer judges and moderators. Thank you!

This year's winner of the Zoetis Award for Veterinary Research Excellence is Dr. Adam Moeser, Matilda R. Wilson Chair, Department of Large Animal Clinical Sciences. The award recognizes researchers whose innovative studies over the last few years have made significant scientific advances.

Centered at the intersection of human and animal health, Dr. Moeser's research focuses on mechanisms of stress-related gastrointestinal (GI) and immune disease risk in animals and people. His research on how early life stress can program the long-term development and function of the intestinal barrier and immune system function in pigs and mice has established a fundamental basis for increased disease risk across the lifespan. This research was recently highlighted as one of the most significant discoveries in the North American pork industry in the past decade and is now being integrated into practice across the swine industry. Dr. Moeser's fundamental research on early life origins of sex differences in the mast cells represents a paradigm shift in the understanding of sex-biased diseases and has formed a foundation for current research in precision medicine relevant to humans and animals. Since 2008, Dr. Moeser's research program has been continually funded by the National Institutes of Health, USDA and industry.

He earned his BS degree from the University of Massachusetts at Amherst and his DVM, MS and PhD from North Carolina State University with joint post-doc fellowship/training at UNC-Chapel Hill and Duke University. Dr. Moeser will present an overview of his current research program today in "Early life adversity on GI immune disease risk across the lifespan" as he accepts the Zoetis Award for Veterinary Research Excellence.

PARTICIPANTS IN THE 2019 SUMMER RESEARCH PROGRAMS

We would like to recognize the students, mentors and the funding sources of our 2019 summer research programs.

Student	Class		Mentor
NIH-Sponsored DVM Students			
	Class	Program	
Kaitlyn Bailey	2022	T35	Jane Manfredi
Olivia Child	2022	T35	Cheryl Swenson & Vilma Yuzbasiyan-Gukan
Carmen-Maria Garcia	2023	T35	Shannon Manning
Taneisha Griggs (Purdue)	2023	R25	Dana Spence
Kimberly Guzman	2023	R25	Cheryl Rockwell
Olivia Lefere	2022	T35	Cheryl Swenson & Vilma Yuzbasiyan-Gurkan
Shelbey Moore	2022	T35	Srinand Sreevatsan
Anna Mukhina	2022	T35	Bonnie Harrington
Nicole Mulready	2021	T35	Linda Mansfield
Hailey Penticoff	2023	R25	Norbert Kaminski
Sara Saikalis	2021	T35	Colleen Hegg
Rachel Sheffler	2022	T35	Margaret Petroff
Taylr Wells	2022	T35	Srinand Sreevatsan
Noah Wolinski	2022	T35	Andras Komaromy
NIH-Sponsored Undergraduate Students			
Maisah Akram	R25		Richard Neubig
Jade Gmitter	R25		Ning Li
Jazmin Johnson (North Carolina A&T University)	R25		Jim Luyendyk
Marlin McKnight (Winthrop University)	R25		Jim Wagner
Sophia Ono-Korkowski (Queen's University of Charlotte)	R25		John LaPres
Yan Pacheco (University of Florida)	R25		Richard Neubig
Amari Parris (Morehouse College)	R25		Adam Lauver
Christina Straham (UM-Flint)	R25		Anne Dorrance
Boehringer Ingelheim/MSU Graduate School Scholars			
Joanna Acosta	2021		Jane Manfredi
Patrick Crannell	2022		Ronald Erskine
Sydney Dudley	2022		Hans Cheng
Ashley Hetak	2022		Brian Petroff
Tim Lin	2022		Vilma Yuzbasiyan-Gurkan
Kellie Rizzolo	2021		Angel Abuelo

Student	Class	Mentor
Bridget Walker	2021	Ronan Eustace
Boehringer Ingelheim Scholar		
Annika-Celine Hollender (University of Veterinary Medicine Hannover)	2021	Stephanie Valberg
NIH T35/Brewer Fund Scholar for Campus Animal Resources		
Joshua Kim	2022	Claire Hankenson
Cincinnati Zoo's Center for Conservation and Research of Endangered Wildlife (CREW)		
Hailee Butler	2021	Dalen Agnew

JAQUA FOUNDATION SYMPOSIUM
IN MEMORY OF DR. SAMUEL POLLOCK

The keynote speaker this year is sponsored by the Jaqua Foundation Symposium in Memory of Dr. Samuel Pollock, which honors the name and career of an esteemed alumnus of Michigan State University's College of Veterinary Medicine, Samuel Pollock, DVM.

In 1996, MSU conferred the Distinguished Veterinary Alumnus Award upon Dr. Pollock, citing his remarkably productive career.

Upon graduation in 1941, Dr. Pollock served with the Bureau of Animal Industry as a cattle tester. After spending nearly four years stationed in the desert region of Ahwaz during World War II, he returned home and established the South Orange Animal Hospital in South Orange, New Jersey. He practiced there for more than 40 years, ably assisted by his beloved wife, Madalyn.

In 1950, he and a group of like-minded individuals established the Metropolitan New Jersey Veterinary Medical Association, which eventually became the largest active group in the state. Dr. Pollock was a strong advocate of continuing education, attending hundreds of such courses himself and developing new and exciting programs for other veterinarians. Among other things, he became keenly interested in molecular genetics.

The monthly meetings of the veterinary association provided him with the stimulus for documenting challenging cases, and he eventually published 35 papers. One such paper led to his appointment as veterinary consultant for research facilities at the Newark Beth Israel Medical Center, a relationship that lasted for 20 years.

Dr. Pollock's activities also earned him the 1972 Practitioner of the Year Award from the New Jersey State Veterinary Medical Association and the 1973 AVMA Practitioner's Research Award. A photographic essay he published won a medal at the XXI World Veterinary Congress in 1979.

His interest in the humane care of research animals attracted the attention of the Jaqua Foundation, a philanthropic organization, which named him as a trustee in 1982. From this position, he helped carry out the founder's wishes to benefit the veterinary profession and animals.

To honor his memory, the Jaqua Foundation established an endowment at MSU to sponsor symposia that feature leaders, scientists, and scholars from the veterinary profession and the biomedical community. Dr. Jason C. Bartz, PhD and Associate Dean, Academic and Faculty Affairs, Vice Chair of Medical Microbiology and Immunology and Professor at the School of Medicine at Creighton University will present *"Environmental and host factors that contribute to prion strain emergence"* as our Jaqua Foundation Symposium Speaker in Memory of Dr. Samuel Pollock.

2018 Phi Zeta Research Day Award Recipients

Judging Category	Best Oral Presentation	Best Poster Presentation
DVM Professional Student	Kennedy Aldrich	Cailin Harro
Resident, Intern, or MS Graduate Student	Danielle Marturello	Casandra Larrivee
PhD Graduate Student	Kevin Baker	Emily Mackey
Undergraduate Student	Terry Everett	Cassidy Harris

29th ANNUAL PHI ZETA RESEARCH DAY

College of Veterinary Medicine
Michigan State University
Veterinary Medical Center

October 4, 2019

PROGRAM

Time	Event	Location
8:00	**Registration begins**	Second Floor Lobby of G-Building
8:00 – 12:30	**Assembling of posters**	G-201 (Reading Room)
8:15-10:15	**RIMS Student Oral Presentations** (see detailed presentations schedule)	A-214 (Buchanan Room)
9:45-11:45	**DVM Student Oral Presentations** (see detailed presentations schedule)	G-150
10:30-12:00	**PhD Student Oral Presentations, AM Session** (see detailed presentations schedule)	A-213 (Cafeteria)
1:30-3:15	**PhD Student Oral Presentations, PM Session** (see detailed presentations schedule)	A-213 (Cafeteria)

11:45- 12:30	Boxed lunches will be available outside of G-150.

1:30 – 3:30 **POSTER VIEWING in G-201 (Reading Room)**

1:30-2:30	Authors of odd-numbered posters will be present at their posters
2:30-3:30	Authors of even-numbered posters will be present at their posters

Zoetis Award and Keynote Address
G-150 Vet Med Ctr

12:00 – 12:15 **G-150**

Presentation of Zoetis Award
for Veterinary Research Excellence

Adam J. Moeser, DVM, MS, PhD
Matilda R. Wilson Chair,
Department of Large Animal Clinical Sciences,
College of Veterinary Medicine
Michigan State University

"Early life adversity on GI immune disease risk across the lifespan"

12:15-1:15 **G-150**

JAQUA FOUNDATION SYMPOSIUM KEYNOTE SPEAKER

"Environmental and host factors that contribute to prion strain emergence"

Jason Bartz, PhD
Associate Dean, Academic and Faculty Affairs,
Vice Chair of Medical Microbiology and Immunology and Professor at the School of Medicine at Creighton University

4:00-4:15 **G-150**

Presentation of Phi Zeta Student Research Day Awards

2019 Phi Zeta Poster Presentations

Presenter's Last Name	Presenter's First Name	Poster #	Category*	Mentor	Title
Acosta Becosme	Joanna	1	1	Jane Manfredi	DEK in canine cruciate ligament disease and immune mediated polyarthritis: a potential therapeutic target
Cates	Angelica	2	1	Jennifer Haupt	A comparison of heating devices for maintaining body temperature in anesthetized marmosets
Child	Olivia	3	1	Vilma Yuzbasiyan-Gurkan	Limiting feline leukemia virus proviral DNA using BET inhibitor IBET-762
Garcia	Carmen-Maria	4	1	Shannon Manning	Role of intramammary ceftiofur on the resistant bacterial populations in the gut of dairy cattle
Guzman	Kimberly E.	5	1	Cheryl E. Rockwell	The effect of two food additives in ovalbumin-elicited food allergy mouse model
Lefere	Olivia	6	1	Cheryl Swenson & Vilma Yuzbasiyan-Gurkan	Detection of feline leukemia virus proviral DNA in cats with lymphoma
Hetak	Ashley	7	1	Brian Petroff	Serum levels of insulin-like growth factor-1 in canines with primary hypothyroidism and non-thyroidal illness
Lin	Kuan-Ting	8	1	Vilma Yuzbasiyan-Gurkan	Evaluation of key oncogenic pathways in histiocytic sarcoma in Bernese Mountain Dogs
Penticoff	Hailey	9	1	Norbert Kaminski	Expression of toll-like receptors in human primary astrocytes
Mukhina	Anna	10	1	Bonnie Harrington	Expression of SETD2 and miR106b-5p in canine diffuse large B-cell lymphoma
Mulready	Nicole	11	1	Linda Mansfield	Analyzing the fecal microbiome in canine lymphoma patients undergoing a modified CHOP chemotherapy protocol
Moore	Shelbey	12	1	Srinand Sreevatsan	Protease activity of *MAP* MarP across pH conditions
Rizzolo	Kellie	13	1	Ángel Abuelo	Using metabolic stress biomarkers for prediction of colostrum quantity and quality in dairy cattle
Saikalis	Sara	14	1	Colleen Hegg	Diesel exhaust particle induced toxicity on neurons derived from healthy and Alzheimer's diseased humans

*Category: [1]DVM professional student; [2]Resident, intern, or MS graduate student; [3]PhD graduate student; [4]Undergraduate student; [5]Other member of the CVM Community

Presenter's Last Name	Presenter's First Name	Poster #	Category*	Mentor	Title
Sheffler	Rachel	15	1	Margaret Petroff	Characterization of pregnancy-associated changes in naïve human and rhesus macaque T cell populations
Walker	Bridget	16	1	Ronan Eustace	Validation of chemiluminescent assays for measurement of canine TSH in red pandas (*Ailurus fulgens fulgens*)
Wells	Taylr	17	1	Srinand Sreevatsan	Identification of *Mycobacterium tuberculosis* complex specific biomarkers in naturally infected Asiatic Elephants in Nepal
Wolinski	Noah	18	1	Andras Komaromy	Ocular biometry and retinoscopy of ADAMTS-10-mutant dogs
Parales	Jair	19	2	Andres Contreras	Oleic acid supplementation reduces fatty acid catabolism in adipose tissue from early lactation dairy cows
Jones	Sarah	20	2	Nyssa Levy	Evaluation of the respiratory adjusted shock index in dogs diagnosed with hemoperitoneum
Wilson	Neco	21	2	Adam Moeser	Weaning and postnatal age influence the early time course and nature of intestinal mast cell activation in a porcine model of early life adversity
Ahmed	Husnain	22	3	Linda Mansfield	*Campylobacter jejuni* mediates reduced gut microbial diversity during pathogenesis of colitis in a mouse model
Bahal	Devika	23	3	Rupali Das	ADAP is critical for iNKT cell homeostatic proliferation and survival
Bunn	Marcus	24	3	Dana Spence	Emerging role of C-peptide on neutrophils
Chirivi	Miguel	25	3	Andres Contreras	LPS induces lipolysis and reduces insulin sensitivity in adipose tissue from dairy cows
Hadi	Syeda Anum	26	3	Srinand Sreevatsan	Application of pathogen-specific biomarkers to enhance specificity of bovine TB diagnosis
Jacobs	Monica	27	3	Dana Spence	Exploring albumin-zinc binding under diabetic conditions
Kobbekaduwa	Vishvapali	28	3	Jean Tsao	Distribution of *Anaplasma phagocytophilum* in Michigan in questing black-legged ticks from 2018
Kuszynski	Dawn	29	3	Adam Lauver	A comparative analysis of clopidogrel and DT678 on the vasculature
Maradiaga	Nidia	30	3	Adam Moeser	The lifelong effects of early life adversity on bone marrow-derived mast cell progenitors

*Category: [1]DVM professional student; [2]Resident, intern, or MS graduate student; [3]PhD graduate student; [4]Undergraduate student; [5]Other member of the CVM Community

Presenter's Last Name	Presenter's First Name	Poster #	Category*	Mentor	Title
Yang	Ya-Ting	31	3	Vilma Yuzbasiyan-Gurkan	A novel therapeutic strategy for osteosarcoma: targeting c-MYC degradation
Akram	Maisah	32	4	Richard Neubig	Inhibition of PLK, AURK, and tubulin in BRAF-i resistant melanoma
Calhoun	Philip	33	4	Janani Ravi	A computational approach to identify unique pathogenic miRNA signatures in infected hosts
Faryean	Joe	34	4	Alexandra Nelson	Treatment-related changes in striatal activity in a mouse model of Parkinson's Disease
Gmitter	Mikayla Jade	35	4	Ning Li	Does organic chemicals in PM2.5 and vapor-phase pollutants influence the induction or exacerbation of lung tumors?
Kos	Noah	36	4	Jessica Fortin	Small molecules break down islet amyloid polypeptide fibrils
Leary	Kelly	37	4	Andras Komaromy	Topically administered netarsudil and latanoprost ophthalmic solution (Rocklatan™) in normal and glaucomatous dogs
Liddicoat	Amanda	38	4	Simon Petersen-Jones	Exclusion of progressive retinal atrophy candidate genes in two breeds of dogs
Ramsey	Harrison	39	4	Paige Winkler	Identification of a novel mutation causing progressive retinal atrophy in Spanish Water Dogs
Raphtis	Vanessa	40	4	Andras Komaromy	Diurnal intraocular pressures (IOPs) in dogs with ADAMTS10-open-angle glaucoma (ADAMTS10-OAG)
Shareef	Sarah	41	4	James Wagner	Endotoxin-induced mucous cell metaplasia is dependent on innate lymphoid cells in mice
Smith	Madison	42	4	Andres Contreras	Uterine caruncle immune phenotype is modulated by colony stimulating factors in dairy cows
Tindle	Ashleigh	43	4	James Wagner	Livestock-derived PM2.5 dust exposure modulates allergic inflammatory responses in mice
Duque-Wilckens	Natalia	44	5	Adam Moeser	Mast cells as mediators of early-life adversity effects on adult neuroinflammation and behavior
Velez-Irizarry	Deborah	45	5	Stephanie Valberg	Upstream regulators of gene expression influencing glycogen repletion

*Category: [1]DVM professional student; [2]Resident, intern, or MS graduate student; [3]PhD graduate student; [4]Undergraduate student; [5]Other member of the CVM Community

29th ANNUAL PHI ZETA RESEARCH DAY

Oral Presentations Schedule

Session	Buchanan Room (A-214 VMC) **Residents, Interns, & MS Students** **Moderator:** **Dr. Patrick Venta**
8:15-8:30	**Chima Maduka[2, 3]** Intranasal Deformities in Dogs: Comparative Diagnostic and Functional Imaging (Bryden Stanley)
8:30-8:45	**Jess Burn[2]** Aqueous Angiography *ADAMTS10*-Mutant Dogs Before and After Development of Glaucoma (Chris Pirie)
8:45-9:00	**Marisa Henry[2]** Impact of Antioxidant Supplementation of Muscle Proteome and Glutathione in Thoroughbreds (Stephanie Valberg)
9:00-9:15	**Lindsey Johnson[2]** Multicenter Placebo-Controlled Randomized Study of Ethyl Pyruvate in Horses Following Surgical Treatment for Large Colon Volvulus (Susan Holcombe)
9:15-9:30	**Tereza Stastny[2]** Risk Stratification in Dogs with Septic Soft Tissue Infections (Nyssa Levy)
9:30-9:45	**Megan Porter[2]** Relative Agreement of Two Surveillance Systems for *Borrelia burgdorferi* in an Emergent State (Jean Tsao)
9:45-10:00	**Julie Pfeifer[2]** Retrospective Evaluation of the Use of Diuretics in Acute Kidney Injury in Dogs (Nyssa Levy)
10:00-10:15	**Rajab Curtis[2]** Screening and Identification of Novel Plant-Inspired Anti-Microbial and Anti-Cancer Agents (Victor DiRita)

29th ANNUAL PHI ZETA RESEARCH DAY

Oral Presentations Schedule

Session	G-150 VMC DVM Students **Moderators:** **Dr. Jane Manfredi** **Dr. Jean Tsao**
9:45-10:00	**Sydney Dudley[1]** Generation of Inducible CRISPR Interfering System for study of Marek's Disease (Hans H. Cheng)
10:00-10:15	**Emma Stapley[1]** Effect of Stirrup Iron Style on Force Exerted on the Stirrup and Rider Position (Jane Manfredi)
10:15-10:30	**Kaitlyn Bailey[1]** Effects of Wrapping on Skin Colony Forming Units from Common Equine Joint Injection Sites (Jane Manfredi)
10:30-10:45	**Sarah Rich[1]** Comparison of Three Methods to Measure Heart Rate in a Canine Rehabilitation Setting (Sarah Shull)
10:45-11:00	**Joshua Kim[1]** Pre-op Warming Effects on Body Temperature and Anesthetic Recovery in a Rat Surgical Model (Claire Hankenson)
11:00-11:15	**Makenzie McDowell[1]** Evaulation of the Effects of Alpha-Casozepine on Cats Exhibiting Anxiety in the Shelter Setting (Marie Hopfensperger)
11:15-11:30	**Hailee Butler[1]** Hepatic Iron Overload Disorder in Captive Rhinoceros (Dalen Agnew)
11:30-11:45	**Patrick Crannell[1]** Developing an Algorithm to Detect Subclinical Mastitis in Automatic Milking Dairy Herds (Ron Erskine)

29th ANNUAL PHI ZETA RESEARCH DAY

Oral Presentations Schedule

Session	Cafeteria (A-213 VMC) PhD Students AM Session Moderator: Dr. Gisela Hussey
10:30-10:45	**Carsten Walker[3]** Bioactivity of the Endocannabinoid Anandamide in Cultured Bovine Endothelial Cells (Lorraine Sordillo)
10:45-11:00	**Fernanda Miyagaki Shoyama[3]** Elucidating the Regulon of a Fur-Like Protein in *Mycobacterium avian* subsp. *Paratuberculosis* (Srinand Sreevatsan)
11:00-11:15	**Yao Lee[3]** Characterization of Feline Herpesvirus-1 Deletion Mutants in Tissue Explant Cultures (Gisela Hussey)
11:15-11:30	**Jaimie Strickland[3]** Milk Production and Disease are Associated with Select Fat-Soluble Vitamins in the Periparturient Period (Lorraine Sordillo)
11:30-11:45	**Matthew Kuhn[3]** *In vitro* Evaluation of Vitamin E Analogs as Ancillary Antioxidants in Dairy Cattle (Lorraine Sordillo)
11:45-12:00	**Hinako Terauchi[3]** Identifying Risk and Protective Gut Microbiomes and Metabolomes in a Mouse Model of Asthma (Linda Mansfield)

29th ANNUAL PHI ZETA RESEARCH DAY

Oral Presentations Schedule

Session	Cafeteria (A-213 VMC) PhD Students PM Session Moderator: Dr. John Fyfe
1:30-1:45	**Kennedy Aldrich[3, 1]** Proteomic and Transcriptomic Analyses of Horses with Recurrent Exertional Rhabdomyolysis (Stephanie Valberg)
1:45-2:00	**Christopher Kellogg[3]** Impact of Bovine Leukemia Virus on Dairy Cattle Lymphocyte and ELISA Status Over a Lactation Cycle (Bo Norby)
2:00-2:15	**Holden Hutchinson[3]** Longitudinal Analysis of Diagnostic Measures of Bovine Leukemia Virus Infections (Bo Norby)
2:15-2:30	**Ivon Moya-Uribe[3]** Fecal Microbiota from Infants Increased Allergic Responses to House Dust Mite in a Mouse Model (Linda Mansfield)
2:30-2:45	**Paulo Carneiro[3]** Molecular Characterization of *Mycobacterium bovis* in Cattle and Buffalo in Amazon Region, Brazil (John Kaneene)
2:45-3:00	**Azam Sher[3]** Motility Mutations and Loss of the σ54 Regulon During Experimental Evolution of *Campylobacter jejuni* (Linda Mansfield)
3:00-3:15	**Nathan Kauffman[3]** Comparing Truncated Proteins with AnnexinV for Targeting Apoptotic Cancer Cells (Kurt Zinn)

DEK IN CANINE CRUCIATE LIGAMENT DISEASE AND IMMUNE MEDIATED POLYARTHRITIS: A POTENTIAL THERAPEUTIC TARGET

Joanna Acosta Bencosme[1], Vilma Yuzbasiyan-Gurkan[2], Karen Perry[2], Loïc M. Déjardin[2], David M. Markovitz[3] and Jane M. Manfredi[1]. 1) Pathobiology and Diagnostic Investigation, 2) College of Veterinary Medicine, Michigan State University; 3) Internal Medicine, University of Michigan Medical School

Inflammatory diseases, such as cruciate ligament disease (CCLD) and immune mediated polyarthritis (IMPA), are painful clinical diagnoses in canine veterinary medicine. Treatments often involve the use of generalized immunosuppressive and anti-inflammatory drugs that may threaten the quality of life of patients. DEK is a protein recently implicated in the pathogenesis of inflammatory diseases which is crucial in the formation of neutrophil extracellular traps (NETs) across species. The presence of DEK and NETs in dogs remains unknown, but could serve as a new, local target for safer therapy. We hypothesize that dogs with inflammatory conditions will have higher number of NETs and other inflammatory markers when compared to controls. Twelve client owned dogs (ten CCLD, two IMPA) and one healthy control were enrolled. Synovial fluid (SF) collected at the time of surgery or clinical examination was evaluated via Western blotting analysis for citrullinated histone H3 (a marker for NETs) and DEK. Semi-quantitative methods were used to compare presence of NETs via a one-way ANOVA test with a Tukey post hoc test (significant at $P < 0.05$). NETs were present in higher amounts in the SF of dogs with either CCLD or IMPA when compared to control. We anticipate to see similar results when evaluating for the presence of other inflammatory markers in SF of dogs with either condition. The presence of DEK and NETs in canine patients with either CCLD or IMPA could serve as a novel target for therapy that can help develop safe, more localized treatments for both.

CAMPYLBACTER JEJUNI MEDIATES REDUCED GUT MICROBIAL DIVERSITY DURING PATHOGENESIS OF COLITIS IN A MOUSE MODEL

Husnain Ahmed [3], Hinako Terauchi [2], Keenan M. O'Dea [2], Jean M. Brudvig [3], Daniel J. Claiborne [2], Julia A. Bell [1,3], Linda S. Mansfield [1,2,3] 1) Large Animal Clinical Sciences ,2) Microbiology and Molecular Genetics 3) College of Veterinary Medicine

Interleukin 10 deficient mice are a model for investigating inflammatory bowel disease. When exposed to the foodborne pathogen *Campylobacter jejuni*; IL-10 deficiency blocks inflammation-suppressive mechanisms in the gut, resulting in colitis. In a recent experiment, when IL-10 deficient BALB/c and C57BL/6 mice were inoculated with the colitogenic strain *C.jejuni* 11168*,* BALB/c mice developed colitis while those of the C57BL/6 background failed to develop colitis. This result suggested protection from inflammation in the infected C57BL/6 IL-$10^{-/-}$ mice. A *Lactobacillus murinus* strain was isolated from infected C57BL/6, but not BALB/c mice correlating with protection. Thus, we hypothesized that *L. murinus* mitigates *C. jejuni* induced colitis in mice. To test this hypothesis, BALB/c IL$10^{-/-}$ mice were used in 4 groups: 1) sham-inoculated, 2) *L. murinus* only, 3) *C. jejuni* 11168 only, and 4) *L. murinus* inoculation 32 days before *C. jejuni* challenge. At day 30 post- *C. jejuni*-challenge, all mice were humanely euthanized, and colon tissues were collected to assess *C. jejuni* colonization and to score for colitis by histopathology. Mice given *C. jejuni* 11168 alone or with *Lactobacillus* had severe colitis, thus *L. murinus* did not protect against colitis. Interestingly, 16S sequence analysis of cecal DNA revealed that bacterial taxonomic diversity was significantly lower ($P<0.001$) in both *C. jejuni* infected groups compared to the *Lactobacillus* only and negative control groups. Thus, understanding how *C. jejuni* mediates drops in diversity of the microbiome could lead to strategies for protection based on maintaining robust microbial communities.

INHIBITION OF PLK, AURK, AND TUBULIN IN BRAF-I RESISTANT MELANOMA

Maisah Akram[1], Sean Misek[1,2] and Richard Neubig[1,2,3], 1) Pharmacology and Toxicology, Michigan State University

Melanoma is the deadliest form of skin cancer and results in approximately seven thousand deaths annually. More than 50% of human melanoma tumors contain mutations in the BRAF gene, most mutations occur in valine 600. While BRAF-mutant tumors are initially responsive to BRaf inhibitors, most tumors develop resistance within months or years. In our prior work we discovered that BRAFi-resistant melanoma cells are more sensitive to inhibitors of the mitosis-associated kinases PLK and AURK. In an effort to understand why, we engineered a system which allows for the observation of mitosis in real time in both the drug resistant cells and the parental non-resistant cells. We hypothesize that the resistant cells have inherently dysregulated mitotic progression which we hypothesize would make the resistant cells more sensitive to inhibitors which disrupt mitotic progression. Melanoma cell lines were engineered to express GFP-tubulin which labels the mitotic spindle and Scarlet-histone 2A (H2A) to label the chromosomes. Fluorescent live-cell microscopy is used to visualize and track cells undergoing mitosis. In our preliminary experiments, I developed methodology to perform live-cell imaging of these cells with images taken at two-minute intervals. While the parental cells and the resistant cells were equally sensitive to inhibition of successful mitosis, the parental cells exhibited an ability to exit from a stalled mitosis and survive while the resistant cells fragmented their nuclei and died. Characterizing compounds that are selective for resistant cells may yield new mechanisms to prevent or reverse drug resistance in melanoma.

PROTEOMIC AND TRANSCRIPTOMIC ANALYSES OF HORSES WITH RECURRENT EXERTIONAL RHABDOMYOLYSIS

Kennedy Aldrich, Deborah Velez-Irizarry, Clara Fenger and Stephanie Valberg. Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University.

Recurrent exertional rhabdomyolysis (RER) in Thoroughbred racehorses causes muscle contracture and pain with exercise preventing consistent training. A definitive cause for RER has not been found, however, research supports a role for intramuscular calcium dysregulation. We hypothesized that analysis of gene and protein expression in skeletal muscle from control and affected horses housed in the same race training environment would elucidate the molecular mechanisms at play in horses with RER. Using RNA and protein isolated from muscle samples, we identified differentially expressed genes and proteins between control and RER horses and identified significantly enriched gene ontology (GO) pathways incorporating these genes and proteins. We found 812 differentially expressed (DE) genes and 125 DE proteins in RER vs control horses. Gene ontology (GO) pathway enrichment analysis for cellular components identified pathways for sarco(endo)plasmic reticulum that were upregulated in both transcriptomic and proteomic datasets. The calcium release channel RYR1, which is responsible for malignant hyperthermia, had significant DE in transcriptomic (decreased vs control) and proteomic datasets (increased). Cellular component pathways involving mitochondrial energy metabolism were upregulated in transcriptomic and down regulated in proteomic analyses. The opposing direction of DE in transcriptomic and proteomic data in mitochondrial pathways suggests that there may be transcriptional compensation (up-regulation) for detrimental changes occurring at the proteomic level, emphasizing the importance of integrating transcriptomic and proteomic data.

ADAP IS CRITICAL FOR INKT CELL HOMEOSTATIC PROLIFERATION AND SURVIVAL

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Invariant natural killer T (iNKT) cells are innate T lymphocytes that play pivotal role in modulating immune response in cancer, infection and inflammation. However, the mechanisms that regulate iNKT cell development and functions are not fully known. Our previous studies have established a critical role for SAP (SLAM-associated protein) and the tyrosine kinase Fyn in iNKT cell development. As ADAP (adhesion and degranulation promoting adaptor protein) is a known binding partner of Fyn, we hypothesized that ADAP regulates iNKT cell development. However, we found that ADAP is dispensable for iNKT cell development but is required for homeostatic maintenance of iNKT cells in peripheral organs such as the liver. To investigate the role of ADAP in peripheral maintenance, we used C57BL/6 (B6) and ADAP-deficient (*Adap*$^{-/-}$) mice. Using flow cytometry, we examined the expression of surface receptors (CXCR6, CD122, and CD127) as well as intracellular proteins (BcL-2 and BcL-xL) that are known to promote iNKT cell homing to the periphery and/or promote survival. We also examined for iNKT cell proliferation and apoptosis *in vivo* by incorporation of BrDU and annexin staining respectively. We observed that both B6 and *Adap*$^{-/-}$mice express comparable levels of CXCR6, CD127, CD122, Bcl-2 and Bcl-xL. Strikingly, iNKT cells from the livers of *Adap*$^{-/-}$ mice exhibit significantly reduced BrDU incorporation but increased annexin staining in comparison to those from B6 mice. Collectively, these results suggest that ADAP regulates iNKT cell peripheral maintenance by modulating their proliferation and survival.

EFFECTS OF WRAPPING ON SKIN COLONY FORMING UNITS FROM COMMON EQUINE JOINT INJECTION SITES

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Joint injection for treatment of osteoarthritis is common practice in equine medicine. Introducing bacteria in concentrations above 10^5 bacteria/gram of tissue inadvertently as part of the procedure can cause a potentially lethal joint infection. Wrapping the joint after injection could prevent contamination or could cause adverse effects. To observe the effect that wrapping has on colony-forming units (CFU) on the surface of a joint injection site, six horses had four joints (bilateral front distal interphalangeal joints and tarsi) swabbed within a 1" x 1" outlined square before (PRE) and after (SCRUB) being aseptically prepared. The animals were randomly assigned which two legs, front and hind, were wrapped. The horses were stalled overnight, the wraps removed, and the outlined site was swabbed again (POST). Swabs were cultured in order to quantify CFU's for comparison between sites. A one way ANOVA with Tukey's post hoc test was performed (significant at $P< 0.05$). Our results showed the mean ± SD CFU's for PRE was 2,621 ±2,881 (front) and 136 ±87 (hind), SCRUB CFU's were 9 ±20 (front) and 0 (hind), and POST CFU's were 320 ±451 (front, wrapped), 3,036 ±4,074 (front, unwrapped), 1,620 ±3,270 (hind, wrapped) and 283 ±379 (hind, unwrapped). No significant differences between mean CFU's for either wrapped/unwrapped sites or PRE/POST were noted. Based on these results, joint wrapping does not appear to significantly decrease site contamination, as all values correlate to below the 10^5 bacteria/gram of tissue threshold, allowing equine practitioners to forgo wrapping as a safety precaution.

EMERGING ROLE OF C-PEPTIDE ON NEUTROPHILS

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Neutrophils are the most abundant immune cell in the body and are the first responders to infections. Important neutrophil functions include chemotaxis, migration, and bactericidal function. However, in individuals with diabetes, key functions do not occur properly. Individuals with diabetes are not only at a higher risk for infection, but the severity of the infections also increases. It is hypothesized that neutrophils in a diabetic environment cannot uptake glucose properly leaving them energy depleted. C-peptide is a 31-chain amino acid peptide produced in the pancreatic beta cells. Previous work in the Spence lab has shown that interactions with C-peptide increase glucose absorption into, and ATP release from, red blood cells. However, we have also shown that for C- peptide to interact with RBCs to elicit a biological response, both Zn^{2+} and albumin are necessary. Preliminary work has been performed showing that C-peptide also binds to neutrophils in a specific manner. Here, we show C-peptide interactions with neutrophils in the presence or absence of albumin and Zn^{2+}. Neutrophils were collected from whole blood samples from consenting adults and separated by immunomagnetic separation. Neutrophils were incubated with varying combinations of C-peptide, Zn^{2+}, and albumin. C-peptide binding was determined using enzyme-linked immunosorbent assay (ELISA). Zn^{2+} binding was determined radiometrically using $^{65}Zn^{2+}$, and radiation was counted using a gamma counter. Our results preliminarily suggest that albumin is required for delivery of C-peptide, but not Zn^{2+}. However, there appears to be additional Zn^{2+} added to the neutrophils when in the presence of albumin and C-peptide.

AQUEOUS ANGIOGRAPHY IN *ADAMTS10*-MUTANT DOGS BEFORE AND AFTER DEVELOPMENT OF GLAUCOMA

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Aqueous angiography (AA) is a novel technique demonstrated in normal and *ADAMTS10*-open angle glaucoma (*ADAMTS10*- OAG) dogs. AA findings were compared to conventional intravenous (IV) scleral angiography (SA). *In-vivo* AA was performed in one eye of 12 normal and 10 *ADAMTS10*-OAG dogs via intracameral (IC) administration of 0.1ml of 0.25% indocyanine green (ICG) under sedation. SA was subsequently performed under sedation, following a wash out period, using 1mg/kg ICG administered IV via the cephalic vein. Scleral quadrants from the same eye of each dog were imaged for comparative purposes. Imaging was performed using a Heidelberg Spectralis ® Confocal Scanning Laser Ophthalmoscope (cSLO) in all eyes. AA permitted visualization of the conventional aqueous humor outflow (CAHO) pathways, demonstrating segmental and dynamic outflow patterns. SA permitted visualization of deep scleral vessels, consistent with the results from AA. Fluorescence occurred with a mean ±SD of 35.0±4.3 seconds (AA) and 34.6 ± 4.5 seconds (SA) post ICG administration in normal dogs, respectively. Fluorescence was most commonly visualized within the superior-temporal quadrant (10/12 eyes). CAHO pathways were observed in all *ADAMTS10*- OAG eyes with AA. The time to fluorescence post ICG administration was a mean ±SD of 34.3±11.0 seconds (AA) and 35.8±10.6 seconds (SA), respectively. Segmental and dynamic outflow patterns were observed in 9/10 *ADAMTS10*-OAG eyes in the superior-temporal quadrant with AA. No obvious differences in CAHO morphology and/or flow patterns were noted in *ADAMTS10*-OAG as compared to normal dogs.

HEPATIC IRON OVERLOAD DISORDER IN CAPTIVE RHINOCEROS

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All species of rhinoceros are critically endangered, and some even extinct in the wild, making captive breeding programs especially important to maintaining these species. Captive populations of rhinoceros also act as ambassadors for their wild counterparts, both educating and inspiring the public about important conservation issues. Iron overload disorder, a commonly reported condition in captive browsing rhinoceros, has the potential for causing severe morbidity and even mortality. The potential for iron overload disorder to cause disease on its own is currently under debate, though it is likely that it predisposes or exacerbates other conditions in rhinos. Currently the most common antemortem diagnostic test that is performed to diagnose iron overload is to look at ferritin levels in serum, but recent studies have shown that this may not be a reliable diagnostic tool. This deficit is a challenge to caretakers because it is often difficult to determine whether an animal should be treated or not. The interaction of other dietary minerals such as copper and nickel are also an unexplored area of interest. In the hopes of developing a better understanding of the pathogenesis of iron overload disorder, we are performing post-mortem analyses on the liver tissue of multiple species of rhinoceros; including histopathology, special stains for iron, nickel, and copper, and correlating those findings with species, age, sex, mineral analysis, and antemortem serum ferritin levels. With this knowledge we can help improve long term health in captively managed populations and the wild populations of rhinoceroses.

A COMPUTATIONAL APPROACH TO IDENTIFY UNIQUE PATHOGENIC MIRNA SIGNATURES IN INFECTED HOSTS

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Early diagnosis of many agriculturally relevant zoonotic diseases has always been problematic since the hosts often remain asymptomatic until it's too late, resulting in late diagnoses post-slaughter. The second problem has been with achieving an accurate and sensitive diagnosis often confounded by closely-related/ environmental bacteria. We, therefore, focus on developing a computational workflow to identify molecular targets unique to the pathogen of interest, that can be detected in a wide range of host species. Here, we focus on pathogen-specific sRNA targets such as miRNA for early detection. When a pathogen infects a host, changes occur in miRNA expression and it becomes possible to detect bacterial miRNA alongside host noncoding RNA in the host sera. Although intra- and extra-cellular miRNA have been used extensively in cancer detection, very few studies have addressed miRNA as a diagnostic target for the detection of pathogens in the host. Therefore, we propose to use computational approaches to detect pathogenic miRNA in host samples. We will use a recently published dataset (Mycobacterium avium paratuberculosis-infected cows; Wang 2019) to optimize and demonstrate our workflow. We will use miRNA databases, published targets, and reference genomes to help us discern uniquely bacterial miRNA isolated from infected host samples. Going forward, we will also use de novo approaches to predict miRNA in uncharacterized pathogens of interest. Taken together, our approach will help us identify miRNA signatures unique to pathogens in infected hosts. The computational workflow we develop can be used for any host-pathogen combination with a focus on early diagnosis.

MOLECULAR CHARACTERIZATION OF *MYCOBACTERIUM BOVIS* IN CATTLE AND BUFFALO IN AMAZON REGION, BRAZIL

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The aim of this study was to characterize *Mycobacterium bovis* from cattle and buffalo tissue samples, from two Brazilian states, and to analyze the genetic diversity of them by spoligotyping. Tissue samples from tuberculosis suspect animals, 57 in Amazonas State (12 cattle and 45 buffaloes) and six from Pará State (5 cattle and one buffalo) from slaughterhouses under State Veterinary Inspection, which were isolated in culture medium Stonebrink. The positive cultures were confirmed by PCR and analyzed by the spoligotyping technique and the patterns (spoligotypes) were identified and compared at the *Mycobacterium bovis* Spoligotype Database (http://www.mbovis.org/). There was bacterial growth in 44 (69.8%) of the tissues of the 63 animals, of which PCR for RD4 identified 35/44 (79.5%) as *Mycobacterium bovis*. Six different spoligotypes were identified among the 35 *Mycobacterium bovis* isolates, of which SB0295, SB1869, SB0121, and SB1800 had already been described in Brazil, SB0822, and SB1608 had not been described. The most frequent spoligotype in this study (SB0822) had already been described in buffaloes in Colombia, a neighboring country of Amazonas state. The other identified spoligotypes were also described in other South American countries, such as Argentina and Venezuela, and described in the Brazilian states of Rio Grande do Sul, Santa Catarina, São Paulo, Minas Gerais, Mato Grosso do Sul, Mato Grosso, and Goias, indicating an active movement of *Mycobacterium bovis* strains within Brazil.

A COMPARISON OF HEATING DEVICES FOR MAINTAINING BODY TEMPERATURE IN ANESTHETIZED MARMOSETS

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Common marmosets (*Callithrix jacchus)* are non-human primates that have grown in popularity as research models due to their small size, ease of handling, and high reproductive rates. Despite their increasing popularity, few publications exist describing surgical support for these emerging research models. It has been described in several species that anesthesia can lead to hypothermia if thermal support is not provided. The consequences of hypothermia are well documented in several species and they include decreases in heart and respiratory rate in correlation with a decreased body temperature. The purpose of this study was to evaluate thermal support systems in use for marmosets following injectable anesthesia for maintenance of body temperature, recovery of sedation, and changes in heart and respiratory rates. This was done by first determining how long the standard heating device, a heating pump with attached warm water blanket, reached a stable temperature. We then determined if there was a difference in recovery times between animals that did or did not receive thermal support by testing three common thermal support systems. We hypothesized that marmosets that did not receive thermal support during sedation would have longer recovery times compared to animals that did receive thermal support. We found significantly different total recovery times and total sedation times between animals that did and did not have thermal support. We also observed a slight correlation between body temperature and heart and respiratory rate. The results of this study highlight the importance of providing thermal support to marmosets undergoing sedation or anesthesia.

LIMITING FELINE LEUKEMIA VIRUS PROVIRAL DNA INTEGRATION USING BET INHIBITOR IBET-762

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Feline leukemia virus (FeLV) is a retrovirus with a prevalence of 2-3% in the cat population. FeLV infection can have multiple negative consequences, including lymphoma, anemia, and immunosuppression, making it a virus of great clinical importance in cats. While routine testing usually detects p27 antigen levels in circulation, seemingly healthy cats may have detectable FeLV proviral DNA integration without concurrent antigenemia. These “regressively infected” cats carry the risk of potential reactivation and transmission of the virus. In order to prevent the integration and re-integration of FeLV DNA into the host genome, we targeted inhibition of bromodomain and extraterminal domain (BET) proteins, which effectively assist in viral integration at important insertion sites in the genome by binding to viral integrase. IBET-762 was chosen as a treatment alternative to JQ1, the archetypal BET inhibitor, as IBET-762 has a superior bioavailability profile. Cells from a noninfected feline fibroblast cell line (81C) were exposed to virus obtained from a FeLVpositive feline lymphoma cell line (FL-74). Experimentally infected cells were treated with increasing concentrations of IBET-762 or DMSO as a negative control. Cell growth and viability were assessed using automated cell counts and viability measures by the Invitrogen Countess. The number of provirus copies per genome was then assessed in each sample using qPCR and absolutely quantified using normalization with feline albumin copy number. Viral antigen levels were assessed using ELISA for p27. We hypothesize that treatment with IBET-762 will reduce proviral integration into the host genome, thus curtailing the viral infection.

LPS INDUCES LIPOLYSIS AND REDUCES INSULIN SENSITIVITY IN ADIPOSE TISSUE FROM DAIRY COWS

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Lipopolysaccharide induces lipolysis and insulin resistance in human and rodent adipose tissue (AT). Excessive lipolysis and AT inflammation predispose transition cows to metabolic diseases that often are comorbidities of inflammatory disease. Subcutaneous AT (SCAT) explants were collected from 12 Holstein dairy cows at -14d prepartum and 6d and 12d post calving. Explants were incubated with LPS (0 or 20µg/ml). The effect of LPS on stimulated lipolysis was determined using 1µM of isoproterenol (ISO) and LPS plus ISO (LPSISO). The impact of LPS on insulin anti-lipolytic responses at late gestation (1µL/L, LPS-IH) and early lactation (0.2µL/L, LPS-IL) concentrations was determined by comparing it to the effect of insulin on lipolysis during ISO stimulation (ISO-IH; ISO-IL). Lipolysis was determined by quantification of glycerol release. mRNA expression was quantified by RT-qPCR. LPS increased glycerol release from SCAT by 73±18% across all time points ($P<0.001$) compared to basal release. Lipolytic responses to LPS tended ($P=0.09$) to be affected by time relative to parturition with higher glycerol release at -14 d (87±2%) compared to +6 d (70±2%) and +12 d (63±2%). LPSISO increased the lipolytic response by 40±17% compared with ISO ($P<0.05$) and by 255±37% compared with basal release ($P<0.001$). IH reduced the lipolytic effect of ISO and LPS by -70±3% and -40±4% respectively ($P<0.05$). LPS increased mRNA expression of lipolytic (*ABHD5*, *LIPE*) and inflammatory (*NFKB1,* and *CCL2)* markers in AT ($p<0.05$). LPS triggers lipolysis and reduces IS in SCAT. LPS exposure during the transition period may exacerbate lipolysis and inflammation and reduce Insulin sensitivity.

DEVELOPING AN ALGORITHM TO DETECT SUBCLINICAL MASTITIS IN AUTOMATIC MILKING DAIRY HERDS

Patrick Crannell, Ronald Erskine

Mastitis is an economic burden in the dairy industry that reduces milk yield and quality, and compromises cow health. Dairy farms that have automatic (robotic) milking systems (AMS) must rely on sensor technology to detect cows with clinical mastitis (abnormal milk and/or swelling of the udder)—that detect inflammatory responses (milk electroconductivity; EC) temperature and color of milk, milk yield and cow motility. As designed, these parameters are fairly robust for cows with more pronounced symptoms of udder inflammation. However, the largest proportion of economic loss from mastitis occurs in cows that are infected, but do not display clinical symptoms; i.e., subclinical mastitis. Despite the sensor technology, previous literature suggests the ability to detect subclinical mastitis in AMS is not standardized. Also, herd managers struggle with monitoring subclinical mastitis because of lack of knowledge on how to interpret sensor information. Our primary objective was to determine if EC can be used to detect subclinical mastitis, or if other parameters need to be considered. 323 random milk samples were collected at a Michigan farm milking with AMS’s from cows in various lactations and processed for individual somatic cell counts (SCC) at an analytical laboratory. In addition, when samples were collected, individual cow data (EC, milk color and temperature, milk yield) from the same milking were retrieved and interpreted in relation to SCC findings. Results indicate EC alone is ineffective for detecting both subclinical or clinical cases, however, a relationship appears to exist between reduced milk yield and high EC when evaluated on a per quarter basis. Future studies in sensor technology will help clarify the criteria for an algorithm to best detect cows with subclinical mastitis.

SCREENING AND IDENTIFICATION OF NOVEL PLANTINSPIRED ANTI-MICROBIAL AND ANTI-CANCER AGENTS

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The bacteria *Enterobacter cloacae* and *Staphylococcus aureus* pose major threats to human health. According to the Centers for Disease Control both pathogens are among the biggest antibiotic resistant threats facing the United States of America, naming carbapenem resistant *Enterobacteriaceae* (CRE) as urgent threats and methicillin resistant *Staphylococcus aureus* (MRSA) as serious threats. *Enterobacteriaceae* are highly opportunistic and cause significant morbidity and mortality in hospital settings particularly among patients with compromised immune systems. Complicating this is their high rate of antimicrobial resistance: currently we have very few compounds that can effectively inhibit these bacteria, and complete resistance has been reported in some strains. The *Staphylococcus* genus is the most common cause of skin and soft tissue infections. This is especially problematic when the infecting strain is resistant to methicillin as the cassette for methicillin resistance often carries resistance to other widespread and effective antibiotics. Even more troublesome are small colony variants (SCV), isolated from persistent infections. These variants are physiologically altered and more difficult to treat than the wild type strains because of their altered growth characteristics. My work aims to identify novel pharmaceutical compounds that can be used to inhibit the growth of or kill these two problematic bacteria. We collaborate with groups in Pharmacology/Toxicology and Plant Sciences to develop novel chemical matter as well as more traditional compounds to screen in my research. Preliminary screens of small molecule chemical libraries have identified potentially promising leads against especially MRSA and MRSA/SCV, and these are being studied further.

GENERATION OF INDUCIBLE CRISPR INTERFERING SYSTEM FOR STUDY OF MAREK'S DISEASE

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RATIONALE: Marek's disease, a lymphoma causing condition caused by Marek's Disease Virus (MDV), has the potential to produce high economic losses within the poultry industry. Current methods of disease control consist of early vaccination that is only capable of preventing tumor progression but fails to prevent virus shedding and infection. This persistence allows for viral mutations and outbreaks. The CRISPRi/dCas9 system allows for novel approaches to host-viral interaction and biological pathway studies that will allow for development of more permanent control solutions.

METHODS: The CRISPRi system was designed using traditional cloning coupled with Gibson assembly techniques and transformed into E coli cells. Cells with successful transformation were selected for and used for transfection into the DF-1 cell line followed by homology directed repair techniques to incorporate the system into the host DNA. The CRISPRi/dCas9 system contains a single gRNA targeting TICAM1 and will effectively block translation of the TICAM1 protein.

RESULTS: We anticipate creating DF-1 cell lines with a functional inducible CRISPR interfering system. CRISPRi/dCas9 should turn on with the addition of doxycycline and downregulate TICAM1 expression, leading to increased MDV viral load.
Conclusion: The proposed CRISPRi/dCas9 system would provide a reversible knock out model to study host interactions relative to MDV as well as any gene or biological pathway of interest with an available gRNA sequence.

MAST CELLS AS MEDIATORS OF EARLY LIFE ADVERSITY EFFECTS ON ADULT NEUROINFLAMMATION AND BEHAVIOR

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Early life adversity (ELA) is linked to an increased susceptibility to adulthood major depressive disorder (MDD), a highly common and debilitating psychiatric disease. Neuroinflammation is a key mechanism underlying the pathophysiology of MDD. Studies across species suggest that ELA increases vulnerability to depression by priming the immune system for an amplified and more persistent inflammatory response to environmental stressors, but the underlying mechanisms are not understood. Mast cells (MCs) are innate immune cells uniquely positioned to drive ELA-mediated inflammatory responses that lead to MDD. Using a mouse model of ELA consisting of neonatal maternal separation combined with early weaning (NMSEW), we showed that exposure to a mild adult stressor reduced social interaction behavior (SI) and sucrose preference (SP) in NMSEW but not normal handled (NH) males and females, respectively, and that meningeal MC from NMSEW mice exhibited a more activated phenotype compared with NH mice. To test the role of MCs in ELA-driven increased susceptibility to stress, we administered Ketotifen, a mast cell stabilizer, before exposure to adult stress. Surprisingly, Ketotifen had different effects in NMSEW vs. NH animals: in males, Ketotifen prevented stress-induced social avoidance in NMSEW but had the opposite effect on NH. In females, Ketotifen rescued SP in NMSEW but had no effect on SP in NH. Finally, preliminary data suggest that Ketotifen prevents stress-induced increased in TNF-alpha levels in the hippocampus NMSEW mice. Together, these data implicate MCs as important modulators of the long-lasting effects of ELA on adult stress-induced behavioral phenotypes.

TREATMENT-RELATED CHANGES IN STRIATAL ACTIVITY IN A MOUSE MODEL OF PARKINSON'S DISEASE

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Parkinson's Disease (PD) is a neurodegenerative disorder characterized by bradykinesia (slowing of movement) and rigidity. The dopamine precursor levodopa is a commonly prescribed pharmacological treatment for PD patients and relieves core motor symptoms. However, chronic treatment with levodopa can lead to several complications, including development of drug-induced involuntary movements known as levodopa-induced dyskinesia (LID). How levodopa treatment changes neural activity, particularly over the course of chronic treatment, is not well understood. As the striatum is the site of dense dopamine projections and high dopamine receptor expression in healthy animals, it is a presumed major site of action. Altered activity of neurons in the striatum may mediate the therapeutic effects of levodopa and also underlie the abnormal movements seen in LID. Striatal projection neurons can be divided into two types: direct and indirect pathway medium spiny neurons (dMSNs and iMSNs, respectively). A prevailing hypothesis predicts levodopa evokes bidirectional changes in the activity of these two pathways, but how these changes evolve during chronic levodopa treatment is poorly understood. Previous work has found that, in response to levodopa, dMSN firing increases and iMSN firing decreases. Using optical recordings of dMSNs and iMSNs in a mouse model of PD, we tested whether repeated exposure to levodopa alters the acute responses of dMSNs and iMSNs, and how these responses relate to behavior. During chronic treatment, we expect dMSN responses to potentiate and iMSN responses to diminish. These long-term celltype specific recordings may shed light on the development of chronic treatment complications in Parkinson's Disease.

ROLE OF INTRAMAMMARY CEFTIOFUR ON THE RESISTANT BACTERIAL POPULATIONS IN THE GUT OF DAIRY CATTLE

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Overuse of antibiotics in human and veterinary medicine has contributed to resistance in bacterial pathogens. Understanding the factors associated with resistance and reducing its spread is essential for new prevention strategies. The goal of this study was to determine the effect that ceftiofur, a common antibiotic used to prevent mastitis, has on resistance levels in select intestinal coliform populations in healthy dairy cattle. We hypothesize that intramammary administration of ceftiofur (Spectramast DC) in dairy cattle will enhance the abundance of resistant gramnegative bacteria relative to cattle treated with a non-antibiotic teat sealant (Orbeseal) at dry off. Fecal samples are being collected and sampled weekly for cultures. The medias being used are MacConkey (MAC) broth with agar, MAC with ceftiofur, and MAC with ampicillin; media is made at concentrations in accordance with clinical breakpoints to quantify the abundance of resistant bacteria. Variations in antibiotic resistance levels are likely due to treatment, but treatment and control groups must remain blinded until after all data is collected. These data will be used to identify those samples and sampling that have the greatest abundance of resistance for future microbiome analyses. In all, this study will help determine the impact of intramammary-administered antibiotics on resistance within bacterial communities in the gut, which is critical to identify new ways to combat resistance.

DOES ORGANIC CHEMICALS IN PM2.5 VAPOR-PHASE POLLUTANTS INFLUENCE THE INDUCTION OR EXACERBATION OF LUNG TUMORS?

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Outdoor particulate matter (≤ 2.5 μm, PM2.5) is a major risk factor for lung cancer, one of the leading cancers worldwide with 2.1 million cases and 1.8 million deaths in 2018. While it is established that generation of cellular oxidative stress by redoxactive organic chemicals is a major mechanism for the adverse health effects of PM2.5, little is known about the effects of coexisting vapor-phase organic pollutants (vapor). We hypothesized that PM2.5 and vapor would exert additive or synergistic effects in promoting the growth of non-small cell lung cancer (NSCLC), which accounts for 80% of all lung cancers, by inducing oxidative stress. Organic extracts of PM2.5 and vapor samples from five locations in California were prepared. Organic extracts of diesel exhaust (DE), a known carcinogen, are included for comparison. Dose-response and kinetic studies are performed by stimulating NSCLC cells with extracts of PM, vapor, PM+vapor or DE. Cell proliferation, oxidative stress and activation of pro-inflammatory cytokines were analyzed by cell counting kit-8 assay, western blot and enzyme-linked immunosorbent assay (ELISA), respectively. We demonstrate that cell proliferation and activation of pro-inflammatory cytokines are positively correlated to pro-oxidant potential of air pollutants and there's an additive or synergistic effect between PM2.5 and vapor. These results provide important insights into how "real-life" multi-pollutant environment contributes to lung carcinogenesis.

THE EFFECT OF TWO FOOD ADDITIVES IN OVALBUMINELICITED FOOD ALLERGY MOUSE MODEL

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Food allergy reactions have been increasing recently and it's not completely understood why. Many factors could contribute to food allergies, such as environmental chemical exposure, genetics etc. Recent findings show that there could be a link between a common food additive, tBHQ, and food allergies. Our study investigated butylated hydroxytoluene (BHT) and 3-hydroxytyrosol (3-HT), other common food additives, which play a similar role as tBHQ in an OVA sensitized mice model. Female BALB/c mice were given a control or food additive specific diet and exposed to OVA transdermally weekly for four weeks. OVA specific IgE and IgG1 levels were determined through ELISAs. Decreased OVA-IgE levels for diet specific groups were found, as compared to the control, and similar levels of IgG1 were found between the control and diet specific groups. We then challenged the mice orally. Systemic anaphylaxis clinical symptoms were quantified. The diet specific groups presented a decrease in symptoms. A change in rectal temperature showed the diet specific groups have the lowest decrease in temperature and quickest return to normal body temperature. Mouse mast cell protease-1 (mMCP-1) levels from BHT, 3-HT, and control group were determined through ELISA, indicating a less mast cell degranulation level in diet specific groups. BHT and 3-HT may have a protective effect in regards to OVA-elicited food allergy mice model, in contrast to tBHQ. These results could potentially help industry decision makers reconsider which food additives to be used in the preservation and will be vital to mitigate the rise in food allergy.

APPLICATION OF PATHOGEN-SPECIFIC BIOMARKERS TO ENHANCE SPECIFICITY OF BOVINE TB DIAGNOSIS

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Bovine tuberculosis (bTB) is a zoonotic disease, causing a global economic and health-burden and necessitating urgent control. Towards this end, we propose a rapid, inexpensive, yet highlyspecific diagnostic tool for bTB that also differentiates infection from non-tuberculous mycobacteria using *Mycobacterium tuberculosis* complex (MTBC)-specific biomarkers.

Previously, 16 mycobacterial proteins in the serum of *M. bovis*-infected animals were identified. Of these, 3 proteins (Pks5,MB2515c, MB1895c) were selected for high-precision DNA ligand selection. Two short peptides per biomarker were selected. A 96-well plate was coated with Pks5 peptides and incubated with a DNA aptamer combinatorial library. High affinity aptamers bound to peptide were eluted by salt gradient. The final eluate was tested for the presence of aptamers by PCR. Validation & identification of aptamers was performed by dot blot, followed by TA-cloning and Sanger sequencing. Specificity analysis for selected aptamers was done via two modified forms of ELISA using aptamers (ELASA and ELA-ASA).

Selection resulted in four redundant anti-Pks5 aptamers. Two aptamers were selected for high GC content and presence of G-tetrads. Specificity analysis failed to show aptamer specificity. A sandwich ELA-ASA performed using monoclonal anti-Pks5 antibodies suggested that small peptide size confounded effective plate-binding, impairing the aptamer selection process.

Currently, larger peptides are being produced, which will permit proper aptamer-selection for ultimate use in field diagnostic device to test for bovine tuberculosis.

IMPACT OF ANTIOXIDANT SUPPLEMENTATION ON MUSCLE PROTEOME AND GLUTATHIONE IN THOROUGHBREDS

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Maximal exercise generates reactive oxygen species (ROS) through the mitochondrial electron transport chain (ETC) with the potential for ROS to overwhelm the muscle's antioxidant capacity causing oxidative stress. A portion of the horse's total antioxidant potential is made up of cysteine bound thiols which includes glutathione (GSH). Supplementing horses with Coenzyme Q10 and N-Acetyl-Cysteine could support the ETC and provide the cysteine required for GSH synthesis. To determine the effect of oral Coenzyme Q and N-Acetyl-Cysteine supplementation (AOX) on plasma and tissue GSH concentrations as well as the muscle proteome. Thirteen Thoroughbred horses in training received AOX or placebo for 30 days in a randomized crossover designed study. On day 30, blood samples were collected and immediately centrifuged and plasma frozen. Percutaneous gluteus medius needle biopsies were also obtained and flash frozen in liquid nitrogen. GSH concentrations were measured in plasma and tissue samples by HPLC. TMT11 proteomic analysis was performed on muscle from 5 horses with and without AOX. Muscle GSH concentrations increased significantly 24.4% ($p<0.05$) with AOX whereas plasma GSH showed large individual variation and no significant difference. Forty out of 387 identified proteins were differentially expressed following AOX including upregulation of 13 mitochondrial proteins and downregulation of 9 glycolytic proteins. Muscle GSH concentrations increases with AOX which is not reflected by plasma concentrations. AOX appears to promote oxidative metabolism by increasing mitochondrial oxidative enzymes in conjunction with decreasing glycolytic enzymes.

SERUM LEVELS OF INSULIN-LIKE GROWTH FACTOR-1 IN CANINES WITH PRIMARY HYPOTHYROIDISM AND NON-THYROIDAL ILLNESS

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Primary hypothyroidism is a common endocrine pathology found in canines, but it is commonly misdiagnosed due to effects of non-thyroidal illness (NTI) on thyroid function. Previous research has postulated that growth hormone signaling may be increased during canine hypothyroidism. Using GH as a biomarker to distinguish the two diseases would be unreliable because of its pulsating release. Insulin-like growth factor-1 (IGF-1) is a more reliable distinguishing measure because of its stable concentrations in blood and its GH stimulated release. To determine the validity of using IGF-1 as a diagnostic biomarker for hypothyroidism in canines, a retrospective study was done using canine thyroid testing submitted to Michigan State University Veterinary Diagnostic Laboratory between January 2019 and May 2019. Selected residual samples (n=164) were then radioimmunoassayed (RIA) for IGF-1 concentrations. IGF-1 concentrations will be analyzed using a multiple regression model with a 95% confidence interval. Weight and sex will be further assessed as being confounding variables that could affect IGF-1 concentrations. It is assumed that IGF-1 concentrations will be higher in patients with hypothyroidism, when correlated to weight, than patients with NTI or that are normal. This study should support the use of IGF-1 being used as a diagnostic biomarker to definitively differentiate NTI and canine primary hypothyroidism.

EXPLORING ALBUMIN-ZINC BINDING UNDER DIABETIC CONDITIONS

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Type I Diabetes (T1D) is a pathology in which insulin and its hormone counterparts are depleted due to the pancreatic b-cells being destroyed. A common therapy for T1D is exogenous insulin to help facilitate glucose control in the blood stream. Despite this therapy, individuals with T1D have high levels of advanced glycated end products (AGEs), which contribute to diabetic complications. Human serum albumin is the most prominent plasma protein and undergoes glycation in a high glucose environment, resulting in its decreased ligand binding capabilities. A vast majority of zinc (Zn^{2+}) is complexed with HSA in the blood stream and if this homeostasis is disrupted it results in diabetic pathologies such as thrombotic complications, poor blood flow and hyperzincuria. Examining the interaction between HSA and Zn^{2+} in hyperglycemic environments will aid in determining contributing factors of diabetic complications. Immunoprecipitation of HSA from diabetic (n=4) and control (n=3) samples was performed using antibody-coated magnetic beads. Ultrafiltration devices were fabricated on a 3D-PolyJet printer, and free radioisotopic $^{65}Zn^{2+}$ was measured on a 2480 WIZARD automatic gamma counter. Results show a significant inverse linear correlation between nK_a and percent glycation, inferring that glycation is interfering in HSA-Zn^{2+} binding (R^2 =0.7227, $p<0.05$). The use of 3D-printed devices enabled the binding of HSA-Zn^{2+} to be investigated in diabetic conditions. This discovery helps explain abnormalities in Zn^{2+} homeostasis in diabetic patients and allows insight to the mechanism of delivery in the diabetic bloodstream.

LONGITUDINAL ANALYSIS OF DIAGNOSTIC MEASURES OF BOVINE LEUKEMIA VIRUS INFECTIONS

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Recent control programs for bovine leukemia virus (BLV), a prevalent retroviral disease among the U.S. cattle population, have focused on removing infected cattle with high proviral loads (PVL) or high lymphocyte counts (LC). The success of these programs can be enhanced by a greater understanding and identification of cattle which will progress to these disease conditions. Therefore, the objective of this study was to utilize a database from a BLV-intervention field trial to describe observed changes in measures of BLV infection (BLV-ELISA optical density, LC, and PVL). During a two- year- long field trial, 49% (380/779) of sampled cows were ELISA+. A wide range in LC per ul (1,800 to 24,000) was observed in ELISA+ cows. Median change in LC between sample timepoints was 0 LC per ul (IQR: -1,000 to 1,300; min: -8,800; max: 10,600). The PVL ranged from 0 to 180,000 copies per 100,000 cells. Median change in PVL between sample timepoints was 0 proviral copies (IQR: -2,200 to 7,200; min -50,000; max: 116,000). These results suggest that a majority of cows experience relatively small fluctuations in BLV diagnostics during the observed period. Further analysis of cow-level factors associated with the large observed fluctuations in a subset of cows may contribute to ongoing control programs.

MULTICENTER PLACEBO-CONTROLLED RANDOMIZED STUDY OF ETHYL PYRUVATE IN HORSES FOLLOWING SURGICAL TREATMENT FOR LARGE COLON VOLVULUS

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Large colon volvulus (LCV) is a painful, often fatal form of surgical colic in horses. Death is associated with rapid deterioration of the colon, subsequent vascular injury, and development of endotoxemia/sepsis. Identifying therapies that mitigate ischemic colonic injury and improve intestinal healing is crucial to improving survival in affected horses. The objective of the following study was to determine the effects of ethyl pyruvate administration on systemic indices of colon viability, expression of inflammatory genes, and survival after surgical correction of LCV. A prospective, randomized clinical trial was initiated where horses received either 150 mg/kg ethyl pyruvate in 1 liter lactated Ringer's solution or 1 liter LRS every 6 hours for 24 hours following surgical recovery for correction of LCV. Ethyl pyruvate treated horses and controls were compared based on colic duration, perioperative heart rates, packed cell volumes, total solids, blood L-lactate concentrations, surgical time, intraoperative episodes of hypoxemia and hypotension, expression of inflammatory cytokine genes, and survival to hospital discharge. Twenty-two horses, 12 receiving ethyl pyruvate and 10 controls, were enrolled. Increased heart rate, PCV and blood L-lactate concentrations at the time of hospital admission and after surgery were associated with death. Seven of 12 ethyl pyruvate treated horses and 5/10 controls survived to hospital discharge. Ethyl pyruvate was safely administered to horses following surgical correction of LCV but was not associated with significant changes in any variables measured other than improved post-operative manure consistency. Additional investigation of potential clinical uses of ethyl pyruvate may be warranted.

EVALUATION OF THE RESPIRATORY ADJUSTED SHOCK INDEX IN DOGS DIAGNOSED WITH HEMOPERITONEUM.

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Recent focus in both human and veterinary medicine has been on early detection and risk stratification of patients in occult shock. The respiratory adjusted shock index (RASI) is gaining utility as a rapid bedside tool for detecting occult shock in human medicine but has not yet been evaluated in dogs. The purpose of this study was to investigate how RASI performs compared to shock index (SI) and lactate in identifying shock and predicting in-hospital mortality among dogs with hemoperitoneum.

Medical records of dogs diagnosed with hemoperitoneum between 2017-2019 were reviewed. Signalment, admission vital signs, blood pressure, lactate, and cause of hemoperitoneum were obtained. SI and RASI were calculated. Patients were classified into 1 of 4 categories of shock by admission lactate values.

One hundred eleven patients met inclusion criteria. Overall inhospital mortality was 64.0%. Median lactate values were significantly different between survivors and nonsurvivors (p=0.0033), and patients with admission lactate values >2.5mmol had increased odds of nonsurvival (OR 4.046, p=0.0127). No difference was noted between SI or RASI in survivors compared to nonsurvivors in this population. No association between lactate and RASI or SI was present at any level of shock in this population.

In this population no association or added benefit of using RASI or SI to identifying shock or predict outcome was identified. Patients with admission lactate >2.5mmol/L may be at higher risk for death, though further studies evaluating changes in plasma lactate concentration following resuscitation are needed.

IMPACT OF BOVINE LEUKEMIA VIRUS ON DAIRY CATTLE LYMPHOCYTE AND ELISA STATUS OVER A LACTATION CYCLE

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Bovine leukemia virus (BLV) is a delta-retrovirus which infects the B-lymphocytes of cattle. Approximately 46% of U.S dairy cattle are infected and ~30% of BLV infected animals develop persistent lymphocytosis. Our objective is to document changes in BLV antibodies, lymphocytes, and new infections over a lactation period in dairy cattle naturally infected with BLV to determine critical time points for new infections within a herd. Two cohorts of 44 animals were enrolled 150 days prior to calving. Blood samples were collected at enrollment, every 2 weeks until calving, and then every 4 weeks until the next dry-off. BLV ELISA testing was performed at each collection time point. Complete blood counts were run every ~4 weeks from enrollment to ~60 days after calving and once after peak milk production. Mean lymphocyte counts (x10e3/uL) at dry-off were 6.43 for BLV+ and 3.54 for BLV- animals which was significantly different
(p<0.01). Using a repeated measures linear mixed model, BLV status (p<0.01), time (p<0.01), and lactation of 3+ (p<0.01) all had significant effects on lymphocyte count. ELISA optical density (OD) values increased at dry-off and ~30 days post calving in all BLV+ animals. Five animals sero-converted over the first 8 months and qPCR in sero-converting and BLV+ animals showed proviral load (PVL, # viral copies/10^3 leukocytes) fluctuations over time. This study shows lymphocyte count, OD, and PVL change throughout a lactation cycle and may be caused by the impact of stress on viral reactivation and replication followed by immune system activation.

COMPARING TRUNCATED PROTEINS WITH ANNEXINV FOR TARGETING APOPTOTIC CANCER CELLS

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Binding of AnnexinV (A5) to Phosphatidylserine (PS) has been used for in vivo imaging of apoptosis. Targeting dying cells could allow the local release of therapeutic agents to kill residual cancer cells or cancer initiating cells. ProteinS (PrS) is another PS binding protein involved in blood coagulation and immune regulation. A truncated version (TPrS) was compared with A5 for binding of untreated and apoptotic induced cancer cell lines *in vitro*. Both proteins were also compared for *in vivo* clearance and biodistribution in normal and tumor-bearing mice. HYNIC conjugation was used to label TPrS and A5 with Tc99m radionuclide. In vitro binding assays were performed using breast, liver, and melanoma cell lines and analyzed using fluorescent microscopy, flow cytometry and a gamma counter. In vivo studies were done using the same cell lines in nude mice and imaging on gamma camera. Tc99m-A5 showed 10-15x more binding of apoptotic cancer cells compared to Tc99m-TPrS. Tc99m-TPrS showed 1.5-2x more binding when incubated with excess unlabeled TPrS and 0.3-0.6x reduced binding with excess unlabeled A5. Tc99m-A5 showed 100x reduced binding when incubated with unlabeled A5 and no change in binding when incubated with unlabeled TPrS. Tc99m-A5 had 9% more of its total dose cleared in the kidneys over the first 13 minutes compared to Tc99m-TPrS. Tc99m-A5 had more healthy tissue deposition and less final dose in kidneys compared to Tc99m- TPrS. TPrS has much less healthy tissue deposition and exhibits unique binding characteristics that could make it a much better imaging agent than A5.

PRE-OP WARMING EFFECTS ON BODY TEMPERATURE AND ANESTHETIC RECOVERY IN A RAT SURGICAL MODEL

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Rats are common models for intracranial surgeries, yet their small size can potentiate the risk of hypothermia under gas anesthesia. Mitigating hypothermia improves animal welfare and limits variability in physiologic data outcomes. Thermal support of laboratory rodents often occurs using a warming device (e.g., circulating warm water blanket (WWB)) during surgery. This study assessed 3 preoperative warming treatments and use of a novel skin prep agent to mitigate hypothermia. Rats (n=24) were donated from an institutional research colony. Group 1 rats were provided a WWB (~42?C) for 30 min before surgery, Group 2 received warmed (36-38?C) fluids intraperitoneally, and Group 3 received warmed fluids and a WWB before surgery; a control group had no preoperative thermal support. Animals were maintained on a heated platform (~38?C) during surgery with continuous monitoring of body temperatures. While generally perioperative body temperatures were similar across all groups, on average, Group 1 animals had the fastest anesthetic recovery time (6.7 min average). Further, it was determined that the novel prep agent was effective for disinfection of the surgical site, as determined by MALDI-TOF. Specifically, 62.5% of animals had positive bacterial cultures at the clipped site prior to agent application (~ 4 colony forming unit (CFU)/100µL). Only 4.2% (with ~1 CFU/100µL) and 12.5% (with~ 1 CFU/100µL) of animals were positive after agent application and site skin closure, respectively. Results of this study and shared videos of these perioperative surgical approaches will contribute to recommendations for thermal support that can be readily implemented by the scientific community.

DISTRIBUTION OF ANAPLASMA PHAGOCYTOPHILUM IN MICHIGAN IN QUESTING BLACK-LEGGED TICKS FROM 2018

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Anaplasma phagocytophilum (Ap) is a tick-borne pathogen vectored by the black-legged tick, *Ixodes scapularis*, the same tick species that transmit the Lyme disease pathogen, *Borrelia burgdorferi* (Bb) in the eastern USA. There are two main strains of Ap circulating sympatrically, the human pathogenic strain (Apha) causing anaplasmosis in humans and dogs, and the deer strain (Ap-V1) found in deer and not known to cause disease. According to the Michigan (MI) Disease Surveillance System, there has been an increasing trend in human anaplasmosis cases since 2014. The main objective of this study was to obtain an understanding of the spatial distribution of anaplasmosis risk throughout the state of MI and also improving public health risk assessment. Questing ticks were sampled by drag cloth in 195 sites throughout Michigan. Sites were sampled in summer and sampled at least once. Ticks were identified using dichotomous morphological keys. Total DNA was extracted, and a real-time PCR was performed to screen the samples. A nested PCR was performed to confirm the Ap positive samples. These samples were sequenced for Ap strain typing. A total of 1396 *I. scapularis* ticks were collected. Overall infection prevalence of questing adults (6.8%, n=251) were significantly greater (Chi-square pvalue= 0.0152) than questing nymphs (2%, n=254) in MI. Strain typing of the Ap infected ticks indicated all of the Ap infected questing ticks carried Ap-ha. Our current findings indicate the importance of further studies in the trend of distribution and spread of Ap risk in Michigan, given the important public health implications.

SMALL MOLECULES BREAK DOWN ISLET AMYLOID POLYPEPTIDE FIBRILS

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Amyloid deposition is a recognised feature of the islets in the majority of type 2 diabetic patients. These deposits originate from islet amyloid polypeptide (IAPP). Normally, IAPP is secreted along with insulin from pancreatic β-cells and has hormonal functions related to satiety. However, IAPP is one of the most potent amyloidogenic proteins. Deposition of IAPP is associated with β-cell death and diabetes progression. Drugs to inhibit pancreatic amyloidosis are not available. We have prepared a small molecule screening library to inhibit pancreatic amyloidosis. Our central hypothesis is that IAPP fibril formation (fibrillization) and deposition can be modulated by IAPP-interactive compounds. We aim to establish structure-activity relationships by which our small molecules impede IAPP fibrillization. Our primary approach, thioflavin T (ThT) assay, represented a major tool for the determination of IAPP fibrillization and inhibition by small molecules. For the most potent molecules, the abrogation of cytotoxic intermediate species (i.e. oligomerization leading to fibril formation) was determined with photo-induced cross-linking of unmodified protein. To confirm the inhibitory effect morphologically, electron microscopy analysis was performed to detect IAPP fibrils directly. Disaggregation of preformed fibrils by different compounds was studied with ThT assays and electron microscopy. The 1st-tier screening method (ThT assay) identified several lead compounds to target IAPP. The oligomerization of IAPP was abrogated by JF-19-029. JF-19-029 and NBMI-19-013 deteriorated preformed fibrils. Few molecules have been reported to break down mature fibrils. We hope to open a new therapeutic approach to reduce type 2 diabetes progression.

IN VITRO EVALUATION OF VITAMIN E ANALOGS AS ANCILLARY ANTIOXIDANTS IN DAIRY CATTLE

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Oxidative stress predisposes dairy cattle to disease during the transition period resulting in significant veterinary costs, production losses, and welfare concerns. Oxidative stress is caused by a concurrent increase in reactive metabolite production and a decline in circulating antioxidants, such as α-tocopherol, at this time. Decades of focus placed on increasing plasma α-tocopherol through supplementation to meet or exceed NRC recommendations has reduced the severity of oxidative stress, yet it remains a substantial problem. Further, supplementation three-times NRC recommendations has increased disease and oxidative stress in some herds. This suggests that a new approach is necessary, potentially through the addition of non-α-tocopherol analogs of vitamin E whose shorter half-life may allow for greater supplementation. Currently, the antioxidant capabilities of such analogs are incompletely understood in cattle. This study aimed to understand the antioxidant capacity of γ-tocopherol and γ-tocotrienol compared to α-tocopherol. Distinct reactive oxygen (ROS) and nitrogen species (RNS) generators were utilized within in vitro models of the bovine mammary gland. Compared to α-tocopherol, γ-tocopherol and γ-tocotrienol had a greater antioxidant capacity towards RNS and had significant antioxidant abilities at lower concentrations; however, α-tocopherol more readily reduced ROS production albeit at higher concentrations. These data show that indeed, γ-tocopherol and γ-tocotrienol contribute additional antioxidative capacity especially towards reducing the production of RNS. Further research should focus on the use of γ- tocopherol and γ-tocotrienols ability to reduce cellular damage from reactive metabolites and determine what impacts this may have on inflammatory signaling.

A COMPARATIVE ANALYSIS OF CLOPIDOGREL AND DT678 ON THE VASCULATURE

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The most common cause of death in the developed world is due to ischemic complications caused by arterial thrombosis. Dual antiplatelet therapy using low-dose aspirin with a purinergic receptor 2Y12 (P2Y12) antagonist is frequently prescribed as a preventative therapy. Patients taking antiplatelet agents, like clopidogrel, have an increased risk of cerebral microbleeds and intracerebral hemorrhage (ICH). ICH is associated with impaired cerebral blood flow and loss of vascular integrity. Clopidogrel, a prodrug, is converted into the active metabolite (AM) by cytochrome P450 enzymes (CYP450's), however, only 5% of the dose is activated and loss of function polymorphisms in CYP450's reduce this further. DT678 is a conjugate of the clopidogrel active metabolite that is activated by a non-enzymatic thiol exchange reaction with glutathione. DT678 only produces the AM that covalently binds to P2Y12. We reported that clopidogrel induces a 1 to 2-fold increase in bleeding time while DT678 has no significant effect. We also demonstrated that clopidogrel induces bleeding before the inhibition of platelet aggregation. This suggests some adverse effects of clopidogrel are not mediated by P2Y12 inhibition. Instead, the parent compound, or one of the many metabolites, may be responsible. The purpose of the present study was to characterize the effect clopidogrel metabolites have on the cerebral vasculature and determine how clopidogrel promotes bleeding. To do that we determined how spontaneous myogenic tone generation and purinergic vasodilation in the middle cerebral artery (MCA) are altered. These results suggest ADP-induced vasodilation is inhibited in MCAs from clopidogrel-treated, but not DT678-treated rabbits.

TOPICALLY ADMINISTERD NETARSUDIL/LATANOPROST OPHTHALMIC SOLUTION (ROCKLATAN™) IN NORMAL AND GLAUCOMATOUS DOGS

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Netarsudil is a recently approved Rho kinase inhibitor for lowering intraocular pressure (IOP) in human patients with glaucoma. We have previously shown in normal and glaucomatous dogs with *ADAMTS10*-open-angle glaucoma (*ADAMTS10*-OAG) that topically administered netarsudil 0.02% ophthalmic solution (Rhopressa™; Aerie Pharmaceutical) only has a marginal and clinically irrelevant effect on IOP. The purpose of the current study was to evaluate safety and efficacy of topically administered netarsudil/latanoprost 0.02%/0.005% ophthalmic solution combination (Rocklatan™;Aerie Pharmaceutical) compared to latanoprost 0.005%. Five normal and 5 dogs with *ADAMTS10*-OAG were enrolled. Once (q24h) and twice daily (q12h) treatments were evaluated. Differences in least square means of diurnal IOPs were compared between netarsudil/latanoprost- *vs*. latanoprost-treated eyes by linear Gaussian model. Safety was assessed by routine ophthalmic examination, gonioscopy and pachymetry. Baseline IOPs were 13.8 ± 0.7 mmHg (mean ± SEM) in normal and 28.4 ± 1.4 mmHg in OAG dogs. IOPs decreased significantly in both netarsudil/latanoprost- *vs*. latanoprost-treated eyes ($p<0.05$), but there was no significant difference between treatments (q24hnormal: 11.7 ± 0.7 mmHg *vs*. 11.6 ± 0.7 mmHg; q24hr-OAG: 14.2 ± 1.3 mmHg *vs*. 13.6 ± 1.2 mmHg *vs*. treatment; q12hr-normal: 9.7 ±0.9 mmHg *vs*. 9.5 ± 0.9 mmHg; q12hr-OAG: 10.7 ±1.4 mmHg vs. 10.7 ±1.3 mmHg), indicating no added treatment effect by netarsudil. The netarsudil/latanoprost was well tolerated but resulted in significant, moderate to severe conjunctival hyperemia ($p<0.001$).

CHARACTERIZATION OF FELINE HERPESVIRUS-1 DELETION MUTANTS IN TISSUE EXPLANT CULTURES

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Feline herpesvirus-1 (FHV-1) is the primary cause of viral respiratory and ocular disease in cats. While commercial vaccines provide clinical protection, they do not protect from infection or prevent latency. Moreover, they are not safe for intranasal administration. Our objective is to develop a new mucosal vaccine against FHV-1 disease to address these shortcomings. FHV-1 deletion mutants of glycoprotein C (gC-), gE (gE-), protein kinase (PK-), and both gE and thymidine kinase (gE-TK-) were generated by bacterial artificial mutagenesis. Tracheal tissue explants from eight cats were used to compare virulence and severity of tissue damage following inoculation with wild-type virus (WT), gE-, gE-TK-, PK-, and gC-. Tissues were collected at 24, 48, or 72 hours post-inoculation (hpi) and stained using immunohistochemistry (IHC) targeting FHV-1. A score was derived by evaluating staining intensity and distribution as well as tissue damage. WT-inoculation resulted in maximal scores at 72 hpi at both MOI 1 and 0.1. Inoculation with gE- exhibited similar scores compared to WT at 24 and 48 hpi but by 72 hpi scores were significantly decreased. Explants inoculated with gE-TK- showed significantly decreased scores at all time points. Further, the majority of explants inoculated with PK- showed negative scores at all time points, regardless of MOI. We concluded that gE-TK- and PK- mutants exhibited reduced virulence and spread in the tracheal explant system compared to WT suggesting they might be good candidates for further in vivo testing in the natural host.

DETECTION OF FELINE LEUKEMIA VIRUS PROVIRAL DNA IN CATS WITH LYMPHOMA

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Feline leukemia virus (FeLV), a gammaretrovirus, is one of the most prevalent causes of feline infectious disease. Integration of the reverse transcribed viral RNA genome into the host cell genome as a provirus can cause activation of host oncogenes and/or inhibition of tumor suppressor genes, potentially inducing neoplastic transformation. Beginning in the 1970s, researchers found an association of FeLV infections with lymphomagenesis in cats. However, to this day this association remains poorly characterized in household cats and is the focus of this study. The aim of this investigation is to determine the prevalence of FeLV proviral DNA in cats with lymphoma. 55 cases submitted to the MSU Veterinary Diagnostic Laboratory in 2018 for suspected lymphoma were examined and 14 well-characterized cases that met histological, immunohistochemical, and antigen receptor clonality criteria consistent with a diagnosis of enteropathy associated T cell lymphoma were included in this study. Using semi-nested PCR as well as qPCR specific for the U3 long-terminal repeat region of the exogenous FeLV genome, FeLV provirus was detected in 9 of the 14 cases. Characterization and evaluation of additional feline gastrointestinal and multicentric lymphoma cases for the presence of FeLV proviral DNA, as well as expression of FeLV glycoprotein gp70, are currently in process. These preliminary findings are supportive of a potential role for FeLV in feline lymphoma.

EXCLUSION OF PROGRESSIVE RETINAL ATROPHY CANDIDATE GENES IN TWO BREEDS OF DOG

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Purpose: To utilize a previously developed panel of 96 polymorphic microsatellite markers to investigate the 48 candidate genes flanked by these markers for association with recessively inherited Progressive Retinal Atrophy (PRA) in two breeds of dog - Old English Sheepdogs (OES) and Belgian Tervurens (BT).

Methods: The microsatellite markers were amplified through PCR, fluorescently tagged and genotyped. These genotyping results were analyzed according to the assumption that the markers for the locus corresponding to the candidate gene harboring the PRA-causing variant are in linkage disequilibrium with the mutation. Exclusion analysis was used to rapidly eliminate potential candidate genes as containing the pathogenic variant when the affected dogs of the respective breeds demonstrated variability for the linked markers.

Results: 17 of the total 48 genes were tested. Eleven of the 17 candidate genes in the OES samples and 9 candidate genes in the BT samples were excluded.

Conclusion: The 6 unexcluded OES genes and 8 unexcluded BT genes require further investigation to assess if one of these genes contains a disease causing mutation.

EVALUATION OF KEY ONCOGENIC PATHWAYS IN HISTIOCYTIC SARCOMA IN BERNESE MOUNTAIN DOGS

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Histiocytic sarcoma (HS) is a malignant neoplasm of dendritic cells and macrophages. It is a rare neoplasm but has a high frequency in certain breeds, especially Bernese Mountain Dogs (BMDs). About 25% of them succumb to this disease. There are currently no effective treatments for disseminated HS, regardless of any treatments. The majority of canine HS is seen in the lung, spleen, liver, and lymph nodes. Histiocytic diseases are rare but do occur in humans. The etiology for HS is currently unknown. Recently, the contributions of the RAS/MAPK pathway has been highlighted in canine HS from studies in our laboratory and in human HS by others. In addition to the RAS/MAPK pathway, we hypothesize that the PI3K/AKT/mTOR pathway, which works parallel to the RAS/MAPK pathway may play a role in HS tumorigenesis. PTEN is a tumor suppressor gene and loss of PTEN may lead to activation of the AKT-pathway and provide additional targets for future therapeutics. We hypothesize that loss of PTEN and activation of the PI3K/AKT/mTOR may be involved in driving tumorigenesis in some cases of HS. Protein samples from tumor tissues of dogs are used for the analysis of PTEN, AKT, p-AKT, ERK, and p-ERK by Western blotting. We will investigate the activation of RAS/MAPK and AKT/MTOR pathways and the presence or absence of key mutations in matched normal and tumor DNA. In tumors with PTEN loss we expect increased p-AKT protein expression, and with tumors with PTPN11 and KRAS mutations, we expect increased levels of pERK.

INTRANASAL DEFORMITIES IN DOGS: COMPARATIVE DIAGNOSTIC AND FUNCTIONAL IMAGING

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Brachycephalic Airway Syndrome (BAS) is a debilitating condition affecting some of the most populous dogs in the USA. Caused by the developmental foreshortening of the skull, BAS constitutes a major welfare concern in affected breeds as they lead an overall poor quality of life. It is not clear how intranasal anatomical deformities contribute to the pathophysiology underlying BAS. We applied high resolution 3-D imaging (computed tomography, CT) to simulate function (flow pattern) using computational fluid dynamics (CFD). We report that measuring functional respiratory air volume to cross sectional area ratio on CT scans is a reliable and novel preoperative assessment index, as it reproducibly relates intranasal structure to function. Our study reveals that the fraction of total air which the functional respiratory air volume comprises is lower in English bull dogs but higher in French bulldogs and pugs. This provides novel insight, currently lacking in literature, into the severity in the clinical presentation of English bulldogs over pugs and French bull dogs. Using CFD, our findings suggest that there is stationary flow in the frontal sinus and maxillary recesses in all dogs studied. Therefore, preoperative assessment of CT scans of the nasal cavity should focus on the functional nasal volume, which excludes the frontal sinus and maxillary recess. The intranasal flow pattern differed from brachycephalic to mesaticephalic dogs, providing evidence for intranasal surgical interventions to be patient-specific, targeting anatomical locations where there is significant pressure drop.

THE LIFELONG EFFECTS OF EARLY LIFE ADVERSITY ON BONE MARROW-DERIVED MAST CELL PROGENITORS

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Early life adversity (ELA) is a risk factor in the onset and exacerbation of inflammatory disorders later in life. Mast cells (MC) are innate immune cells that play a key role in driving inflammation. Our previous studies using murine and porcine models have shown MCs displaying a hyperactive phenotype in adult animals exposed to ELA, exhibiting increased serum histamine levels, tissue MC degranulation, colonic tryptase and heightened MC activation following adult chronic stress. However, the mechanism by which ELA induces MC hyperactivity in adulthood remains unknown. Our hypothesis is that ELA induces transcriptional changes on bone marrow derived MC progenitors programming them toward hyperactivity. Pups (C57BL/6) were either raised normal handled (NH) or subjected to 3 h of daily NMS plus early weaning (NMSEW). At 10 wks, bone-marrow derived MCs (BMMCs) from adult mice were harvested and stimulated with IgE DNP, IL33 and LPS to assess degranulation and cytokine release. BMMCs from NMSEW mice exhibited greater histamine and MC protease-1 release ($P<0.05$) upon IgEDNP stimulation. Higher IL6 release ($P<0.001$) upon IL33 stimulation and higher TNFα release ($P<0.05$) upon LPS stimulation. RNAseq analysis identified an upregulation of genes that encode for cytokines and receptors of the TNF family, toll-like receptors, metalloproteases, and genes related to MC storage and proteases. Taken together, these new data suggest there are ELA induced factors promoting long-lasting functional changes in MC progenitors. Future studies will investigate the mechanisms by which ELA drives lasting hyperactivity of MC progenitors and its role in MC-related disorders in adulthood.

EVALUATION OF THE EFFECTS OF ALPHA-CASOZEPINE ON CATS EXHIBITING ANXIETY IN THE SHELTER SETTING

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Shelter cats exhibiting signs of stress, such as hiding and freezing, are less likely to be adopted than those that appear friendly and approachable. Therefore, treating stressed shelter cats with anxiolytics may increase the likelihood of adoption. Controlled anxiolytics, such as gabapentin, are difficult to manage in the shelter environment, while over-the-counter anxiolytic nutraceuticals, such as Zylkene (alpha-casozepine), are readily available. The aim of this study was to evaluate the effectiveness of Zylkene in adult shelter cats. Twenty-seven cats exhibiting behavioral signs of stress as determined by the validated cat stress score (CSS) were enrolled in the study. Cats were randomly assigned to receive Zylkene or a placebo once daily for 14 days or until adoption. There was no difference in baseline CSS between treatment and control groups (Mann-Whitney U, $p = 0.25$). A researcher filmed approach to the cage once daily, and two blinded observers assessed the CSS from video. The majority of enrolled cats exhibited decreased stress over time (69% of treatment cats; 78% of control cats). Of the cats exhibiting a decline in CSS, three treatment cats (33%) and four control cats (36%) achieved a two-point decrease in CSS, which was deemed clinically meaningful a priori. Six treatment cats (46%) and eight control cats (57%) were adopted during the study (mean length of stay 6.8 and 6.0 days, respectively). Further statistical analysis is pending; however, based on preliminary data analysis, there is no clear benefit to Zylkene supplementation as observed in this population.

ELUCIDATING THE REGULON OF A FUR-LIKE PROTEIN IN *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS*

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Intracellular iron concentration must be tightly regulated for bacterial survival. *MAP* carries a putative metal transport operon that includes *MAP3773c*, a *fur*-like protein, on a genomic island. The objective of this study is to define functions of *MAP3773c,* in iron homeostasis. ChIP-seq was performed to identify the Fur regulon under iron replete and deplete conditions in-vitro. Sequences were analyzed using CLC Genomic Workbench. Confirmation of physical binding of *MAP3773c* to Fur box was carried out by EMSA, using a labeled Fur box and a recombinant *MAP* Fur-like protein. A total of 5,381 (replete) and 4,960 (deplete) binding sites of Fur on the *MAP* genome were identified. Applying a false discovery rate at ≤ 10^{-50} we homed in on 43 enriched regions (replete) localized either between (27%) or within ORFs (73%). In contrast, in a deplete condition, 11 enriched binding-sites within ORFs were identified. Four binding sites under both conditions simultaneously, located either between (75%) or within ORF (25%), were also identified. Binding was sensitive to iron availability. Under replete condition, Fur Box 2 (located between 4159132 and 4159456) site presented peak score 33.46 compared to 19.63 in box 1(located between 4158681 and 4158966), while in deplete condition the highest peak score of 38.57 was in Fur box 1, against 12.54 in box 2. EMSA showed that Fur-Fur box 1 binding is regulated by the availability of Mn^{2+} and a competitive binding assay confirmed specificity. Characterization of *MAP3773c* is ongoing. Fur binding to Fur box 1 and its iron dependence has been confirmed. Results generated from this project are expected to lead to a better understanding of iron regulation in *MAP*.

PROTEASE ACTIVITY OF MAP MARP ACROSS PH CONDITIONS

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Previous research has identified a gene encoding a serine protease, MarP*,* that contributes to the survival of *Mycobacterium tuberculosis* in hostile conditions, like those inside a phagosome after immune response. In order to understand the physiology of infection, this pathway must be further characterized. Kugadas *et al.* have identified a homologue of *marP* on the *MAP* genome. To characterize this homologue and confirm prior results, a study of the protease activity at different pH levels was performed on recombinant MarP that contributes to the intracellular survivability of *MAP* and other pathogenic mycobacteria. We posit that MarP will be most biologically active at the known physiologic pH and oxidative states inside phagosomes. MarP is expressed from a plasmid vector transformed into *E. coli*. Once the cultures are grown and protein expression induced, MarP is extracted by bacterial lysis and purified by immobilized metal affinity chromatography and confirmed by SDS-PAGE. After the proteins are extracted and purified, the Pierce Protease Activity Assay is used that quantifies protease activity by colorimetry. We anticipated that the MarP protein will show the greatest activity at pH and oxidative states similar to those found in maturing phagosomes. However, we were unable to optimize conditions that allowed us to test both the pH and oxidative states with the protease. This could have been from the buffers used in the assay or from self-digestion from MarP. For further direction, the colorimetric assay needs to be optimized for further evaluation.

FECAL MICROBIOTA FROM INFANTS INCREASED ALLERGIC RESPONSES TO HOUSE DUST MITE IN A MOUSE MODEL.

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Recent literature suggests that gut microbiota modulates adaptive and innate immune responses in the lungs and impacts allergic airway disease (AAD). We transplanted germ free mice with *Enterobacteriaceae*-dominant microbiota from eczemic infants (risk) or with *Bacteroidaceae*-dominant microbiota from noneczemic infants (protective). After transplantation, microbiota remained stable for at least two generations. We hypothesized that the offspring of mice transplanted with risk microbiota would exhibit increased allergy-associated responses, while offspring of mice transplanted with protective microbiota would exhibit decreased allergy-associated responses when exposed to house dust mite antigen (HDM) intranasally for 12 days. Mice of both groups were sensitized with HDM for 10 days and phenotyped at day 14. Lung function measurements were taken to evaluate airway resistance and compliance in response to a cholinergic agonist (Mch). Inflammatory responses were assessed through evaluation of lung inflammation, eosinophil infiltration in bronchoalveolar lavage fluid, and total and specific-HDM IgE levels in blood. Results showed mice carrying risk or protective microbiota had increased allergic responses to HDM represented mainly by significantly higher levels of total and specific-HDM IgE compared to control mice carrying conventional mouse microbiota. Exacerbated airway responses to Mch were seen in both groups compared to negative controls, similar to responses of positive controls; surprisingly, the “protective” microbiome group showed the highest responses. We conclude both putative risk and protected microbiotas are associated with AAD exacerbation. Further studies are needed to determine causal relationships between type of microbiota and AAD exacerbation.

EXPRESSION OF SETD2 AND MIR106B-5P IN CANINE DIFFUSE LARGE B-CELL LYMPHOMA

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Set-domain containing protein 2 (SETD2) is a tumor suppressor gene that is frequently lost or mutated in many cancers, including human and canine diffuse large B-cell lymphoma (DLBCL). SETD2 is a histone methyltransferase functioning in DNA damage response and transcriptional regulation. In addition to mutations, some human cancers exhibit negative regulation of SETD2 mRNA levels by miR106b-5p. Though previous research has shown that SETD2 is frequently mutated in canine DLBCL, mRNA levels and post-transcriptional regulation of this gene have not been investigated. To address this deficiency, we assessed expression of SETD2 and miR106b-5p in canine DLBCL cells, correlating these variables to ascertain whether this miR represents an alternative mechanism of SETD2 suppression in DLBCL. mRNA expression levels of SETD2 and miR106b-5p were compared across canine patient lymph node and liver samples using qPCR. Preliminary data did not show that miR106b-5p expression is negatively correlated to SETD2 in canine DLBCL samples, suggesting that miR106b-5p does not negatively regulate SETD2. Understanding the mechanisms behind inactivation of genes important to lymphomagenesis in DLBCL may lead to new approaches to control or treat this malignancy. In future investigations, we will assess posttranscriptional regulation of SETD2. We will also use SETD2 knockout cell lines to verify the mechanism by which this gene contributes to lymphoma progression and explore mechanisms by which we can exploit alterations in SETD2 therapeutically.

ANALYZING THE FECAL MICROBIOME IN CANINE LYMPHOMA PATIENTS UNDERGOING A MODIFIED CHOP CHEMOTHERAPY PROTOCOL

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Multicentric lymphoma is a common canine cancer often treated with the Cyclophosphamide, Hydroxydaunorubicin, Oncovin® [vincristine], and Prednisone (CHOP) protocol. Some dogs develop chemotherapy-induced gastrointestinal disease (CIGD) with nausea, vomiting, and diarrhea that can result in increased morbidity, decreased quality of life- and life-threatening conditions. Severe gut dysbiosis predisposes them to CIGD. We hypothesized 1) that specific chemotherapeutic agents decrease the richness of certain bacterial taxa, 2) that dogs with CIGD have a less diverse gut microbiome, and 3) that metronidazole treatment of CIGD further amplifies these effects leading to gut dysbiosis. This longitudinal study followed canine patients undergoing chemotherapy for multicentric lymphoma by analyzing their fecal microbiome collected before and at each week of the CHOP protocol. Fecal samples were analyzed by isolating DNA, confirming 16S DNA presence using Qubit and PCR, and sequencing the 16S rRNA gene. In the first ten patients, there was a trend found that clinical manifestation of CIGD results in a less diverse microbiome. Dogs that received antibiotics during chemotherapy had a significant decrease in microbiome diversity. In the bacterial taxa bar plots for individual dogs, there is a trend in taxa shifts during episodes of CIGD and antibiotic treatment. Future studies will determine if fecal transplants of healthy dog microbiota increase treatment success in dogs during CHOP treatment. As dogs are a suitable translational model for human lymphoma, the results are expected to apply to human patients.

EXPRESSION OF TOLL-LIKE RECEPTORS IN HUMAN PRIMARY ASTROCYTES

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Toll-like receptors (TLRs) are part of the innate immune system and play a role in activating the immune cell response. 10 TLR genes are expressed in humans, and each is able to recognize different ligands that are specific to various pathogens, including viruses such as HIV. Astrocytes are glial cells that are a component of the blood-brain barrier, but little is known about which TLRs, if any, they express. The interaction of astrocytes and activated monocytes is involved in the chronic neuroinflammation that is characteristic of HIV-associated neurocognitive disorder (HAND), a progressive disorder with symptoms similar to dementia. The cytokine IL-1β is produced by activated macrophages and is a mediator in the inflammatory response. We hypothesize that astrocytes stimulated with IL-1β will display a higher degree of TLR expression than the control group. Primary astrocytes were cultured on a T75 flask, which were then passaged into 6 T25 culture flasks. 3 of these flasks were left untreated and the remaining 3 flasks were stimulated with a 100 pg/mL solution of IL-1β. One flask of cells from each group was lysed at 6, 24, and 48 hr time points to extract their RNA. TLRs 2-5 and 7-9 were used as probes for qPCR, with IL-6 as a positive control to confirm that the cells were successfully stimulated with IL-1β. Astrocytes stimulated with IL-1β showed higher expression of TLRs 2 and 3 compared to the control group. This supports our hypothesis that IL-1β induces TLR expression in astrocytes. Understanding which toll-like receptors are expressed on astrocytes in the blood-brain barrier may lead to new therapeutic treatments for HAND.

OLEIC ACID SUPPLEMENTATION REDUCES FATTY ACID CATABOLISM IN ADIPOSE TISSUE FROM EARLY LACTATION DAIRY COWS.

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Introduction: Oleic acid (*cis*-9 C18:1; OA) supplementation to early lactation dairy cows improves lactation performance and reduces body weight loss and plasma NEFA levels. In rodents, feeding OA enhances lipogenesis in adipose tissue (AT). However, responses of dairy cow AT to OA supplementation are unknown. The objective of our study was to determine changes in AT transcription patterns in response to OA supplementation.
Methods: Twelve multiparous dairy cows were abomasally infused with 60g/d of OA (n = 6) or vehicle (n = 6) from 1 to 15 d after parturition. Subcutaneous AT samples were collected at −14, +6, and +12 d relative to parturition. RNA was extracted and bulk RNA sequencing analysis was performed. The resulting transcripts (Fragments per kilobase of exon model per million reads mapped, FPKM) were analyzed using Biojupies (Torre, Lachmann & Ma'ayan, 2018). Lipid metabolic processes altered by OA supplementation were analyzed for functional enrichment and network visualization using Cytoscape.
Results: At +6 and +12 d OA supplementation decreased the expression of genes involved in fatty acid (FA) beta-oxidation (+6 and +12 d), FA catabolic process (+6 and +12 d), FA oxidation (+6 d), and FA betaoxidation using acyl-CoA oxidase (+12 d).
Conclusions: These results demonstrate that OA supplementation decreases FA catabolism and AT lipolysis and therefore may reduce the risk to present metabolic disorders in early lactation.

RETROSPECTIVE EVALUATION OF THE USE OF DIURETICS IN ACUTE KIDNEY INJURY IN DOGS

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Acute kidney injury (AKI) is commonly encountered in veterinary medicine, with high mortality rates in patients with oliguria or anuria. Diuretics are frequently used in the management of anuric/oliguric renal failure. This study aimed to evaluate the use of mannitol and furosemide in dogs with AKI. Medical records were reviewed from 2012 to 2018 to identify dogs with AKI. Cases were included if they had a diagnosis of AKI, and a urinary catheter with documentation of UOP. Oliguria was defined as UOP between 0.51-1.0ml/kg/hr, and anuria was defined as UOP of 0-0.5ml/kg/hr. Four hundred and twenty-five dogs were diagnosed with AKI, and 110 met inclusion criteria (25.8%). Sixty-seven cases had oliguria (16/67) or anuria (52/67). Twenty-four dogs (21.8%) received furosemide, 3 (2.7%) received mannitol, 27 (24.5%) received both furosemide and mannitol, and 56 (50.9%) received no diuretics. UOP increased to >1ml/kg/hr within 24 hours in 54.1% (13/24) of dogs receiving furosemide alone, 0 dogs receiving mannitol alone, 18.5% (5/27) of dogs receiving both diuretics, and 50% (8/16) of dogs receiving no diuretics. Change in UOP at 12h was significantly increased (0.52ml/kg/hr, [range 0.0 – 5.62]) in anuric/oliguric dogs receiving furosemide alone, compared with 0.0ml/kg/hr (range 0- 4.69) in dogs receiving no diuretics (p = 0.0062). The overall mortality rate was 57.2%, compared to 71.6% in cases with oliguria/anuria. Oliguric and anuric renal failure carry significant mortality rates. Furosemide may increase UOP when administered to oliguric or anuric dogs, however the efficacy of diuretics and association with survival requires further prospective evaluation.

RELATIVE AGREEMENT OF TWO SURVEILLANCE SYSTEMS FOR *BORRELIA BURGDORFERI* IN AN EMERGENT STATE

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While active surveillance confirmed that populations of the blacklegged tick (*Ixodes scapularis*), vector of the Lyme disease bacterium (*Borrelia burgdorferi*), were established in the western Upper Peninsula and Lower Peninsula of Michigan by 2014, little was known about its distribution in the rest of the state. However, results from a national canine serological database surveying more than 150,000 dogs per year throughout Michigan indicated that the pathogen and vector were emerging in the eastern Lower Peninsula. Thus, a survey was conducted from spring 2015 – fall 2018 among Michigan's healthy canine population in cooperation with veterinary clinics and animal shelters to determine the extent of emergence of *B. burgdorferi* and *I. scapularis.* Information obtained for each dog included zip code of residence, signalment, history of travel, Lyme disease vaccination, and recent use of tick chemoprophylaxis. Ticks were assayed for *B. burgdorferi*, *B. miyamotoi*, and *Anaplasma phagocytophilum* via real time PCR. Over 1800 ticks were submitted from more than 7500 dogs, representing six tick species, with *Dermacentor variabilis* (the American dog tick, 71.6 %) and *I. scapularis* (24.0%) representing the majority of ticks (95.6%). Weighted kappa analysis was used to determine the relative agreement between canine *B. burgdorferi* serologic and tick surveys. Active canine tick and serosurveillance systems can be useful and economical methods for assessing the geographic distribution of tick-borne disease risk for both humans and companion animals in areas of both established and emerging tick populations and pathogens.

IDENTIFICATION OF A NOVEL MUTATION CAUSING PROGRESSIVE RETINAL ATROPHY IN SPANISH WATER DOGS

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As researchers and medical professionals continue to search for causes and cures for human blindness, animal models serve as an invaluable tool for discovery and eventual solution. Retinitis pigmentosa (RP), an inherited disease that affects nearly 200,000 people annually and its canine equivalent, progressive retinal atrophy (PRA) are characterized by degeneration of photoreceptor cells in the retina, leading to eventual blindness. PRA and RP are genetically heterogeneous diseases, which can present with autosomal recessive, autosomal dominant or X-linked modes of inheritance. In the study reported here, we investigated a newly recognized autosomal recessive PRA in the Spanish Water Dog (SWD, N=4) breed by whole genome sequencing. We compiled and filtered variants, cross-listing candidate genes with known expression in the retina. A 6 base-pair in-frame deletion in the gene *PDE6B* (p.Phe740_Trp741del) was identified within a highly conserved functional domain of the gene. Prediction software described this presumptive mutation to have a moderate to severe effect. Additional affected, unaffected, and known carrier SWDs were genotyped for the variant, which was found to segregate perfectly with disease status. This is the third mutation in *PDE6B* to be identified as a cause of PRA in dogs. A DNA-based test is now available for breeders to allow eradication of this form of PRA from the breed.

DIURNAL INTRAOCULAR PRESSURES (IOPS) IN DOGS WITH ADAMTS10-OPEN-ANGLE GLAUCOMA (ADAMTS10-OAG)

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Glaucoma is a leading cause of incurable blindness affecting the optic nerve. The most common form is open-angle glaucoma (OAG). Elevation of intraocular pressure (IOP) is a major risk factor. At the Michigan State University College of Veterinary Medicine, we house a unique, well-established and clinically relevant large animal model: dogs with OAG due to a G661R missense mutation in the ADAMTS10 gene. The purpose of this study was to perform a large-scale diurnal IOP screening in dogs with ADAMTS10-OAG as a function of age/disease and compare the findings with those of normal control dogs. Diurnal (8am,11:30am, 3:30pm) IOPs (via tonometry) were performed over a 7-year period on 37 ADAMTS10-mutant and 31 unaffected control dogs between the ages of 2 weeks and 7 years. Changes of diurnal and average daily IOPs were evaluated as a function of age by linear mixed models ANOVA, followed by pairwise comparison of consecutive means. In contrast to normal dogs with no major age and diurnal effects, there was a significant, gradual, age-related IOP increase in ADAMTS10-mutant dogs (LS mean +/- SEM): year1=19.0+/-0.6, year2=21.2+/-0.7, year3=23.4+/-1.0, year4=24.0+/-0.9, year5=25.2+/-1.0, and year6=29.5+/-1.4 mmHg ($p<0.05$). There was a significant diurnal effect between the ages of 2.7-4.8 years with IOPs being highest at 8am ($p<0.001$). Overall average normal IOP was 13.6+/- 1.6 mmHg. To date, this is the largest scale description of diurnal IOP in dogs with detailed documentation of gradual IOP increase with age and disease progression in ADAMTS10-mutant dogs, an important large animal model of human OAG.

COMPARISON OF THREE METHODS TO MEASURE HEART RATE IN A CANINE REHABILITATION SETTING

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Cardiovascular conditioning and assessment of cardiovascular risk is important in humans before exercising, but it is not routinely assessed in canine rehabilitation patients. The aim of the study was to compare the VET-Scout Holter Monitor (VETScout), the KER ClockIt Heart-Rate Monitor (ClockIt), and auscultation in measuring canine heart rates. We hypothesized that there would be no significant difference between the methods and that all would be easily performed in a clinical rehabilitation setting. Heart rate was simultaneously recorded in 3 adult healthy large breed dogs during rest, immediately before treadmill exercise, during walking on the treadmill (VET-Scout and ClockIt; 2.5 MPH for 6 minutes) and immediately following treadmill exercise. Statistical analysis included Spearman Correlations and repeated measures ANOVAs, with significance set at $P<0.05$. Only the VET-Scout was able to consistently record data at all time points. Although not significant, after 4[f1] [SS2] minutes of walking on the treadmill there was an average increase of 24 b.p.m. as compared to rest. Auscultation strongly correlated to Vet-Scout ($r=0.96$, $P<0.002$), and weakly to ClockIt ($r=-0.55$, $P<0.001$) at rest. At rest, Vet Scout and ClockIt had perfect agreement with each other (N=2), although they both overestimated heart rate as compared to auscultation. Auscultation was not able to obtain heart rate in motion, and ClockIt was inconsistent. Elevations in heart rates during exercise were not reflected in the immediately post-exercise auscultation. Therefore, the VET-Scout but not auscultation or ClockIt, could be used in a clinical setting to get accurate heart rates during exercise reflecting cardiac workload.

USING METABOLIC STRESS BIOMARKERS FOR PREDICTION OF COLOSTRUM QUANTITY AND QUALITY IN DAIRY CATTLE

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Prior to calving, cows mobilize immunoglobulins (Ig) to the udder for colostrum production. Bovine colostrum is exclusively responsible for passive transfer of immunity to calves. High quality and quantity of colostrum is therefore a critical prerequisite to neonatal health and future development. Preparturient factors, like metabolic stress, may influence colostrum development, yet colostrum can only be analyzed after parturition, and existing research only focuses on postpartum effects. This study's objective was to identify the potential of using metabolic stress blood indicators during the dry period to predict colostrum volume and Ig concentration. Serum concentrations of non-esterified fatty acids, Beta-hydroxybutyrate, glucose, albumin, calcium, haptoglobin, reactive oxygen species, and antioxidant potential were analyzed weekly from dry cows during the last 6 weeks of gestation to determine their degree of metabolic stress. Following parturition, colostrum volume was recorded and analyzed for IgG concentration ([IgG]). For each biomarker, cows were classified into "low" and "high" concentration groups (high volume > 8L, high [IgG] > 60g/dL). Colostrum volume and [IgG] were compared between groups using student t-test. Low serum BUN concentration and high cholesterol were significantly associated with low colostrum volume. Serum antioxidant potential was significantly associated with low colostrum [IgG]. The future of this study is to determine cut-off points for these biomarkers so producers can identify dry cows expected to not produce adequate colostrum. This would allow them to intervene with management changes to improve colostrum quality of that cow, thereby increasing transfer of passive immunity to calves and improving calf health.

ENDOTOXIN-INDUCED MUCOUS CELL METAPLASIA IS DEPENDENT ON INNATE LYMPHOID CELLS IN MICE

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Inhalation exposure to environmental pollutants, such as bacterial endotoxin (lipopolysaccharide; LPS), cause mucous overproduction and hypersecretion that may contribute to the severity of inflammatory airway diseases such as rhinitis and bronchiolitis. Airway responses to LPS include the accumulation of neutrophils, mucin gene expression and airway wall remodeling, resulting in a hypersecretory epithelium. In the present study, we determined quantitative and qualitative changes in inflammatory responses in mice after repeated exposure to LPS, as a short-term model of chronic obstructive pulmonary disease (COPD), to test the hypothesis that cellular remodeling in the airways is dependent on innate lymphoid cells (ILCs). To determine the role of T-, B-, and innate lymphocytes in the pathogenesis of LPS-induced mucous cell metaplasia (MCM), we compared responses in lymphoid cell-sufficient C57BL/6 mice, ILC-sufficient Rag2-/- mice (devoid of T- and B- cells), and ILCdeficient Rag2-/-Il2rg-/- mice (devoid of all lymphoid cells). C57BL/6 mice were exposed intranasally to 1 or 10 µg of E. coliderived LPS for 2, 4 or 9 installations. Rag2-/- and Rag2-/-Il2rg-/- knockout mice were given the same treatment for 9 consecutive weekdays. Airway exposure to LPS induced MCM in the lungs of ILC-sufficient mice only, (i.e., C57BL/6 and Rag2-/-). LPS exposure caused a decrease in Club Cell Secretory Protein (CCSP) in conducting airways and an increase in neutrophils in all mouse strains. These results suggest that LPS induced MCM in mice airways is dependent on ILCs, but is not associated with neutrophil inflammation.

DIESEL EXHAUST PARTICLE INDUCED TOXICITY ON NEURONS DERIVED FROM HEALTHY AND ALZHEIMER'S DISEASED HUMANS

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A progressive and irreversible disease, Alzheimer's disease (AD) poses a worldwide threat as the most common cause of dementia. While a genetic component, the apolipoprotein E4 allele (ApoE4), significantly increases the risk of developing AD, genetic causes alone represent less than half of all cases. Limited studies have examined gene-environment interaction as a causative factor responsible for the majority of AD cases. Diesel exhaust particles (DEP) are often used as a model for ambient air pollution, and preliminary studies support that exposure to DEP decreases neurogenesis and cognitive functions, which suggests a negative effect on neural progenitor cells and worsening of AD progression. A model using human induced pluripotent stem cells (hiPSCs) can thus be a useful tool in performing a toxicological risk assessment on the ApoE4 population susceptible to AD. I hypothesized that the ApoE4-derived cells would be more susceptible to DEP-mediated toxicity compared to the control, which would support a gene-environment interaction. To investigate the relationship between DEP and ApoE4 populations, I differentiated control and ApoE4 hiPSC-derived neural progenitor cells into neurons and verified maturity via immunocytochemistry. Next, I measured the effects of DEP on the production of nitric oxide and reactive oxygen species, markers of toxicity. Statistical analyses were performed using one-way and two-way ANOVA. The results indicate that although DEP caused dose-dependent toxicity, there was no difference in susceptibility between control neurons and ApoE4 neurons. This study provides preliminary data on how exposure to environmental toxicants, like DEP, can affect neurons in populations susceptible to AD.

CHARACTERIZATION OF PREGNANCY-ASSOCIATED CHANGES IN NAÏVE HUMAN AND RHESUS MACAQUE T CELL POPULATIONS

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The thymus is the site of T cell development and is critical for ensuring self-immune tolerance; however, little is known about its role in maternal-fetal tolerance during pregnancy. We have found that progesterone-induced thymic atrophy is required for normal pregnancy in mice, and we hypothesize that this is conserved across species. We will quantify T cell receptor excision circles (TREC) in women as a surrogate metric of thymic output. Additionally, a pilot experiment will be completed in collaboration with the Wisconsin National Primate Research Center (WNPRC) to determine the effect of overnight shipment of blood on TREC measurement in rhesus macaques. Blood samples were collected from nonpregnant and uncomplicated term pregnant women. Duplicate blood samples were collected from nonpregnant and pregnant rhesus macaques. Of the duplicates, one was shipped overnight at room temperature from WNPRC, and the other was subject to immediate isolation of peripheral blood mononuclear cells (PBMC) then shipped. PBMC from women and macaques were isolated using density gradient centrifugation. Eight-parameter flow cytometric analysis was used to characterize naïve T cells in human samples, and TREC were quantified in both human and macaque samples using commercially available qRT-PCR-based assays. TREC copies per ug DNA were not observed to be different in pregnant women ($p=0.4$) and rhesus macaques compared to nonpregnant individuals. Overnight shipment did not affect the ability to quantify TREC with the assay; therefore, future collaborative study with a larger macaque population is possible. While we did not observe a significant difference in naïve T cell phenotype expression nor TREC quantity in peripheral blood, future study may find a role of progesterone-induced thymic involution in maternal-fetal tolerance.

MOTILITY MUTATIONS AND LOSS OF THE σ54 REGULON DURING EXPERIMENTAL EVOLUTION OF *CAMPYLOBACTER JEJUNI*

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Previous work showed the importance of slipstrand mutation in adaptation of *C. jejuni* to a novel murine host. We hypothesized that other genetic mechanisms would also allow adaptation of this highly mutable organism to a novel environment. An experimental evolution study was conducted by: 1) evolving mammalian hostadapted *C. jejuni* experimentally by serial passage in rich broth medium and observing phenotypic changes by motility assay; 2) re-sequencing all final evolved populations to assess genetic variation, and 3) using colony PCR assay to confirm these ORFdisrupting mutations in genes over the 35-day time course.

Experimental evolution of *C. jejuni* in rich broth medium produced loss of flagellar motility—an essential function for efficient host colonization. Motility assays showed that motility was irreversibly lost in 5 independently evolved populations after 35 days. Genome re-sequencing analysis revealed numerous disruptive mutations to regulatory genes in the *C. jejuni* flagellar transcriptional cascade, including genes known to affect expression of the σ54 (*rpo*N) regulon and chromosomal deletion of all or part of the *rpo*N locus in all evolved lines. Parallel loss of the σ54 regulon and accumulation of non-revertible mutations over the course of the experiment was confirmed by colony PCR. A phase variable (reversible) motility mutant was deficient for colonization in a murine disease model. These results suggest a stepwise mechanism of *C. jejuni* genetic adaptation to a novel environment by high rate, small mutations for rapid phenotype selection, followed by irreversible large genetic deletions during prolonged *in vitro* selective pressure.

EFFECT OF STIRRUP IRON STYLE ON FORCE EXERTED ON THE STIRRUP AND RIDER POSITION

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The stirrup iron is a major point of contact between horse and rider, influencing the forces experienced during exercise. Many stirrup designs are available but the effect of different configurations remains anecdotal. Improved rider position and decreased forces on the stirrups would directly impact the comfort and performance of horses and riders. Four riders (one advanced, two intermediate, and one beginner) rode with three types of iron: traditional (T), flexible (F), and flexible and rotatable (FAR). At the highest and lowest points of the posting trot, the hip, knee, and ankle angles and toe position from film and the normal force exerted on force sensors located on the stirrups' tread were evaluated; rider preference for type of irons was assessed. Bilateral forces on the stirrup were described for the first time at the trot and canter. No significant difference was seen in joint angles, toe position, or forces between the types of irons. Riders were generally asymmetrical in the right vs left joints, which may have been compensatory for chronic pain. The highest forces always occurred at the highest part of the post. The advanced rider had the lowest peak forces; the beginner rider had the highest forces at the lowest part of the post. Less experienced riders applied force to one stirrup before the other. 3/4 riders preferred the FAR irons but advanced experienced the highest forces with the FAR. Stirrup style does not appear to significantly impact rider position or the forces experienced by horse and rider.

UTERINE CARUNCLE IMMUNE PHENOTYPE IS MODULATED BY COLONY STIMULATING FACTORS IN DAIRY COWS

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Uterine diseases affect 25% of dairy cows in the US and often develop from retention of the placenta (RP). One of the major causes of RP is an impairment of the inflammatory responses at the uterine-placenta junction. The malfunction of certain components of the immune system, including macrophages and neutrophils, appears to trigger RP. Immunomodulatory cytokines such as granulocyte colony stimulating factor (G-CSF) and granulocyte macrophage CSF (GM CSF) improve phagocytic cells function and increase their circulating numbers in dairy cows. Transcriptional studies were performed to determine the effects of G-CSF and GM-CSF on the gene expression of markers of immune cell phenotype and function at the uterine-placenta junction. Uterine caruncles were collected from multiparous cows (n=7) at 1-2 hours after calving. Caruncle apexes were dissected and then exposed to 50-500 ng/gram of caruncle of G-CSF (recombinant bovine CSF, Elanco Animal Health) and GM-CSF (recombinant bovine GM-CSF, Kingfisher Biotech) and a vehicle control (CON) for 3 hours at 37 oC. Caruncles exposed to G-CSF exhibited enhanced expression of the neutrophil activity marker MPO (myeloperoxidase) compared to those exposed to GM-CSF and CON. G-CSF also enhanced the expression of the chemokine IL8 and the T-effector cell related marker CD8a. These findings suggest that G-CSF may promote uterine inflammatory responses by attracting not only neutrophils but also M1 macrophages.

RISK STRATIFICATION IN DOGS WITH SEPTIC SOFT TISSUE INFECTIONS

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Use of the quick Sepsis Related Organ Failure Assessment (qSOFA) score has been proposed in human medicine to rapidly identify septic patients at greater risk for poor outcomes via assessing 3 clinical criteria: systolic blood pressure <100mmHg, respiratory rate > 22, and altered mental status. Bite wounds are a common reason for admission of dogs to veterinary clinics. The purpose of this study was to assess the prognostic utility qSOFA score for prediction of in-hospital mortality among dogs with septic soft tissue infections. Medical records of dogs diagnosed with septic soft tissue infections between 2011-2018 at Michigan State University were reviewed. Inclusion criteria were: diagnosis of septic or necrotizing soft tissue infection (positive culture) in addition to having wound management performed. Signalment, source of infection, length of hospitalization (LOH), vital signs, blood pressure, vasopressor use, and culture growth were recorded, and qSOFA scores calculated. Thirty-six patients met inclusion criteria. Overall in-hospital mortality was 11%. Admission qSOFA scores were significantly different between patients who survived to discharge (median 1, range 0-3) compared with those who died or were euthanized (median 2.5, range 2-3)($p < 0.002$). Admission qSOFA ≥2 was associated with a higher rate of inhospital morbidity and mortality within this study population, suggesting that qSOFA scores may have utility in risk stratification to identify patients at greater risk of poor outcome, however further prospective studies are necessary.

MILK PRODUCTION AND DISEASE ARE ASSOCIATED WITH SELECT FAT-SOLUBLE VITAMINS IN THE PERIPARTURIENT PERIOD

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Current dietary vitamin supplementation recommendations for dairy cows are based on research primarily focused on avoiding deficiency and promoting production rather than health. The objective of this study was to analyze serum concentrations of retinol (RET), α-tocopherol (AT), and β-carotene (BC) in dairy cows on commercial farms and their association to diseases in the periparturient period. Cows (n=353) from 5 commercial dairy herds were enrolled over a 3-year period. Blood samples were collected at dry off (DO; -48±12d pre-calving), close-up (CU;-17±7d pre-calving), and fresh (7±3d post-calving) and analyzed for serum RET, AT, BC, and cholesterol. The health status of each cow was monitored during the study period up to 30 days in milk. Negative health outcomes included milk fever, mastitis, metritis, retained placenta, lameness, displaced abomasum, hyperketonuria, abortion, and pneumonia. A Pearson correlation analyses was performed to assess the correlation between vitamins at each sample point. A linear mixed model was built to describe changes in vitamins over time. Mixed logistic regression models were built for each disease outcome. Increased ME305 was positively correlated with ATCR. Serum BC was associated with higher ME305 in 3+ parity cows only. An increased serum RET was associated with a reduced risk for hyperketonuria. Additionally, higher serum RET at C+7 was associated with lower risk for uterine disease. Serum concentrations of RET, ATCR, and BC may be important biomarkers for fresh cow diseases. Our future research goals are to establish serum vitamin cut-off values which optimize health.

IDENTIFYING RISK AND PROTECTIVE GUT MICROBIOMES AND METABOLOMES IN A MOUSE MODEL OF ASTHMA

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Asthma is a chronic disease with inflammation and constriction of airways, leading to wheeze, cough and airway constriction. Asthma risk factors include family history, obesity, air pollution, and smoking. Recently, gut microbiome was associated with risk for asthma, but the exact etiology is unknown. We transplanted germ free mice with human stool from infants with and without eczema to study effects of the early microbiome on development of asthma because eczema often precedes asthma. We hypothesized that mice transplanted with eczema positive infant feces will have increased asthmatic responses compared to mice transplanted with eczema negative infant feces. Mice from each group along with a negative control mouse microbiome group were sensitized with house dust mite allergen for 12 days followed by phenotyping for lung function and immune responses at 14 days. No significant differences were seen in lung function or cytokine responses between eczema positive or negative groups, however both of these groups had significantly decreased lung function and elevated Type 2 cytokine compared to the mouse microbiota negative control group. Thus, both eczema positive or negative microbiomes were associated with risk for asthma. Based on 16S rRNA sequencing, eczema positive or negative groups had significantly different microbiome composition and diversity; microbiome composition were also distinct from the controls. 16S rRNA sequences analyzed to predict bacterial metabolic outputs using PAPRICA showed panels of metabolites associated with risk and protection from asthma in mice. More work is needed using Koch's postulates to determine how bacterial taxa and metabolites mediate asthma.

LIVESTOCK-DERIVED PM2.5 DUST EXPOSURE MODULATES ALLERGIC INFLAMMATORY RESPONSES IN MICE

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Airborne fine particulate matter (PM2.5) derived from livestock farming is a combination of dusts, biogenic materials and anthropogenic emissions. Allergic individuals that work in or live near high density animal operations may be at risk for adverse health effects associated with PM exposure. We tested the hypothesis that exacerbation of allergic airway responses by inhaled livestock farm particles is dose-and-source-dependent. We compared PM2.5 collected from chicken, pig, and goat farms in the Netherlands. Female BALB/c mice were sensitized and boosted with ovalbumin (OVA; days 0, 10, respectively), and then challenged with intranasal OVA for 2 consecutive days (days 17,18) prior to a single intranasal exposure to 0, 0.9, or 3 µg of farm-derived PM2.5. Twenty-four hours later bronchoalveolar lavage fluid (BALF) was collected for cell analysis and lung tissues were processed for light microscopy to analyze eosinophil density and intraepithelial mucosubstances (IM). OVA sensitization and challenge induced allergic airway inflammation, indicated by accumulation of eosinophils in BALF and lung tissues, and increased IM in conducting airways. PM2.5 alone had no adverse effects in non-allergic mice, but increased BALF and tissue eosinophils in allergic mice (goat >>pig >chicken PM). Chicken farm-derived particles dose dependently enhanced IgE (chicken>>goat>pig), while particles from goat farms were most potent to enhance OVA-induced IM. Microbiome analysis of PM samples revealed distinct bacterial and fungal signatures across livestock sources. Our results suggest that modulation of allergic airway inflammatory responses by livestock-farm associated- PM2.5 may be related to airborne biogenic components unique to each farm.

UPSTREAM REGULATORS OF GENE EXPRESSION INFLUENCING GLYCOGEN REPLETION

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Skeletal muscle glycogen is critical for a horse's successful performance; however, for unknown reasons, replenishment of muscle glycogen following exercise occurs at a slow rate in horses. Our goal was to identify upstream regulators (UR) of genes with altered expression during glycogen repletion. Two isocaloric diets, high- starch (HSD), or low- starch, high- fat (LSD) concentrates were used to elucidate factors impacting glycogen resynthesis. Five Thoroughbred horses fed diets in a cross- over design, exercised to deplete glycogen by 30% then rested on feed for 72h to allow glycogen repletion. Glycogen at 72h- repletion was HSD>LSD. Total RNA and proteins were extracted from gluteal muscle biopsies at pre-depletion, and 72h. Whole gene and protein expression were quantified with Illumina HiSeq4000 and TMT LC/MS/MS, respectively, and mapped to EquCab3.0. Pre- 72h differential expression (DE), determined through weighted least squares/permutation test (FDR≤0.05), identified 240 DE genes in HSD and 324 in LSD, one DE protein in HSD and 53 in LSD. A transcription factor enrichment analysis (RcisTarget) identified 97 enriched UR in the HSD and 77 in LSD (NES≤3.0). Among the UR, only *AKR1A1* and *PGAM2* showed altered expression in both transcriptome and proteome for both diets. KEGG pathways enriched for the target genes of *AKR1A1/PGAM2* include insulin and AMPK signaling (FDR≤0.05), which impact insulin sensitivity, glucose uptake, and glycogen resynthesis. The LSD had greater impact, showing downregulation of target genes involved in glucose (GLUT4) translocation to the cell membrane, which may explain the slow glycogen resynthesis observed for this treatment.

VALIDATION OF CHEMILUMINESCENT ASSAYS FOR MEASUREMENT OF CANINE TSH IN RED PANDAS (*AILURUS FULGENS FULGENS*)

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Red pandas (*Ailurus fulgens fulgens*) are a common species in zoos and, despite their anatomical and physiological specializations, are oftentimes equated with domestic canines and felines for diagnostic tests. Thyroid abnormalities have been anecdotally reported in red pandas. A key diagnostic test for thyroid dysfunction in domestic canines and felines is serum TSH testing. While the canine thyroid stimulating hormone (cTSH) assay has been validated in both domestic canines and felines, there has been no validation of cTSH assays in red pandas. Definitive diagnosis of thyroid dysfunction in red pandas has been hindered by the lack of valid tests. This study aims to validate canine chemiluminescent assays for TSH in red pandas. Validation was tested via serial dilutions of samples and both inter- and intra-assay testing. Based on the data acquired, the cTSH assay is valid in red pandas and can be utilized to accurately detect thyroid dysfunction.

BIOACTIVITY OF THE ENDOCANNABINOID ANANDAMIDE IN CULTURED BOVINE ENDOTHELIAL CELLS

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The period from parturition, throughout lactation, places increased metabolic demands on dairy cattle and increases the risk of disease due a dysregulated immune response. Increased plasma levels of the endocannabinoid (EC) anandamide, or arachidonoylethanolamide (AEA), from early to late lactation in Holstein dairy cows, is accompanied with early lactation downregulation of the hypothalamic fatty acid amide hydrolase (FAAH), which metabolizes AEA into arachidonic acid (AA) and ethanolamide. Meanwhile, the AEA catalyzing enzyme N-acyl phosphatidylethanolamine-specific phospholipase D is upregulated in the hypothalamus during early lactation. The EC system has been implicated in regulation of feed intake in other mammals and proliferation of human endothelial cells. AEA is also metabolized by the cyclooxygenase-2 enzyme to bioactive prostaglandin -ethanolamides. The purpose of this study was to establish the effect of AEA on bovine aortic (BAEC) and mammary endothelial cells (BMEC). Both cell lines were challenged with lipopolysaccharide (LPS) and treated with AEA. Electric cell-substrate impedance sensing (ECIS) was utilized to establish effects on networking over a 24-hour period. Viability was established using a fluorescent plate reader, with treatment times of 1, 6, and 12 hours. Both cell types had increased networking for concentrations <1 μM, with the largest treatment effect at 1-hour. Similarly, both cell types had increased viability during the LPS challenge, but only at the 1-hour time point. Tightly regulated control of AEA concentration is needed to enhance endothelial cell networking and viability. Further research into the full biological effects, including local and systemic regulatory mechanisms of AEA in bovine, is warranted.

IDENTIFICATION OF *MYCOBACTERIUM TUBERCULOSIS* COMPLEX SPECIFIC BIOMARKERS IN NATURALLY INFECTED ASIATIC ELEPHANTS

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Asiatic elephants are a classic case of interspecies transmission that usually get infected with tuberculosis due to their close contact with their mahout's suffering from tuberculosis. A critical need exists for rapid and inexpensive diagnostics capable of detecting and differentiating Mycobacterium tuberculosis complex (MTBC) infection from other pathogenic and environmental mycobacteria to assist in earlier treatment and reduced spread of the disease. Pathogen-specific biomarkers, Pks5, MB2515c and MB1895c, were previously identified and validated in cattle, deer, goat and primate serum samples that were experimentally and naturally infected with *Mycobacterium bovis*. In the current study, these three MTBC proteins were selected for validation in elephant serum. We had access to 26 randomly selected positive (11) and negative samples (15) from the serum bank at Michigan State University where MAPIA and necropsy results ascertained tuberculosis status in the animals. Using a highly sensitive tool of indirect ELISA, we optimized the protocol for all three biomarkers with cattle sera being our positive control and tested for the presence of these circulating proteins. ELISA performed for the three proteins have revealed elevated levels in all MAPIA negative as well as MAPIA positive elephants, suggesting these animals to have been infected with MTBC infection even though the antibody levels in them were not adequate at that time to be detected as positive by MAPIA. The detection of our pathogen-specific biomarkers in MAPIA and necropsy positive animals validates our use of these biomarkers in diagnosis of tuberculosis.

WEANING AND POSTNATAL AGE INFLUENCE THE EARLY TIME COURSE AND NATURE OF INTESTINAL MAST CELL ACTIVATION IN A PORCINE MODEL OF EARLY LIFE ADVERSITY

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Early life adversity (ELA) is a risk factor for later life emergence of functional and inflammatory GI disorders in people and animals. In this study we compared the early GI immune responses in piglets exposed to early weaning (EW) at 16-18 d of age, a form of ELA we've previously shown result in altered GI developmental and health trajectories similar to ELA in humans, with that of late weaned piglets (LW:28 d of wean age). Mast cells (MC), a critical innate immune cell activated by stress and modulator of GI immune regulation, were activated early and differentially expressed in EW and LW pigs. Compared with LW piglets, EW piglets exhibited higher levels of plasma histamine during the first 1h post-weaning which coincided with a no significant change in histamine degrading enzymes DAO and HNMT in the intestinal mucosa. Mast cell tryptase (MCT7) and chymase (CMA1) gene expression were downregulated regulated in ileal mucosa (within 3h post-weaning) in EW; however, LW piglets exhibited a greater induction of chymase expression, compared to EW piglets. These data show weaning stress in piglets induces early GI MC activation which precedes immune cell recruitment and intestinal inflammation and age have a significant impact on the level and nature of this response. Understanding how EW and LW differentially regulate early GI immune responses may lead to a more mechanistic understanding of GI and immune disorders linked with ELA.

OCULAR BIOMETRY AND RETINOSCOPY *ADAMTS-10*-MUTANT DOGS

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A G661R missense mutation in *ADAMTS10* causes open-angle glaucoma (OAG) in beagle dogs, a well-established model for human OAG. This mutation has also been associated with lens zonular dysplasia and abnormal scleral collagen microstructure. The purpose of this study was to determine if *ADAMTS10*-mutant dogs have refractive errors and abnormal ocular dimensions.

Methods. In Part 1, refractive errors were measured in 38 male and female dogs (27 mutant, 11 normal controls; ages 2.48 +/- 1.37 years) by streak retinoscopy. In Part 2, 17 of these dogs were studied in more detail by performing A-scan ultrasound biometry both at the ages of ~1 and ~3 years for the measurement of their eyes' axial length (AXL), anterior chamber depth (ACD), lens thickness (LT), and vitreal chamber depth (VCD). Outcome measures were compared between *ADAMTS10*-mutant and normal controls by Student's t-test.

Results. In Part 1, we found significant myopia in *ADAMTS10*-mutant eyes with a mean refractive error of -5.4 +/- 1.8 diopters (D) compared to -0.4 +/- 0.8 D in normal controls ($p<2.2E-16$). In Part 2, A-scan ultrasound biometry showed that the mutant eyes were significantly longer with mean AXL of 21.4 +/- 0.6 mm compared to 20.8 +/- 0.4 mm in normal controls ($p<1.298E-4$). This difference could be explained by the deeper ACD (4.5 +/- 0.4 vs. 4.3 +/- 0.5 mm) and VCD (9.5 +/- 0.5 vs. 9.3 +/- 0.4 mm) in mutant eyes. LT was similar between mutants and controls (7.2 +/- 0.5 vs. 7.2 +/- 0.2 mm). The G661R missense mutation in *ADAMTS10* results in a significant elongation of the eye compared to normal, age-matched dogs, resulting in significant myopia.

A NOVEL THERAPEUTIC STRATEGY FOR OSTEOSARCOMA: TARGETING C-MYC DEGRADATION

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Osteosarcoma (OS) is the most common bone tumor in both humans and dogs and has a nearly ten-fold higher incidence in dogs than humans. There is a great need for novel therapeutic strategies, as survival rates have not improved much in the last decades. In this study, we evaluated using a novel proteasome modulator, TCH-165, a first in its class compound which induces c-MYC degradation, critical for both initiation and maintenance of tumors and compared it with convention treatments in *in vitro* studies. We demonstrated dose dependent decrease of c-MYC protein expression with TCH-165 treatment. Utilizing Seven canine OS cell lines and three human OS cell line, we determined that the IC_{50} values for TCH-165 range from 2.2 to 11.6 mM in canine OS, 3.1 to 4.5 mM in human OS cell lines, while $IC_{50}$of several primary canine fibroblasts ranged from 22.3 mM to >200mM, suggesting a wide margin of safety. In cell cycle studies, we showed that TCH-165 resulted in G1 arrest after 12 hours of incubation. In addition, we examined the potential of combing TCH-165 with doxorubicin, one of the standard anti-tumor drugs for OS. Combination index calculations using TCH-165 and doxorubicin in the canine D17 cell line support synergistic effects of this combination. The data point to potential novel avenues for treatment of OS which can be further examined in proof of concept studies in dogs with OS, serving as a relevant translational model, and accelerate drug development for human OS patients.