UNIVERSITY OF GEORGIA
College of Veterinary Medicine

Steeve Giguère Science of Veterinary Medicine
symposium

October 10, 2019

University of Georgia

College of Veterinary Medicine

Contents

About the Symposium

Veterinary medicine and veterinary research have critical roles in both animal and human health. While caring for animals is inherent to the profession, veterinary professionals have a much broader impact. By improving the health and welfare of our companion animals, agricultural animals and wildlife animals, as well as by expanding our understanding of animals in health and disease, veterinarians and veterinarian scientists directly and indirectly impact public health. Moreover, veterinarians and research conducted at veterinary institutions are critical to the One Health concept, which recognizes that human, animal and environmental health are interconnected. The University of Georgia College of Veterinary Medicine is home to a spectrum of research training programs for the next generation of veterinary medicine practitioners and researchers. Each year we highlight the cutting edge research conducted in these programs at the Science of Veterinary Medicine Symposium. The symposium provides an opportunity for research trainees at all levels to present their work to faculty and students from across the College of Veterinary Medicine community. The symposium also provides first and second year veterinary students the opportunity to explore the scope of veterinary research and the career opportunities available to them while learning about the rigor involved in scientific inquiry. Each year The Symposium also features a nationally recognized Keynote Speaker from industry, academia or government.

Dr. Steeve Giguère

We are very pleased to announce that from this year forward, the annual SVMS will be officially renamed **"The Steeve Giguère Science of Veterinary Medicine Symposium"** in honor of Dr. Steeve Giguère, former faculty member in the Department of Large Animal Medicine and Marquerite Thomas Hodgson Chair of Equine Studies who passed away suddenly in 2018. Dr. Giguère was, and will remain, a world-renowned researcher in equine infectious diseases and comparative immunology, particularly the development, treatment and prevention of *Rhodococcus equi* pneumonia in foals. His research endeavors and his dedication to sharing his knowledge were deservedly recognized; he received multiple teaching and research awards throughout his career, including the Carl Nordern-Pfizer Distinguished Teaching Award in 2006, the Intervet/Schering Plough World Equine Association Applied Equine Research Award in 2009, and the Zoetis Award for Research Excellence in 2017. He led by example to steer his graduate students and colleagues towards collegiality, research success, and excellence in teaching and clinical practice, and was the epitome of the "clinician scientist" and a true advocate for training veterinary students and residents in evidenced-based medicine and research methods. We are so proud that the Symposium will honor Dr. Giguère's memory and his contributions to the UGA CVM for years to come.

Keynote Speaker

Noah Cohen, VMD, MPH, PhD, DACVIM (Large Animal) is a professor of large animal internal medicine and the Patsy Link Chair in Equine Research at Texas A&M College of Veterinary Medicine & Biomedical Sciences. He also serves as Associate Department Head for Research and Graduate Studies in the Department of Clinical Sciences, and as Director of the Equine Infectious Disease Laboratory at Texas A&M. Dr. Cohen is a longtime friend and collaborator of Dr. Steeve Giguère, and currently also serves as an adjunct professor in the Department of Large Animal Medicine here at the University of Georgia College of Veterinary Medicine. In this role, he continues to closely collaborate with and mentor UGA students and faculty as they work to carry forward Dr. Giguère's legacy of excellence in equine research.

Dr. Cohen received his VMD from the University of Pennsylvania in 1983, and then spent two years in private equine practice in Toronto, Ontario, Canada before completing an MPH (1986) and PhD in epidemiology (1988) at Johns Hopkins University. He then completed a large animal internal medicine residency at Texas A&M, prior to joining the faculty there as an assistant professor in 1991. Dr. Cohen's research interests center on infectious disease epidemiology with emphasis on enteric and respiratory tract bacterial pathogens in horses. His work has made great strides in the prevention and treatment of Rhodococcus equi pneumonia, which can cause severe disease and death in young foals. Dr. Cohen has mentored over 50 graduate students, and his contributions to veterinary research and graduate training have been acknowledged with numerous awards, including the 2013 American Association of Equine Practitioners' Frank J. Milne Honorary Lecture, the 2015 Texas Veterinary Medical Association's Research Award, the 2018 Texas A&M University Distinguished Service Award for Graduate Mentoring, and the 2019 AVMA Clinical Research Award. The AVMA selected Cohen for this latter award because "his knowledge and expertise in the design and analysis of research and clinical trials is well regarded, and he is renowned nationally and internationally in the field of equine health." He has contributed to over 283 publications but considers his most important professional accomplishment to be mentoring students to become successful clinician-scientists.

TIME	LOCATION	EVENT/SPEAKER
8:00 - 8:10 AM	Room H237	**Welcome Address – Dean Lisa K. Nolan and Dr. Kelsey Hart**
8:10 - 9:19 AM	Room H237	**Keynote Presentation - "Falsely positive: why we overestimate what we know"** **Noah Cohen,** VMD, MPH, PhD, DACVIM Texas A&M University, College of Veterinary Medicine and Biomedical Sciences
9:15 - 10:30 AM		**Oral Presentations - Concurrent Sessions I-IV**
Session I: Room H237 Veterinary & Undergraduate Students		**9:15 / Chase Connelly** - Impact of two equine peripheral blood leukocyte enrichment techniques on leukocyte cell recovery, purity, viability, cell surface phenotype, proliferation and TNFα concentration
		9:30 / Bryce Golsen - Preliminary analysis of IBD-like disease and metabolic bone disease in MIT's marmoset (*Callithrix jacchus*) colony
		9:45 / Caroline Hawkins - Investigating the role of equine platelet lysate on wound healing in vitro
		10:00 / Shenise Howard - Comparison of respiratory stimulants during laryngeal exam in canines
		10:15 / Jazz Stephens - Random, Real Time Sequencing of Avian Avulaviruses Isolated From Wild Bird Samples
Session II: Room H203 Graduate Students		**9:15 / Jongsuk Mo** - Mutation E48K in the PB1 gene improves stability of a temperature-sensitive Influenza B live attenuated vaccine and maintains efficacy against homologous challenge.
		9:30 / Brittany Seibert - Development of a swine RNA polymerase I driven influenza reverse genetics system for the rescue of swine, avian and human origin influenza A and influenza B viruses
		9:45 / Alejandro Hoyos-Jaramillo - Health status and endoscopic evaluation of the upper respiratory tract of dairy bull calves inoculated with BVDV2 and BHV1 after vaccination and trace minerals injection
		10:00 / Jacquline Risalvato - A single dose of recombinant PIV5 expressing VP1 of Norovirus protected against human Norovirus challenge
		10:15 / Maria Huertas-Diaz - A parainfluenza virus 5 (PIV5)-based vaccine protects mice against lethal challenge with wild-type strains of *Burkholderia mallei* and *Burkholderia pseudomallei*
Session III: Room 363 Graduate Students		**9:15 / Laura Huber** - Prevalence and Genetic variability of resistant *Rhodococcus equi* in Horse-breeding Farms in Kentucky, USA.
		9:30 / Kate Sabey - Coinfection shapes how pathogens interact with the gut microbiota
		9:45 / Alec Thompson - Development of a diagnostic assay for detection and differentiation of *Theileria* species in white-tailed deer
		10:00 / James Collins - Resistance to fenbendazole in *Ascaridia dissimilis* and its economic impact on the production of turkeys
		10:15 / Pablo David Jimenez Castro - Multiple drug resistance in *Ancylostoma caninum*: an emerging threat to canine health
Session IV: Room 311 Graduate Students		**9:15 / Ahmed Hikal** - The mechanism of copper acquisition by pathogenic mycobacteria
		9:30 / Aline De Oliveira - Assessing the role of the Type 6 Secretion System (T6SS) in extraintestinal pathogenic *Escherichia coli*
		9:45 / Morgan Adkins - Evaluation of serum metabolic parameters as predictors of bovine respiratory disease events in high-risk beef stocker calves
		10:00 / Kara Wyatt - Regulation of early innate immunity to influenza virus within the respiratory mucosa by the serine-threonine kinase, Tumor Progression Locus 2
		10:15 / Ashley Beavis - The roles of serine and threonine residues of Respiratory Syncytial Virus phosphoprotein on viral transcription and replication efficiency
10:40 - 11:40 AM	Reading Room	**Poster Presentations I: odd-numbered posters**
11:40 - 12:40 PM	Coliseum	**Speed Networking - All 1st Year Veterinary Students**
	Free	**Lunch**

TIME	LOCATION	EVENT/SPEAKER
12:50 - 1:50 PM	Coliseum	Speed Networking - All 2nd Year Veterinary Students
	Free	Lunch
2:00 - 3:00 PM	Reading Room	Poster Presentations II: even-numbered posters
3:00 - 4:15 PM		Oral Presentations - Concurrent Sessions V-VIII
Session V: Room H237 Veterinary & Undergraduate Students		**3:00 / Case Hillier** - Using VacSIM® to deliver and stabilize VLP-based vaccines for Ebola Virus vaccines
		3:15 / Lyle Kotsch - Developing PIV5-based AVLP vaccines for Burkholderia mallei and *Burkholderia pseudomallei*
		3:30 / Helen Jones - A survey of state regulations for Brucella testing, RB51 vaccination requirements, and legible tattoo standards in cattle and domestic bison across the United States
		3:45 / Maria Eugenia Orbay-Cerrato - Single-cell transcriptome analysis and 3D light sheet imaging of feline spermatogenesis
		4:00 / Fred Torpy - Efficacy of a thermostable dry powder live attenuated influenza virus in the ferret model
Session VI: Room H203 Residents & PostDocs		**3:00 / Chanel Berns** - Single pedicle advancement flap technique as a novel approach to feline stenotic nares: 4 cases
		3:15 / Leah Moody - Albumin in tear film decreases the bioavailability of topical tropicamide and latanoprost in dogs
		3:30 / Cynthia Xue - Pharmacokinetics of pergolide mesylate in donkeys (*Equus africanus asinus*)
		3:45 / Rodrigo Abreu - Longitudinal Assessment of Memory B cell and Plasmablast Reactivity Against Influenza A in Subjects of Varying Age
		4:00 / Sonsiray Alvarez-Narvaez - A common practice of widespread antimicrobial use in horse production promotes multi-drug resistance
Session VII: Room 363 Graduate Students		**3:00 / Sarah Vaughn** - Associations between systemic oxidative stress and endocrine parameters in horses and ponies
		3:15 / Tara Denley - Effect of diphenhydramine and cetirizine on immediate- and late-phase cutaneous allergic reactions in healthy dogs: a randomized, double-blinded crossover study
		3:30 / Amanda Blubaugh - Comparative transcriptomic disease profiles of canine atopic dermatitis and canine models of house dust mite- and IgE-induced skin lesions
		3:45 / Ileia Scheibe - Characterization of the IgE-independent pro-inflammatory and pruritogenic transcriptome of compound 48/80-mediated skin lesions in healthy dogs
		4:00 / Emily Cook - Efficacy of disinfectant wipes for reducing bacterial contamination on stethoscopes in a veterinary teaching hospital
Session VIII: Room 311 Graduate Students		**3:00 / Kelsey Robinson** - Transcriptome analysis of necrotizing and granulomatous meningoencephalomyelitis in dogs
		3:15 / Kate Birdwhistell - An evaluation of osteochondral xenograft immunogenicity in vitro
		3:30 / Ashley Rasys - CRISPRizing the lizard genome—generating a novel model system to understand fovea development.
		3:45 / Xiaotian Wang - Aurora Kinase A is essential for the maintenance of meiotic spindle stability in ovulated oocytes
		4:00 / Luhan Yang - Bisphenol compounds exert cytoskeletal disrupting activity that perturbs early embryonic development
4:30 - 5:00 PM	Room H237	Closing Remarks/Awards - Drs. Kelsey Hart and Jennifer Smith-Garvin
5:00 PM	CVM Front Steps	Group Photo for Award Winners

Poster Presentations

Odd-numbered abstracts will be presented 10:40 - 11:40 AM, and even-numbered abstracts will be presented 2:00 - 3:00 PM.

Abstract #	Last name	First name	Title
7	Al Mansi	Maryam	Prenatal exposure to Bisphenol A, S and F increases blood pressure in female rats.
3	Austin	Caleb	New Avian Pathogenic *Escherichia coli* O Serogroup Epidemiology for Poultry Health in Georgia.
4	Barton	Kaitlin	Anti-oxidant effects of vitamin C, thiamine, and hydrocortisone on equine leukocyte function in an *ex vivo* sepsis model
5	Bates	Rebecca	*In vivo* effects of pimobendan on platelet function in healthy adult cats
6	Blubaugh	Amanda	The comparison of skin lesion transcriptomes between human and animal models of chronic cutaneous lupus erythematosus
2	Bou Dagher	Josephine	Independent and combined effects of low-dose Bisphenol A and Diethylhexyl Phthalate on pregnancy outcomes and offspring development in Sprague-Dawley rats
8	Box	Erin	Molecular Confirmation of Ranavirus Infection in Amphibians from Chad, Africa.
9	Box	Erin	Rates of Copepod Consumption by Frogs and Fish with Implications for Transmission of *Dracunculus* spp.
10	Cassandra	Margaret	Factor XIII expression in normal canine tissues and perivascular wall tumors
11	Collier	Hannah	*In vitro* serial passage to evaluate selection required for the adaptation of human Influenza A viruses to pigs
12	Corn	Matthew	Construction of recombinant HA for the design of a broadly-reactive H1 influenza antigen
13	Creamer	Danielle	Evaluation of endogenous immunomodulatory genes from stimulated and immunosuppressed adMSCs from cats with and without CKD
14	Day	Katie	A rapid, parasite-dependent cellular response to *Dirofilaria immitis* in the jird (*Meriones unguiculatus*)
15	De Luca	Eliana	Prevalence, pathological aspects and genome heterogeneity of feline morbilliviruses
16	Goins	Faythe	Sea turtles, worms, and blood flukes: an experimental infection study
17	Guest	Kelsea	Effects of trace minerals on the immune response to bovine coronavirus and rotavirus vaccination in dairy cows
18	Harris	Kyle	Hatching optimization and culture media evaluation for *Ascaridia dissimilis*
19	Holt	Vivian	Immunogenicity of the canine influenza A virus (CIV) vaccine in parasitized dogs
20	Irick	Amber	Quantification of Pain from Facial Expression Characteristics Assessed from Photos of Horses and Donkeys
21	Kaimal	Amrita	Prenatal EDC Exposure Followed by Chronic Treatment with Estradiol Affects Behavior and Brain Dopamine Levels in Female Rats
22	Kelly	Davis	Suppression of microglia activation reduces body fat accumulation in rats consuming high fat diet
23	Kemelmakher	Hannah	Full-thickness modeling of inflammation in the synovial membrane *ex vivo*
24	Kim	Gerina	Neural circuitry for maternal behavior and recognizing infant distress in the mouse primary auditory cortex
25	Kirks	Sara	Comparison of pregnancy outcomes in dairy heifers artificially inseminated with sexed semen deposited in the uterine horns versus the uterine body
27	Kunkel	Melanie	Serosurvey for West Nile virus in Ruffed Grouse (*Bonasa umbellus*) across the Midwest and eastern United States
28	Latha	Krishna	Tumor Progression Locus 2 (Tpl2) prevents immunopathology during Influenza infections
29	Lemons	Margare	Effects of intravenous vitamin C on oxidative status in adult horses
30	Li Puma	Maggie	Cataract prevalence in the Large Japanese Field Mouse (*Apodemus speciosus*) following the Fukushima nuclear disaster

31	Lucchetti	Brittany	Effect of a Probiotic on Dysbiosis Index in a Hospitalized, Post-Operative Dog Population
32	McClintock	Dayle	Oral administration of dexamethasone sodium phosphate in feline hypersensitivity dermatitis: an open-label study
34	Newman	Darby	Characterization of Avian Pathogenic *Escherichia coli* (APEC) from poultry lesions in Georgia
35	Ortiz	Lucia	Influenza A virus sequencing directly from swab material using Nanopore technology
33	Patel	Avani	Modulation of microbiome, glucose homeostasis, behavior and cognition in non-obese diabetic mice following daily dosage of LemonGlycerol dietary supplement
37	Rivera	Natalia	Impact of deforestation on tick-borne infections of dogs in communities East and West of the Panama Canal.
38	Sanchez	Rachel	Eosinophils are required for the generation of protective adaptive immunity in *Bordetella bronchiseptica* infections
39	Sanders	Jackson	Pharmacokinetic evaluation of sotalol hydrochloride in cats
40	Sautto	Giuseppe Andrea	A computationally optimized broadly reactive antigen (COBRA) elicits broadly neutralizing antibodies against a conserved influenza virus hemagglutinin B-cell epitope
41	Suzuki-Williams	Lui	Elicitation of protective antibodies against the A/Swine/North Carolina/152702/2015 influenza virus (H1N2)
42	Thevelein	Britt	Effects of potential confounding variables on accuracy and precision of a commercial veterinary hematocrit meter
43	Van Brackle	Will	An online-based survey investigating the experiences of AVMA registered veterinarians with persistent hookworm infection in dogs
44	Wetherly	Patricia	Measuring pyrantel resistance in the canine hookworm, *Ancylostoma caninum*, using the Larval Arrested Morphology Assay (LAMA)
45	Yue	Tiffany	Bisphenol S (BPS) disrupts meiotic spindle organization in ovulated oocytes

Speaker Abstracts

Longitudinal Assessment of Memory B cell and Plasmablast Reactivity Against Influenza A in Subjects of Varying Age

Rodrigo B. Abreu[1], Greg A. Kirchenbaum[1], Emily F. Clutter[1] and Ted M Ross[1,2]

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Influenza is a highly contagious viral respiratory disease that affects millions worldwide each year. Annual vaccination is recommended by the World Health Organization with the goal to reduce influenza severity and limit transmission through elicitation of antibodies targeting the hemagglutinin (HA) glycoprotein. The antibody response elicited by current seasonal influenza vaccines is predominantly strain-specific; however, continuous antigenic drift by circulating influenza virus isolates facilitates escape from pre-existing antibodies and requires frequent reformulation of seasonal influenza vaccine. Furthermore, pre-existing influenza immune responses can greatly impact the serological antibody response to vaccination. However, it remains unclear how B cell memory is shaped by annual vaccination over the course of multiple seasons, especially in high risk elderly populations. Here, we systematically profiled the B cell response in young adult (18-34 year old) and elderly (65+ year old) vaccine recipients that received annual split inactivated influenza vaccination for 3 consecutive seasons. Specifically, we quantified changes in frequency of memory B cell and plasmablast in peripheral blood prior, 7 and 21 days after vaccination over 3 consecutive seasons. Additionally, we tracked the frequency of vaccine-elicited H1 and H3-reactive memory B cells (B_{mem}) by flow cytometry using tetrameric rHA probes ablated for sialic acid binding activity. Moreover, to assess the impact of sequential influenza vaccination on the B_{mem} repertoire, we *in vitro*-differentiated donor PBMCs collected over 3 consecutive years and evaluated the breath of B_{mem}-derived antibodies against a panel of rHA by ELISA. In summary, we show that vaccine elicited B_{mem} and plasmablast expansion is impaired in elderly subjects compared to young adults. Collectively, these studies will shed invaluable insight into how pre-existing immunity shapes the memory B cell response to recurrent influenza.

Evaluation of serum metabolic parameters as predictors of bovine respiratory disease events in high-risk beef stocker calves

Morgan L. Adkins[1], Emmanuel Rollin[1], Brad D. Heins[1], Roy D. Berghaus[1], Brent C. Credille[1]

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Bovine respiratory disease (BRD) is the most common cause of morbidity and mortality in North American beef cattle with substantial economic and welfare impacts. Although BRD is classically considered to be an infectious process, predisposing factors related to weaning, transportation, and commingling increase stocker calf susceptibility to disease. Despite the availability of labeled antimicrobials to treat this disease process, there are challenges associated with current diagnostic procedures, cost of treatment, and concerns regarding antimicrobial resistance. Considering these complications, there is a need for more reliable and cost-effective diagnostic tools to aid in the early detection of BRD. The primary objective of this study was to evaluate the association between serum metabolic parameters at arrival and the risk of bovine respiratory disease (BRD) in high-risk beef stocker calves. We hypothesized that there would be a difference in the serum metabolic parameters in calves that were treated for BRD within 45 days of arrival to a stocker facility and cattle that did not need treatment. Jugular venous blood samples were collected from mixed-breed beef bull, steer, and heifer calves (n=468) at the time of arrival processing at a stocker facility in northeast Georgia. Serum samples were then submitted for determination of serum creatinine, total calcium, phosphorus, magnesium, albumin, serum urea nitrogen (BUN), glucose, cholesterol, beta-hydroxybutyrate (BHBA), non-esterified fatty acid (NEFA), sodium, potassium, and chloride concentrations, as well as sodium:potassium ratio and NEFA:cholesterol ratio. Calves were monitored for the development of signs consistent with BRD for 45 days following arrival. A multivariable logistic regression model was created to evaluate the association between serum variables and subsequent risk of BRD. In this analysis, cattle diagnosed with BRD had higher serum potassium concentrations (Odds ratio: 1.29, P=0.001), lower BUN (Odds ratio: 0.943, P=0.048) and lower BHBA (Odds ratio: 0.634, P=0.001). These data suggest that hydration status, nutrient balance, and rumen development play a role in the development of BRD in high-risk beef stocker calves. These data provide further evidence of the positive effect of preconditioning programs to better prepare a weaned calf to handle the stressful events associated with transportation and cattle markets.

A common practice of widespread antimicrobial use in horse production promotes multi-drug resistance

Álvarez–Narváez, S.[1], Berghaus, L.J.[1], Morris, E.R.A.[2], Willingham-Lane, J.M.[1], Slovis, N.M.[3], †Giguere, S.[1], Cohen, N.D[2],*

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†Deceased

Background: Despite their beneficial properties for treating infections, injudicious antimicrobial use can promote resistance in bacteria in both human and animal populations. *Rhodococcus equi* is a well-known veterinary pathogen for which antimicrobial resistance has become an emerging concern. Our laboratories have documented an increasing prevalence of macrolide- and rifampin-resistant isolates of this facultative intracellular pathogen in foals, and have linked this finding to the practice of mass treatment of subclinical pneumonia with macrolides and rifampin at horse-breeding farms. Here, we report direct evidence of multi-drug resistance resulting from a common practice of treating foals with a macrolide combined with rifampin (MaR). Methods: We performed *in silico* analyses of the fecal microbiome and resistome of 38 subclinically pneumonic foals treated with either MaR (n = 19) or alternative treatment based on gallium maltolate (GaM; n = 19) and 19 untreated controls. Results: Treatment with MaR, but not GaM, significantly decreased fecal microbiota abundance and diversity, and expanded the fecal resistome of 6 classes of antimicrobials (macrolides, aminoglycosides, glycopeptides, phenicols, tetracyclines, and bacitracin). Conclusions: Our results indicate that MaR use promotes multi-drug resistance in *R. equi* and commensals that are shed into their environment where they can persist and potentially infect or colonize other animals.

The roles of serine and threonine residues of Respiratory Syncytial Virus phosphoprotein on viral transcription and replication efficiency

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[2]Division of Allergy and Immunology, Department of Internal Medicine, Morsani College of Medicine, University of South Florida, Tampa, Florida, United States.

Respiratory syncytial virus (RSV) is a single-stranded, negative-sense, RNA virus in the family *Pneumoviridae* and genus *Orthopneumoviridae* that can cause severe disease in infants, immunocompromised adults, and the elderly. In addition to human RSV, the *Orthopneumoviridae* genus includes Bovine Respiratory Syncytial Virus (BRSV) and Murine Pneumonia Virus (MPV). BRSV infects cattle, sheep, and goats, and can cause severe respiratory disease that results in secondary infections like pneumonia. Any advances made to better understand human RSV replication can be applied towards future therapeutic and preventative treatments for all Orthopneumoviruses. To transcribe and replicate its genome, RSV forms a viral RNA-dependent RNA polymerase (vRdRp) complex that is composed of the phosphoprotein (P) and the large polymerase (L) protein. The P protein is constitutively phosphorylated by host kinases and is an essential component of the vRdRp. There are 41 S and T residues within P, which are potential phosphorylation sites. To identify important phosphorylation residues in the P protein, we systematically and individually mutated all serine (S) and threonine (T) residues to alanines (A) and first analyzed their affect on genome activities using a minigenome (MG) system. We found that the mutation of six residues resulted in significantly reduced MG activity compared to wild-type P. We then incorporated these six mutations into full-length genome cDNA and tried to rescue recombinant RSV. We were able to recover three. Comparing these viruses to WT RSV, we have identified residues that play critical roles in RSV viral RNA replication. Most intriguingly, we identified a residue whose mutation resulted in deficient genome replication but increased transcription activity, suggesting that this residue may be critical for controlling replication and transcription switch.

Single pedicle advancement flap technique as a novel approach to feline stenotic nares: 4 cases

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Brachycephalic obstructive airway syndrome is a common cause of upper airway obstruction in dogs, but occurs less commonly in cats. In cats, the lesions typically associated with brachycephalic syndrome in dogs are suspected, but this disease is largely unstudied in cats. Stenotic nares appear to play a major role in cats. However, the axial deviation of the alar wing, a common cause of obstruction in dogs, is typically not present. Brachycephalic cats have a ventral nasal obstruction resulting from redundant skin along the floor of the nares at the junction of the nares and haired skin of the lip. As such, adaptation of surgical techniques developed for dogs may not be optimal. This report describes a novel surgical technique for treatment of stenotic nares in cats and presents initial clinical results. Four brachycephalic cats presenting for clinical signs of stenotic nares underwent surgical repair. In each case, a bilateral single pedicle advancement flap technique was performed. The ventral advancement flaps were based just caudal to the junction of the nares and lip, involving the entire ventral floor of the nasal cavity, and oriented parallel to the long axis of the nasal cavity. The obstructive skin fold was excised and the flaps were undermined and advanced rostrally to close the defect. All cats had positive outcomes, resulting in opening of the nares and resolution of clinical signs associated with stenotic nares. This technique is a novel and effective strategy for surgical treatment of stenotic nares in brachycephalic cats.

An evaluation of osteochondral xenograft immunogenicity in vitro

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Xenografting is increasingly of interest given the scarcity of allogeneic tissue available for transplantation. However, efforts to use live xenogeneic tissue of any type, have resulted in hyper-acute or acute graft rejection. Similar rejection responses are found in allogenic tissue transplantations between MHC or ABO blood group mismatched recipients. Interestingly, osteochondral allografts are routinely transplanted without blood type matching or immunosuppressant administration. These osteochondral allografts are transplanted after a minimum of fourteen to twenty-eight days of *ex vivo* tissue culture. It is thought that the *ex vivo* culture process improves graft performance *in vivo* by maintaining viable chondrocytes while simultaneously eliminating the potentially immunogenic bone cells. As such, we hypothesized that osteochondral xenografts may also be less immunogenic after ex vivo tissue culture. To this end, we evaluated allogeneic and xenogeneic responses to cultured osteochondral grafts in vitro. Briefly, femoral condyles were harvested from eight skeletally mature yorkshire pigs and cultured *ex vivo*. Six millimeter by ten millimeter dowels were harvested from the condyles after 0, 14, or 28 days of culture. Dowels were then placed in co-culture with equine peripheral blood mononuclear cells (n = 8) or equine enriched T cells (n = 8) to model the xenogeneic response, and porcine peripheral blood mononuclear cells (n = 8) or porcine enriched T cells (n = 8) to model the allogeneic response. After six days of co-culture, the cells were pulsed with 5-ethynyl-2'-deoxyuridine (EdU) and assessed by flow cytometry.

Comparative transcriptomic disease profiles of canine atopic dermatitis and canine models of house dust mite- and IgE-induced skin lesions

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Atopic dermatitis (AD) is caused by a complex interplay between immune and barrier abnormalities. Canine models (Immunoglobulin-E induced (IgE) and House Dust Mite (HDM)) have been used to simulate AD for preclinical assessments treatments in their comparative transcriptomic profiles with canine spontaneous AD, but with a lack of stringent criterion. We sought to evaluate the transcriptomic profiles of all canine AD models and determine how they relate to canine spontaneous AD lesional skin. Gene expression data of dogs with spontaneous AD and house dust mite (HDM) patch and tape stripping canine models were obtained and re-analyzed from microarray published cohorts; transcriptomic profiling of IgE-induced model was performed using RNA sequencing. Criteria of False Discovery Rate (FDR)≤0.05 and Fold Change (FC)≥2 were used for analysis across all samples. Gene Set Variation Analysis (GSVA) was performed for phenotypically unbiased analysis of gene sets (Th1, Th2, Th17, Th22). Spontaneous AD, patch and tape stripping HDM, and IgE modeling comparisons to healthy canine skin revealed 41 common DEGs: upregulation of pro-inflammatory (IL8, CCL2, MMP9); IFNγ (CXCL10, ISG15), and T helper-(Th)2 genes (IL13RA2). Transcriptomic comparison of DEGs between canine models revealed IgE contained 63.5%, whereas tape stripping and patch HDM induced model comprised 49%, of spontaneous AD genes. Multipolar pathways were observed among spontaneous and AD models in GSVA, with robust Th1/Th2 activation. Th17 was significantly enriched in IgE and spontaneous AD, but not HDM. Further studies of current canine models and spontaneous AD with larger sample sizes utilizing next-generation RNA sequencing should be conducted.

Resistance to fenbendazole in Ascaridia dissimilis and its economic impact on the production of turkeys

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Ascaridia dissimilis is the most prevalent and one of the most economically important gastrointestinal nematodes of turkeys. Infections are most often subclinical, producing reduced feed conversion efficiency, with heavier infections causing symptoms such as lethargy, intestinal blockage, diarrhea, and enteritis. Currently, fenbendazole (FBZ) is the only FDA approved drug for use against this parasite in turkeys. We recently tested the efficacy of FBZ against 5 field isolates of *A. dissimilis* acquired from commercial farms. 3 of the 5 isolates (Wi, Ow, Po) demonstrated greater than 99% efficacy. However, two (Sn, Ad1018), yielded efficacies of 63.9% and 76.2%, respectively, indicating FBZ resistance in these isolates. Having proven FBZ resistance in *A. dissimilis*, we wanted to determine the economic impact of resistant worms on growth and productivity. 384 1-week old turkey poults were infected with either the Sn (resistant) or the Ow (susceptible) isolates. For each isolate, 8 replicates each of untreated and treated pens were established with 12 birds/pen. Turkeys were grown to 10 weeks of age, changing feed types at the recommended time points as per commercial growing practices. Birds were infected via a trickle dosage of 25 eggs/bird/week sprinkled onto their feed. At 4- and 8-weeks post infection, birds in the treated groups were administered FBZ via water (SafeGuard® Aquasol, 1.25mg/kg) for 5 consecutive days. Weight, weight gain and feed conversion were analyzed weekly for differences between groups. At weeks 7 and 8 post infection, feed conversion in the Ow-treated groups was significantly better than the other three experimental groups; this group used 700 grams less feed/bird than the other three groups. Since this was the only group with an effective treatment, these results strongly suggest that worms infecting the other 3 groups (due to either lack of efficacy or lack of treatment) caused a significant decrease in feed efficiency. Further work is needed to determine the full scope of *A. dissimilis* infections on feed conversion, but based on these data, there appears to be a large economic impact of *A. dissimilis* on turkey production. Consequently, resistance to FBZ also requires further study to investigate its prevalence and distribution.

Impact of two equine peripheral blood leukocyte enrichment techniques on leukocyte cell recovery, purity, viability, cell surface phenotype, proliferation and TNFα concentration

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Peripheral blood is commonly sampled to assess the health status of human and veterinary patients. Venous blood collection is a minimally invasive procedure, and in the horse, the common collection site is the jugular vein. Post blood collection, sample processing for leukocyte enrichment can vary by laboratory with the potential to yield different effects on the enriched cells and their function. The focus of the present study was to compare a common blood dilution leukocyte enrichment technique using a Histopaque gradient medium (His) to a modified leukocyte buffy coat syringe-Histopaque gradient medium technique (Syr-His) with peripheral blood from 12 horses. The endpoints examined included cell recovery/mL of blood, cell viability, leukocyte enrichment purity, leukocyte cell marker subset phenotype, leukocyte spontaneous and mitogen-induced proliferation and secretory TNFα concentrations. Leukocyte cell recovery/mL of whole blood and cell viability was significantly increased in enriched leukocytes from the Syr-His technique. Interestingly, the percentage of $CD8^+$ and $CD21^+$ cells were significantly increased with the His technique as was Con A-induced proliferation. Still, leukocyte cell purity and TNFα secretions from the 72hr cell culture supernatants were comparable across the two enrichment techniques. To summarize, the type of whole blood leukocyte enrichment technique employed does significantly impact the results of select assay endpoints and thus, likely the result interpretations.

Efficacy of disinfectant wipes for reducing bacterial contamination on stethoscopes in a veterinary teaching hospital

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Stethoscopes have long been recognized as a source for potential pathogens in human and veterinary medicine. Despite this, stethoscope cleaning practices among hospital personnel tend to be inconsistent. To mitigate the risk of healthcare-associated infections, widespread use of effective cleaning and disinfection protocols for stethoscopes should be encouraged. While 70% alcohol has been shown to reduce bacterial loads on stethoscopes, to date, its comparative efficacy to that of other commonly used disinfectants has not been evaluated. As such, the objectives of this study were to: 1) compare efficacy of 3 commercially available disinfectants wipes (70% alcohol [ALC], accelerated peroxygen [AHP], quaternary ammonium [QAC]) for reducing bacterial load on stethoscopes; and 2) characterize current stethoscope disinfection practices among personnel in a veterinary teaching hospital. AHP was hypothesized to be the most effective disinfectant, and stethoscope bacterial loads were expected to reflect frequency of disinfection indicated by study participants. An experimental study was undertaken where all hospital personnel routinely using stethoscopes were eligible to participate. For each stethoscope (N=48), an aerobic culture was performed by pressing the bell onto a contact plate (RODAC™). The stethoscope then randomly received 1 of 3 disinfectant wipe treatments (ALC, AHP, or QAC) for 10 seconds, was allowed to dry for 3 minutes, then was re-cultured. All plates were incubated at 35°C for 24 hours, and bacterial colony counts were performed. Each participant completed a brief survey regarding current stethoscope use and disinfection practices. In general, stethoscopes cleaned using an AHP wipe had the greatest reduction in bacterial count, followed by the QAC wipe, and had the least reduction with the ALC wipe. While there was a statistically significant reduction in bacterial count for AHP and QAC wipes compared to ALC wipes, the AHP and QAC wipes were not significantly different from each other. Participants indicated that product availability throughout the hospital was key to encouraging their use. This study used a short 10-second wipe and 3-minute dry time, suggesting that the use of either AHP or QAC disinfectant wipe may be a viable method to reduce bacterial counts on commonly use instruments in veterinary practice.

Assessing the role of the Type 6 Secretion System (T6SS) in extraintestinal pathogenic *Escherichia coli*

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Extraintestinal pathogenic *Escherichia coli* (ExPEC) are a pathotype of *E. coli* responsible for extraintestinal infections in humans and other animals. ExPEC includes the sub-pathotypes Neonatal meningitis-causing *E. coli* (NMEC) and Avian Pathogenic *E. coli* (APEC), both representing a significant public health concern. NMEC is the leading cause of neonatal meningitis caused by Gram-negative bacteria with mortality rates up to 50%, and survivors suffering lifelong neurologic sequelae. APEC causes extraintestinal infections in poultry, resulting in multi-million dollars loss for the industry, and serves as a reservoir of pathogenicity genes that can lead to the emergence of human pathogens.

Despite the identification of several pathogenicity determinants in NMEC and APEC, the involvement of secretion systems in their virulence remains poorly understood. The Type 6 Secretion System (T6SS) functions as a molecular syringe that secretes proteins into the external milieu or directly into the host cells, and is composed of a membrane complex, a baseplate and a tail-like structure. Although the T6SS is involved in virulence mechanisms by several human and animal bacterial pathogens, current knowledge on the role of T6SS in NMEC and APEC pathogenesis is limited. Here, we aim to expand our knowledge of the role of T6SS in ExPEC pathogenesis. To gain insight into the relationship between the T6SS and ExPEC pathogenicity, we screened a collection of 92 NMEC, 454 APEC, and 102 avian fecal *E. coli* isolates for the presence of 7 T6SS genes including effector and uncharacterized structural components. The prevalence of these genes was considerably higher in NMEC and APEC than in fecal isolates, providing evidence that the T6SS contributes to virulence in NMEC and APEC. To further characterize T6SS in ExPEC, we have generated mutants for the aforementioned T6SS components in NMEC15 and APECO18. Eleven mutants have been generated thus far. Functionality of the T6SS, biofilm formation, adherence and invasion of host cells, and Hcp secretion will be analyzed. With the data generated we expect to significantly enhance our understanding of the role of the T6SS in NMEC and APEC virulence, and potentially provide insights on the development of new treatment strategies for controlling these pathogens.

Effect of diphenhydramine and cetirizine on immediate- and late-phase cutaneous allergic reactions in healthy dogs: a randomized, double-blinded crossover study

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In veterinary medicine, oral histamine type-1 antagonist diphenhydramine (DPH) is frequently administered extra-label to dogs to control mild allergic reactions and to prevent mast cell degranulation and histamine release during surgical excision of mast cell tumors. However, there is insufficient evidence to confirm oral DPH efficacy and the inhibitory effect on the histamine-induced reactions in dogs. The objective of this study was to determine and compare the effects oral of DPH and cetirizine on the immediate- and late-phase cutaneous allergic reactions in healthy dogs. An additional objective was to determine if the oral DPH and cetirizine administration would produce plasma drug concentrations that in people are considered therapeutic and inhibit the histamine-induced reactions. Twelve healthy male Beagles were randomized to receive oral DPH at 2.2 mg/kg or cetirizine at 2 mg/kg twice daily for six days with a 2-week washout period. Ten days before (baseline) and after six days of oral antihistamine administration, intradermal injections of histamine (5 micrograms/site), compound 48/80 (10 micrograms/site; positive control) and saline (negative control) were performed on the right thorax. Global wheal scores (GWS) at 20 min and late-phase reactions (LPR) at 6h post-injection were evaluated by an investigator blinded to the drug and the interventions. Treatment with cetirizine reduced histamine and compound 48/80 GWS ($p=0.0002$ for both, respectively) compared to baseline; there was no significant difference for DPH (histamine; $p>0.99$ for both, respectively). Late phase reactions of histamine and compound 48/80 were significantly inhibited during cetirizine treatment ($p=0.0004$ and $p=0.0005$); DPH showed no significant effect ($p>0.99$ for both, respectively). Oral DPH (mean 114 ng/mL) and cetirizine (mean 10.9 μg/mL) reached plasma concentrations considered therapeutic in people in all dogs. No adverse effect or behavioral changes were observed during the study. In conclusion, oral DPH failed to show an inhibitory effect on the cutaneous allergic reactions despite attaining plasma drug concentrations that are considered effective in people. Oral cetirizine at 2 mg/kg twice daily is effective in preventing wheal formation induced by intradermal histamine injections without any obvious unwanted effect and should be preferred over DPH in dogs.

Preliminary analysis of IBD-like disease and metabolic bone disease in MIT's marmoset (*Callithrix jacchus*) colony

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Common marmosets (*Callithrix jacchus*) are petite new-world nonhuman primates used as translational models in biomedical research, including but not limited to modeling osteoporosis.[1] A common issue in marmoset colonies is the spontaneous incidence of inflammatory bowel disease (IBD)-like syndrome, a gastrointestinal disease characterized by lymphocytic enteritis. Additionally, many marmosets with IBD-like disease suffer from simultaneous metabolic osteopathies including rickets, fibrous osteodystrophy (FOD), and osteopenia. We hypothesize that the IBD-like marmosets at MIT also suffer from metabolic bone disease.

For phase I of this study, complete blood count (CBC) and serum chemistry values of twenty-five marmosets with inflammatory bowel disease (IBD)-like disease were surveyed to determine the presence and severity of bone degeneration. Over the course of two years from intake to necropsy, the final calcium levels of the marmosets were significantly lower than the initial serum calcium levels ($P < 0.0078$). The difference between initial and final albumin levels over the same time period were not significant ($P = 0.40$). However, severity of bone disease varied upon diagnosis at necropsy.

During phase II, IBD-like populations were separated by age, above three years (n= 4) and below three years (n=5). A control group (n=2) was chosen with no signs of IBD-syndrome and under 3 years of age. Each individual received digital radiographs and were scored based on an internal four-point scale based on the severity of their bone lesions. We determined the radiographic bone lesion severity of the living colony members significantly differed with age ($P<0.029$).

This MIT pilot study will add to a developing body of literature on marmoset bone disease started by authors at Johns Hopkins University (JHU) and the Wisconsin National Primate Research Center (WNPRC).

Investigating the role of equine platelet lysate on wound healing in vitro

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The aim of this study was to determine if replacing fetal bovine serum (FBS) with equine platelet lysate (ePL) in cell culture media would enhance wound healing using an in vitro scratch assay with primary equine dermal fibroblasts. In addition to our "insult" by physically injuring the cells, we also challenged wound healing by adding killed *Staphylococcus aureus* into the media. Skin biopsies were taken from the cannon bone region of 7 healthy horses and fibroblasts were isolated from the samples. Fibroblasts were passaged to P2 before being plated onto 4-well collagen-coated slide plates at a density of 100,000 cells per well. After incubating and reaching 80% confluence, a scratch assay was performed and media was replaced with treatment groups containing: 10% FBS, 10% FBS with *S. aureus*, 10% ePL, and 10% ePL with *S. aureus*. Infected groups were at a 1:1500 dilution. Cell supernatant was obtained pre-scratch, 12h, 24h, 48h, and 72h following the scratch assay. The supernanants were frozen in -80°C until further analysis via ELISAs for the cytokines TGF-β1 and IL-6. At these same time points, slides were fixed, stained, and digitally scanned for further analysis. Cell morphology was analyzed for presence of myofibroblasts as well as cell organization around the "wound". Wound closure was determined using Image J Software in which the area of the scratch at 12h, 24h, 48h, and 72h was compared to the area of the scratch at 0h. The difference in scratch closure in terms of area healed were not significantly different between groups infected with the *S. aureus* antigen in either the 10% ePL groups or 10% FBS groups. A significant difference in closure was detected between cultures containing FBS in the media versus ePL.

The mechanism of copper acquisition by pathogenic mycobacteria

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Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis (TB), one of the deadliest infectious diseases worldwide1. The world health organization estimated 10 million people had active TB infection and 1.6 million died from this disease in 2017[1]. The emergence of multiple drug resistant (MDR) and extensively drug resistant (XDR) TB complicates treatment efforts. copper is an essential trace element that functions as a cofactor for several enzymes involved in diverse biological reactions. It plays a vital role in bacterial growth as a cofactor of cuproenzymes[2]. Thus, copper uptake during infection is vital for *Mtb* survival. It still unclear how *Mtb* acquire copper. Studying the mechanism of copper uptake will yield important insights into the metallobiology of mycobacteria and possibly aid in the design of novel drug or vaccines targeting copper acquisition.

Recently, it was shown that the nonribosomal peptide synthase (*nrp*) operon (*PPE1-nrp*) of *Mycobacterium marinum* expressed in *E. coli* resulted in the production of isonitrile lipopeptides (INLPs) after supplementation of culture medium with long-chain fatty acids such as 2-deconoic acid[4]. Further, *a M. marinum nrp* operon mutant was found to have lower cytoplasmic zinc levels, suggesting a role for this operon in metal transport[4].

Our hypothesis is that when starved of copper ion, *Mtb* and *M. marinum* synthesize chalkophores (copper-binding molecules) to facilitate copper acquisition across the outer membrane.

Our data show that deletion of *Mtb PPE1* or *Rv0097* results in growth defects when the mutants were grown in copper-deficient media. Transposon-mediated *M. marinum nrp* operon mutants were also defective for growth when copper was chelated from the media.

A 365.2167 Dalton peak, which corresponds to diisonitrile compound (chalkophore) that was previously shown to be produced by *Streptomyces thioluteus*, was detected by high resolution mass spectrometry (HR/MS) after expression of *Mtb Rv0097-nrp* genes in *M. marinum PPE1* mutant.

The combined results show that the *nrp* operon of *Mtb* or *mycobacterium marinum* is required for growth in copper-starved media.

Using VacSIM® to deliver and stabilize VLP-based vaccines for Ebola Virus vaccines

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Ebola Virus Disease (EVD) is a fatal disease found primarily in Africa. There is no approved vaccine for EVD, but many are being developed. One currently effective vaccine contains Ebola virus-like particles (eVLPs), but highly reactogenic adjuvants are needed for acceptable efficacy levels. VacSIM is a non-immunostimulatory adjuvant which uses (RADA)4 polypeptide. VacSIM increases vaccine persistence, enhances immune responses to antigens, and eliminates reactogenicity caused by adjuvants. Vaccines with VacSIM can be lyophilized and rehydrated without loss of immunogenicity. The objective was to evaluate VacSIM as a delivery method for eVLPs-based vaccines and to determine if VacSIM can be used to maintain effectiveness after lyophilization. Mice were immunized with lyophilized or freshly prepared formulations of eVLPs +/- 0.5% VacSIM + 75ug CpG by subcutaneous (SC) or intradermal (ID) routes. Mice received 2 vaccinations within a 3-week prime-boost interval. Blood was collected 3 weeks post prime and 1-week post boost to evaluate humoral response. Data showed high IgG endpoint titers (10^5) after one dose for both ID and SC routes when using VacSIM. Titers reached 10^6 via SC and 10^6 via ID 1-week post boost. Initial testing did not detect neutralizing antibodies after prime immunization, however neutralizing antibodies were detected 1-week post-boost in the ID group using VacSIM. These results demonstrate that a single vaccination with eVLPs in VacSIM, fresh or lyophilized, can induce high antibody titers. Further study of neutralizing antibodies on lyophilized groups and T-cell response will be performed 3 weeks post boost. If effective, these experiments will aid in generating functional thermostable human vaccines.

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Comparison of respiratory stimulants during laryngeal exam in canines

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Laryngeal examination is performed in anesthetized dogs and can diagnose laryngeal paralysis. Respiratory stimulants might improve the quality of the exam. We compared the administration of 0.55mg/kg (L-DOX) or 2.20mg/kg (H-DOX) of doxapram with the inhalation of 10% carbon dioxide (CO_2). Six dogs were anesthetized three times in a latin-square design. The laryngeal motion was recorded to obtain the normalized glottic gap area (NGGA). Mean arterial blood pressure (MABP) and heart rate (HR) were recorded continuously. All treatments increased NGGA, in average from 0.220 ± 0.077 to 0.315 ± 0.069 ($P < 0.001$). H-DOX (0.351 ± 0.037) increased more than L-DOX (0.304 ± 0.072) and CO_2 (0.290 ± 0.084, both $P < 0.013$). Treatments H-DOX, L-DOX increased BP by 41 ±30% and 36 ± 23%, respectively. The increase in BP with CO_2 (18 ± 10%) was lower than H-DOX and L-DOX (both $P <0.001$). H-DOX (39 ± 20%) increased more HR than L-DOX and CO2 (both < 0.016). L-Dox (26 31%) increased the HR more than CO2 (8 ± 7%) ($P < 0.008$). Doxapram has a dose dependent effect on the glottic opening and L-Dox and CO2 have similar effects on NGGA. However, doxapram caused more cardiovascular effects.

Health status and endoscopic evaluation of the upper respiratory tract of dairy bull calves inoculated with BVDV2 and BHV1 after vaccination and trace minerals injection

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Bovine respiratory disease (BRD) is a major illness that affects cattle industry worldwide. *Bovine viral diarrhea virus* (BVDV), and *Bovine herpes virus 1* (BHV1) are pathogens commonly involved in BRD. Prevention of BRD includes multivalent vaccination and early diagnosis. The concurrent use of multivalent vaccination with injectable trace minerals (ITM) has demonstrated beneficial effects. Endoscopy is a diagnostic tool that permits prompt evaluation of the upper respiratory tract (URT). Thus, we hypothesize that administration of ITM at the time of vaccination enhances protection against BVDV2 and BHV1 infection. Twenty-four calves were administered a modified-live virus (MLV) intranasal (IN) vaccine containing BHV1, BRSV, PI3V, and randomly assigned to subcutaneous administration of ITM (Multimin®90 containing Se, Cu, Zn & Mn; ITM, n=12) or saline (Sal, n=12). Ten weeks later, the calves received a booster vaccination, and a second dose of ITM, or saline, according to treatment. Additionally, 12 calves did not receive vaccine or treatment (Unvacc, n=12). Forty-nine days after booster vaccination, all calves were intranasally inoculated with BVDV2 (5 x10^5 $CCID_{50}$); seven days later with BHV1 (8 x10^6 $CCID_{50}$). Health status was evaluated daily. Five days after BHV1 inoculation, a random subset of calves (ITM= 5; Sal= 5 & Unvacc= 3) were selected for an endoscopic evaluation of the URT (nasal cavity, pharynx, larynx, trachea, and bronchi). Three evaluators blinded to treatments, assessed each section of the URT for vascularization, integrity of the mucosa, and secretions. An endoscopic score (ES) from 0 to 3 (0: normal; 1: mild; 2: moderate; 3: severe) was assigned for each characteristic. An overall ES was calculated for each calf and compared among groups using SAS®. Health score was higher ($P < 0.05$) in Unvacc compared with the vaccinated groups after BVDV2 inoculation. Calves treated with ITM showed significantly lesser URT ES (18.2 ± 1.25) compared with Sal (24.9 ± 1.0) and Unvacc groups ($P < 0.01$). Unvaccinated calves had the highest mean URT ES (27.5 ± 4.47) after BHV1 challenge. In conclusion, administration of ITM concurrent with MLV IN vaccination resulted in lower level of inflammation and tissue damage compared with Unvaccinated and Saline groups.

Prevalence and Genetic variability of resistant Rhodococcus equi in Horse-breeding Farms in Kentucky, USA.

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The combination of a macrolide and rifampicin has been the mainstay of therapy in foals with *Rhodococcus equi* pneumonia for decades. Recent studies suggest that mass antimicrobial treatment of subclinically affected foals over time has selected for antimicrobial resistance. The increasing resistance of bacteria to macrolides and rifampicin extends beyond equine rhodoccocosis because these drugs are widely used in humans. Our laboratories have documented emergence of macrolide and rifampin resistance in isolates of *Rhodococcus equi* from foals and their environment. Although the resistance mechanisms and the genetic variability of the resistant isolates in the environment was ill-defined. The objective of this research is to estimate the genomic diversity of *R. equi* strains resistant to macrolides or rifampicin horse-breeding farms. *R. equi* isolates (n=158) collected in 2017 form soil samples were submitted for single molecule real-time (SMRT) sequencing. Reads were trimmed and assembled (Canu version 1.7), and phylogenetic relatedness was determined (Harvest version 1.1.2) and portrayed (FigTree version 1.4.4). Resistant *R. equi* were found at 76% of farms, and 98% were resistant to both macrolides and rifampicin. Three distinct phenotypes were submitted for sequencing: 1) dual resistance (macrolides and rifampicin) with the *vapA* virulence gene and the *erm*(46) gene (d++), known to confer resistance to macrolides, lincosamides and streptogramins B in *R. equi*; 2) dual resistance lacking the *vapA* and the *erm*(46) genes (d--); and, 3) sensitive (S) to both macrolides and rifampicin. At least 2 different mechanisms confer resistance in *R. equi* to macrolides and isolates within resistance type appear to be highly related genetically (Figure 1). Preliminary analysis of the whole genome sequencing data has indicated that the novel resistance mechanism present in the d-- phenotype is inserted in a mobile element that could be transferred to susceptible strains via horizontal gene transfer. The dissemination and maintenance of resistance genes in the environment where many other pathogenic bacteria are encountered presents a large-scale concern for both animal and human health. Further investigation regarding the resistance mechanisms present in the sequenced isolates will be shown at the meeting.

Keywords: antimicrobial resistance, phylogenetic, whole genome sequencing

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A parainfluenza virus 5 (PIV5)-based vaccine protects mice against lethal challenge with wild-type strains of *Burkholderia mallei* and *Burkholderia pseudomallei*

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Burkholderia mallei and *Burkholderia pseudomallei* are highly pathogenic bacteria that affect both animals and humans causing glanders and melioidosis, respectively. Due to their ability to be used as biological warfare agents, the intrinsic resistance of the organisms to antibiotics, and the lack of a licensed vaccine for either disease, both bacteria have been categorized as Tier 1 organisms by the U.S. Federal Select Agent Program. The similarities between *B. mallei* and *B. pseudomallei* make it possible to create a vaccine that protects against infection with either organisms. With this in mind, our lab sought to developed a vaccine for *B. mallei* and *B. pseudomallei* by using parainfluenza virus 5 (PIV5) as a vaccine platform. We incorporated the novel antigen outer membrane protein 7 (OMP7) into the PIV5 genome and tested it in a mouse model. We hypothesized that a PIV5-based vaccine expressing the OMP7 protein (PIV5-OMP7) will protect mice against lethal challenge with wild-type strains of *B. mallei* and *B. pseudomallei*, while also reducing bacterial burden in target organs. The results of this study showed that mice vaccinated with a single-dose of PIV5-OMP7 were protected against lethal challenge with a wild-type strain of *B. mallei*, with surviving mice also showing higher percentages of bacterial clearance in target organs. We also tested a prime-boost vaccination regimen with the PIV5-OMP7 vaccine in combination with the previously published vaccine candidate PIV5-BatA (PIV5 expressing autotransporter protein BatA). Results showed that the PIV5-OMP7 plus PIV5-BatA bivalent vaccine induces higher levels of protection against lethal challenge with *B. mallei* and *B. pseudomallei* when used in a prime-boost vaccination regimen as opposed to a single-dose vaccination. This study highlights and further demonstrates the potential of OMP7 and BatA as antigens for generating vaccines to protect against glanders and melioidosis, as well as the versatility of PIV5 as a viral vector for vaccine development.

Multiple drug resistance in Ancylostoma caninum: an emerging threat to canine health

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The canine hookworm, *Ancylostoma caninum* is the most prevalent and important intestinal nematode parasite of dogs in the United States. Hookworms are typically well controlled by treatment with all commonly used anthelmintics that are approved for this use in dogs. However, in the past few years, cases of recurrent/persistent canine hookworm infections appear to have dramatically increased, suggesting that anthelmintic resistance (AR) may have evolved in this parasite. These cases are highly overrepresented by greyhounds, but multiple other breeds are also represented. The aim of this study was to characterize several of these suspected resistant isolates using *in vitro*, genetic, and clinical testing to determine if these cases represent true anthelmintic resistance in *A. caninum*. Fecal samples containing hookworm eggs from three cases of persistent hookworm infections; one from a greyhound, one from a miniature schnauzer, and one from a hound-mix, were received by our laboratory. These were then used to establish infections in laboratory dogs, and to perform egg hatch assays (EHA) and larval development assays (LDA) for detecting resistance to benzimidazoles and macrocyclic lactones, respectively. Additional EHA and LDA were performed on eggs recovered from the laboratory-induced infections. Fecal egg count reduction tests were performed to detect resistance to pyrantel. Deep amplicon sequencing assays were developed to measure the frequency of non-synonymous single nucleotide polymorphisms (SNP) at codons 167, 198 and 200 of the *A. caninum* isotype-1 β-tubulin gene. Resistance ratios for the three *A. caninum* isolates tested ranged from 6.0 to >100 and 5.5 to 69.8 for the EHA and LDA, respectively. Following treatment with pyrantel, reduction in faecal egg counts ranged from -323 to 0%. Deep amplicon sequencing of the isotype-1 β-tubulin gene identified a high frequency of resistance-associated SNPs at codon 167 in all three resistant isolates and in two additional clinical cases. These data conclusively demonstrate multiple anthelmintic resistance in multiple independent isolates of *A. caninum*, strongly suggesting that this is an emerging problem in the United States. Furthermore, evidence suggest that these resistant hookworms originate from racing greyhound farms and kennels, though additional research is needed to confirm this.

A survey of state regulations for Brucella testing, RB51 vaccination requirements, and legible tattoo standards in cattle and domestic bison across the United States

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Historically, brucellosis resulting from infection with *Brucella abortus* was a devasting disease that resulted in economic loss to producers with affected herds. As part of a comprehensive eradication program in partnership with the United States Department of Agriculture, each state developed regulations to control the spread of and ultimately eliminate *Brucella abortus*. Regulations vary widely and are still in place despite the successful eradication of *Brucella abortus* from domestic livestock in the United States. Utilizing an email and phone questionnaire, this study aimed to document each state's requirements for *Brucella abortus* testing and vaccination in cattle and domestic bison for movement purposes, and legible tattoo standards as a proof of vaccination. The questionnaire also assessed each state's willingness and process to change their testing and vaccination requirements. The questionnaire was administered to State Veterinarians in each of the 50 states. Responses were received from 47 out of 50 states. Among respondents, 62% (29/47) of states had requirements for *Brucella abortus* testing or vaccination when entering the state and 28% (13/47) had requirements when moving cattle or domestic bison within the state. Entry requirements were primarily for animals originating from the Greater Yellowstone Area (GYA), a state Designated Surveillance Area (DSA), or if the animals originated from a state that was not brucellosis free. States either had variable requirements for a legible tattoo or there were no standards for a legible tattoo. Among states that had entry requirements for *Brucella abortus*, 15 out of 30 were willing to make changes to their current requirements. Among states with intrastate *Brucella abortus* movement requirements, 10 out of 14 were willing to make changes to their current requirements. Remaining states indicated that they would keep their *Brucella abortus* testing and vaccination requirements for movement purposes. In order to make any changes, 40 states indicated that a rulemaking or legislative process would be required. Despite the absence of Brucella abortus in domestic livestock, there are states that maintain movement testing and vaccination requirements for cattle and domestic bison.

Developing PIV5-based AVLP vaccines for *Burkholderia mallei* and *Burkholderia pseudomallei*

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Burkholderia mallei and *Burkholderia pseudomallei* are highly pathogenic zoonotic bacteria that cause glanders and melioidosis, respectively. Both bacteria are categorized as Tier 1 organisms by the U.S. Federal Select Agent Program due to the potential threat they pose to human and animal health and the lack of a licensed vaccine. Our lab has previously shown that a parainfluenza virus 5 (PIV5)-based vaccine against *B. mallei* and *B. pseudomallei* expressing the autotransporter protein BatA protects mice against lethal challenge with either organism, primarily through a cell-mediated immune response. To improve this vaccine, we constructed a new vaccine candidate by incorporating the novel antigen outer membrane protein 7 (OMP7) gene into the PIV5-based amplifying virus-like particle (AVLP) genome plasmid. AVLPs contain the safety qualities intrinsic to traditional virus-like particles, with the inherent immunogenicity seen with live-virus vaccines. More importantly, studies performed using the AVLP platform as a vaccine for respiratory syncytial virus show that this system is capable of inducing robust cellular immune responses. After constructing the AVLP-OMP7 plasmid, we generated a stable cell line containing the AVLP genome. Our results confirmed that we successfully produced a stable cell line expressing the AVLP-OMP7 genome. This cell line will then be used to produce AVLPs and we will test its efficacy as a vaccine in a mouse model. The results from this study will take us one step closer towards producing an effective vaccine against glanders and melioidosis.

Mutation E48K in the PB1 gene improves stability of a temperature-sensitive Influenza B live attenuated vaccine and maintains efficacy against homologous challenge.

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Influenza B virus (IBV) is a major respiratory pathogen that accounts for 30% of all human seasonal influenza infections occurring worldwide. IBV can be categorized into two major lineages based on the hemagglutinin (HA) surface glycoprotein, the Victoria and the Yamagata lineages. While there are several licensed seasonal vaccines against IBV and influenza A viruses (IAV), including live attenuated influenza virus (LAIV) vaccines, recent efficacy issues of licensed LAIVs highlight the need for the development of alternative LAIV vaccines. We developed a live attenuated (*att*) IBV vaccine strain by introducing 2 temperature-sensitive (*ts*) mutations (E580G and S660A) into the PB1 gene along with a modified HA epitope tag in the C terminus of PB1. The engineered *att* strain was shown to be safe and protect against virulent IBV challenge in mice. A third *ts* mutation (K391E) was attempted in PB1 of the att IBV strain but was not stable in SPF eggs. However, we found that a small subpopulation retained the K391E mutation in combination with a new mutation (E48K), which may have served as a compensatory mutation for the stability of the K391E. To test this hypothesis, we introduced the E48K mutation into the PB1 gene by site-directed mutagenesis and produced the mutant virus containing E48K, K391E, E580G, and S660A in the PB1 by reverse genetics. We tested the stability of the strain containing 4 mutations (E48K, K391E, E580G, and S660A) compared to the unstable strain containing 3 mutations (K391E, E580G, and S660A) in MDCK cells and SPF eggs. Our results showed that the K391E mutation in the PB1 was stable in the strain containing E48K while the strain containing only 3 mutations reverted to 391K after only 3 passages. The reconstructed 3D structure of PB1 confirmed the interactions between positions 48 and 391 that could be involved with the stability of the att strain. Immunization of mice with the new att IBV vaccine strain (containing 4 mutations) provided complete protection against homologous challenge as evidenced by reduced weight loss and increased survival rate compared to unvaccinated controls. Our results show that an additional mutation was able to stabilize the K391E temperature-sensitive mutation in the PB1 gene of our LAIV vaccine, potentially increasing the safety of this vaccine while maintaining its efficacy.

Albumin in tear film decreases the bioavailability of topical tropicamide and latanoprost in dogs

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The purpose of this study was to investigate the effects of albumin in tears on the bioavailability of topically administered drugs in dogs. Eight healthy female Beagles (16 eyes) were used. In each dog, one eye received 20 microliters (μL) of artificial tears (control) or canine albumin (0.2 or 1%) at random, immediately followed by 20μL of tropicamide (days 1-2) or latanoprost (days 3-4) in both eyes. Pupil diameter (digital caliper) and intraocular pressure (IOP; rebound tonometry) were recorded at various times following drug administration (0, 2, 4, 6, 8, 10, 15, 20, 30, 45, 60, 90, 120, 180, 240 and 480min) and compared between both groups with the Student t-test. For tropicamide, differences in pupil size were noted from 8min up to 240min with a significant decrease in maximal pupillary dilation noted with 0.2% albumin (11.9mm) and 1% albumin (11.7mm) compared to control (12.8mm; $P \leq 0.006$). Similarly, the overall drug exposure (area-under-the-curve of pupil diameter over time) was significantly reduced with both albumin concentrations ($P<0.001$) by 7.1% on average. For latanoprost, differences in pupil size were noted from 4min up to 20min, but no significant changes in maximal pupillary constriction or overall drug exposure were noted between 0.2 and 1% albumin and controls ($P \geq 0.663$). Similarly, IOP did not change between groups at any time point ($P \geq 0.351$). In conclusion, albumin in tear film, previously shown to leak from the blood in eyes with conjunctivitis, likely binds to topically administered drugs and reduces their intraocular penetration and bioavailability. Further investigations in diseased eyes and other commonly used ophthalmic medications are warranted.

Single-cell transcriptome analysis and 3D light sheet imaging of feline spermatogenesis

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Large populations of free roaming cats in the world raise both ethical and public health concerns, yet trapping, neutering, & releasing the estimated 87 million feral cats in the US alone would cost 9-14 billion dollars. As such, we must develop rapid non-surgical sterilization techniques that reduce the cost needed to keep these populations under control. Directly targeting spermatogenic cells would provide an effective approach for sterility, yet little is known about feline spermatogenesis. In this study, we used a multi-level approach from genetic analysis to whole organ imaging to learn more about feline spermatogenesis. First, we used single cell resolution RNA sequencing to characterize different cell populations at the transcriptome level in the feline testis. Bioinformatic analysis identified 12 clusters corresponding to distinct testicular cell populations. We also employed state of the art light sheet microscopy to analyze the expression of SYCP3, a prominent marker of meiotic cells, and generated a high content 3D map of marker localization in the entire testis. Using superresolution structured illumination, we validated the expression of SYCP3, the cohesin protein Rad21, and the double strand DNA break marker γH2AX. Importantly, treatment with a histone deacetylase inhibitor (TSA) induced a significant increase ($p < 0.05$) in the levels of histone acetylation and DNA damage during meiosis. Our results indicate that TSA disrupts spermatogenesis by causing errors in chromatin organization. Overall, we have shown that state-of-the-art imaging and transcriptome analysis are viable and powerful tools that will empower us to evaluate the effect of pharmacological compounds in the control of fertility.

CRISPRizing the lizard genome—generating a novel model system to understand fovea development.

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Critical for the finer aspects of vision, the fovea—a pit-like depression in the retina with a high density of visual cells, is specialized area that is important to the vision of primates and many non-mammalian amniotes. Despite its importance, very little is understood about its development, as currently used model systems—like the chick, mouse, frog, and zebrafish, lack a fovea. To address this, we are establishing the *Anolis sagrei* lizard as a novel foveated model system. Towards this aim, we have developed a surgical approach (i.e. mirco-injecting CRISPR-cas9 into immature lizard oocytes) that enables genome editing for the first time in reptiles. Recently, we targeted the pigmentation genes—*tyr and oca2*, implicated in human fovea development. We report here the morphological findings on fovea development in our albino $tyr^{-/-}$ mutant lizards.

A single dose of recombinant PIV5 expressing VP1 of Norovirus protected against human Norovirus challenge

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Human norovirus (NoV) is the leading causative agent of acute nonbacterial gastroenteritis worldwide. No vaccine is currently available for this virus. Parainfluenza virus 5 (PIV5) is an excellent viral vector for vaccine development. In this work, we generated a recombinant PIV5 expressing VP1 of human NoV (PIV5-VP1) (GII.4, GenBank accession number FJ537136, named as 1988-NoV in this work). PIV5-VP1 was safe and generated robust antibody responses in gnotobiotic piglets. Most importantly, PIV5-VP1 immunized piglets were protected against human NoV virus challenge: immunized animals had minimal diarrhea and a reduction over 1,000 fold of NoV RNA genome shedding after infection. Interestingly, while the challenge virus (GenBank accession number JX126912.1, named 2012-NoV) was a GII.4 strain, all five known epitopes of VP1 are different between these two strains of human NoV. The fact that PIV5 expressing VP1 of 1988-NoV protected challenge from 2012-NoV suggests that PIV5-VP1 is a promising vaccine candidate for NoV and PIV5-based NoV vaccine may provide broad protection.

Transcriptome analysis of necrotizing and granulomatous meningoencephalomyelitis in dogs

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Necrotizing and granulomatous meningoencephalitis (NME and GME, respectively) are common inflammatory diseases affecting the central nervous system of dogs. They are presumed to be immune mediated in nature with environmental and genetic components both contributing to development. Their etiopathogenesis is poorly understood and they are associated with significant morbidity and mortality. As a pilot study, we performed RNA transcriptome analysis via next-generation sequencing using 3 biologic samples from histopathologically confirmed cases of GME, NME, and non-neurologically affected dogs. In total, there were 3552 differentially expressed genes (DEGs) for GME and 2433 DEGs for NME with 1408 total shared DEGs between subsets as compared to non-neurologic illness. Assessment of gene ontology found 199 (22.8%) of shared upregulated genes were involved in the immune response. Enriched functional pathways included NF-kB signaling, T and B cell receptor signaling, cell adhesion, antigen processing and presentation, and apoptosis. Both subsets contained upregulated genes which are involved in T-helper (Th) cell 1, Th2, and Th17 processes. Comparatively, gene ontology evaluation for downregulated genes included neuronal development and differentiation as well as synaptic signaling. Enriched pathways included glutamate receptor signaling, calcium signaling, neuroactive ligand-receptor interaction, and Wnt signaling. Given the enrichment of cell signaling as well as multiple immune mechanisms, the results support previous considerations that NME and GME are immune-mediated and suggest an imbalance in regulatory mechanisms controlling inflammation. Key DEGs will be validated and pursued for development of biomarkers and targets for both diseases.

Coinfection shapes how pathogens interact with the gut microbiota

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Since the gastrointestinal microbiota provides many benefits to host health, gut microbial communities are often predicted to shift during host infection, and may even influence disease pathogenesis. However, studies exploring whether pathogens induce such microbiota shifts in humans and lab animals have yielded inconsistent results, suggesting that variation in the infection process, rather than the presence of infection alone, might influence pathogen-microbiota interactions. For example, most humans and animals are co-infected with multiple pathogens simultaneously, which may amplify or diminish microbial shifts induced by each infection alone. We therefore examined the role of coinfection with bovine tuberculosis (*Mycobacterium bovis*, TB) and gastrointestinal helminths in shaping the gut microbiota of free-ranging African buffalo. Female buffalo were repeatedly captured in Kruger National Park, South Africa from 2009-2011 to administer anthelmintic treatment and assess TB infection status. Fecal samples were then processed for 16S rRNA gene sequencing to determine whether TB-helminth coinfection explained more variation in the buffalo gut microbiota than each infection alone. Only weak differences in microbial diversity were present due to either TB infection or anthelmintic treatment. However, associations between microbial abundance and anthelmintic treatment were significantly altered by host TB infection status. For example, the abundance of select bacterial taxa decreased with anthelmintic treatment in TB-negative buffalo, but increased with anthelmintic treatment in TB-positive buffalo. These patterns suggest that coinfection can modify the microbial shifts induced by single pathogens, and emphasize that accounting for host coinfection status is key to understanding pathogen-induced alterations in the gut microbiota across a range of disease systems.

Characterization of the IgE-independent pro-inflammatory and pruritogenic transcriptome of compound 48/80-mediated skin lesions in healthy dogs

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Classical allergic studies have focused on immunoglobulin E (IgE)-mediated mast cell activation. Recently, activation of Mas-related G-protein-coupled (Mrgp) receptors have been identified to cause itch and IgE-independent mast cell and eosinophil degranulation. Compound 48/80 functions by activating mast cells via Mrgp receptor member X2 (MrgprX2) in humans and dogs. Intradermal injections of compound 48/80 have been proposed histologically to resemble canine AD, but the activation of the associated inflammatory and pruritic pathways is unclear. The objectives of this study were to characterize the inflammatory and pruritogenic transcriptome of compound 48/80-induced skin lesions. All 16 healthy male castrated Beagles were intradermally injected with compound 48/80 (10 micrograms/site) and buffered saline (diluent). Biopsies were collected 24h after intradermal injections of compound 48/80; healthy (non-injected) and saline-injected skin served as controls. One half of the biopsy was used for histopathology while the second half was immediately placed in RNALater solution for transcriptome analysis. We extracted total RNA from all skin biopsies then analyzed transcriptome using RNA-sequencing. Intradermal injection of compound 48/80 resulted in positive wheal and erythema reactions on the injected thoracic side in all 16 dogs. Histologically, there was an influx of inflammatory cells at 24h post-injection with marked tissue eosinophilia and a mixed infiltrate of neutrophils and lymphocytes. Acute compound 48/80-mediated lesions had significant upregulation of pro-inflammatory (e.g., LTB, PTX3, CCL2, IL6, IL8, IL18) and T helper-(Th)2 (e.g., IL4R, IL13RA1, IL25, IL33) genes, as well as Th2 chemokine CCL17. The Th1 (e.g., IFNγ, OASL, MX-1, CXCL10, CXCL11) and Th22 (e.g., IL32, LOR, KRT1, S100A9) signal, as well as the T-cell trafficking chemokine (CCR7) and its ligand (CCL19), were also among the upregulated genes. Significant upregulation of genes encoding known pruritogenic proteins and pathways, such as cathepsin S (CTSS) and CTSC, nerve growth factor (NGF), and histamine-synthesis enzyme and receptors (HDC, HNMT) were additionally seen. Pathway analysis revealed strong significant upregulation of JAK-STAT, IL-4, and TREM1 signaling. In conclusion, acute compound 48/80-mediated skin lesions exhibit a multipolar immunological axis upregulation (Th1, Th2 and Th22) in healthy dogs, resembling spontaneous canine AD lesions.

Development of a swine RNA polymerase I driven influenza reverse genetics system for the rescue of swine, avian and human origin influenza A and influenza B viruses

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Influenza viruses (IV) are one of the most devastating pathogens for swine production. Swine have close contact with humans providing optimal conditions for zoonotic transmission of IVs. Vaccination is the most efficient method to prevent and control influenza in swine. However, the high diversity of IVs hinders the selection of swine IV vaccine strains. Generation of IVs by reverse genetics allows for the development of fast, tailored vaccines that need to be consistently updated to combat circulating strains. The most common reverse genetics methods use bi-directional vectors containing a host polymerase (pol) I promoter to produce virus-like RNAs and a pol II promoter to direct the synthesis of viral proteins. Given the species-dependency of the pol I promoter, we explored the potential of using the swine RNA pol I promoter (*spolI*) in a bi-directional vector for rescuing influenza A viruses (IAVs) and an influenza B virus (IBV). Using expression plasmids containing the human pol I promoter (*hpolI*) or *spolI*, we compared reporter activity in human cells (HEK293T) and vaccine approved swine cells (PK-15). The expression plasmid was co-transfected with viral ribonucleoproteins (vRNPs) of swine-, avian-, and human-origin IAVs. The expression of the reporter gene was used as a readout of the ability to generate viral-like RNA and thus, the pol I promoter activity. Our results showed that the activity of the *spolI* was higher than the *hpolI* for the human-origin IAV and similar in the swine- and avian-origin IAV in swine PK-15 cells. Meanwhile, the *hpolI* had higher activity than the *spolI* for all IAVs in HEK293T cells as expected. Since we observed differential activity between the *hpolI* and the *spolI*, we assessed whether a bi-directional swine vector carrying the *spolI* could be used to rescue IVs. We found that swine-, avian- and human-origin IAVs and an IBV could be successfully generated employing an eight-plasmid transfection system in swine PK-15 and HEK293T cells. In conclusion, the *spolI*-based reverse genetics system could be used as a new platform to produce live attenuated vaccines.

Random, Real Time Sequencing of Avian Avulaviruses Isolated From Wild Bird Samples

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Wild animals are known reservoirs for many viruses that can transfer into domestic animals or humans. For example, wild fowl have been observed to carry virulent and non-virulent strains of avian avulavirus 1 (AAvV1), an economically impactful disease within the poultry industry. Therefore, the monitoring of wild animal populations for potential transfer of virulent viruses is imperative to protect domestic animal populations. Current methods for diagnosing viruses rely on agent-specific assays, such as PCR. While effective, the inherently high specificity of these assays can result in failure to detect mutants or novel viruses. MinION sequencing of samples is emerging as an effective way to quickly sequence whole genomes of viruses and to genotype viruses, without prior knowledge of the virus present in the sample. This novel technology could potentially provide both the accuracy and speed required to diagnose important viruses, such as avian AAvVs. The goal of this project was to determine the ability of MinION to effectively identify RNA viruses from hemagglutination-positive, influenza-negative viral cultures, isolated from wild bird populations using a random hexamer strand switching protocol. MinION sequencing was used to genotype egg-cultured viral isolates derived from cloacal/oropharyngeal swabs from North American ducks and shorebirds. This study shows that the MinION results align with the pan-AAvV and AAvV1 PCR results, while also detecting avian avulaviruses other than AAvV1 including AAvV2 and AAvV4. Additionally, MinION sequencing allowed for the detection of multiple viruses in several of the samples which would normally require multiple PCRs.

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Development of a diagnostic assay for detection and differentiation of Theileria species in white-tailed deer

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The spread of non-native parasites with the movement of animals is a major concern for disease emergence and native species health/conservation. *Haemaphysalis longicornis* (Asian longhorned tick) is native to eastern Asia, but has become invasive in several countries including Australia, New Zealand, and now the United States. Within its established range, *H. longicornis* is a vector of the piroplasm parasite, *Theileria orientalis* subtype Ikeda, which until recently was not known to occur in the United States. In 2017, clinical disease resulting in cattle mortality was reported in a herd in Virginia. *Theileria orientalis* Ikeda was determined to be the causative agent of disease and further investigation at the index site revealed *H. longicornis* infestations. Within the United States, white-tailed deer (WTD, *Odocoileus virginianus*) serve as suitable wildlife hosts for *H. longicornis* and are also infected with several genotypes of a *Theileria* sp. (often called T. *cervi*) that are distinct from *T. orientalis*. It is currently unknown if deer are susceptible to *T. orientalis*, a pathogen of agricultural concern. In this study, we developed a restriction fragment length polymorphism (RFLP) assay that can distinguish between the *Babesia* spp. and *Theileria* spp. commonly found in WTD as well as the exotic *T. orientalis*. Using this assay, conducted a survey assessing the diversity of piroplasm parasites found in WTD from the eastern United States using archived and recently collected cervid blood samples. So far, we have sampled 350 deer and found 287 (82%) positive for the WTD *Theileria*. sp., 28 (8%) positive for Babesia spp., and 6 (2%) positive for exotic *Theileria* spp. Twenty of the 264 samples had insufficient quantities of DNA to visualize RFLP results. Sequencing of 50 selected samples confirmed RFLP results validating the assay as a screening tool. Sequences of the six 'exotic' Theileria amplicons were >99% similar to a Theileria spp. isolated a Gray brocket deer from Brazil. Our data indicate that piroplasm infections are common in WTD and that they can possibly maintain a higher diversity of *Theileria* spp. than previously recognized.

Efficacy of a thermostable dry powder live attenuated influenza virus in the ferret model

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Influenza is a pathogen of widespread concern and impact, affecting millions of individuals every year. Vaccination is the most effective tool for protection against infection and disease. Live-attenuated influenza vaccines (LAIV) are particularly appealing as they elicit multiple arms of the immune system to provide protection. These vaccines, however, have implementation limitations as their effectiveness can be greatly diminished if they are not appropriately refrigerated. Here, we assessed the efficacy of an intranasal dry powder LAIV which has been made thermostable through a technique called preservation by vaporization (PBV). The ferret model, considered the gold standard for influenza vaccine efficacy studies, was used to determine the immunogenicity and efficacy of the stabilized LAIV as measured by serum and nasal wash (NW) antibody responses, and reductions in viral shedding and clinical signs after virus challenge. The ferrets were vaccinated using liquid LAIV or PBV LAIV with or without an adjuvant (AdVax) to assess the vaccine and the potential effect of an added adjuvant. Four weeks after vaccination, ferrets were challenged with a homologous influenza A virus, assayed for virus shedding and monitored for clinical signs. Virus loads in nasal washes were measured by EID_{50} assay, while serum and NW antibody responses were measured by hemagglutination inhibition assay (HAI) or ELISA. The study will demonstrate the immunogenicity and efficacy of the thermostable powder LAIV vaccine to advance the vaccine and adjuvant combination towards clinical trial with the goals of eliminating the need for a cold-chain and facilitating the stockpiling and distribution of influenza vaccines.

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Associations between systemic oxidative stress and endocrine parameters in horses and ponies

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British native ponies are predisposed to obesity and insulin dysregulation (ID) and healthy ponies have increased circulating insulin compared to horses. Obesity and ID are associated with oxidative stress in other species and oxidative damage is integral to the development of Pituitary Pars Intermedia Dysfunction (PPID) in equids, to which certain pony breeds may be predisposed. The objective of this study was to compare systemic oxidative stress between Welsh ponies and Quarter horses to determine if oxidative status is associated with breed, endocrine parameters, or obesity. We hypothesized: 1) there is a pro-oxidant bias in ponies, and 2) pro-oxidant markers are positively correlated with insulin concentration and obesity. 28 healthy Quarter horses (2-20 years) and 58 healthy Welsh ponies (1-30 years) were used. Plasma total cortisol, ACTH, insulin, and leptin concentrations were measured with validated immunoassays, and plasma reactive oxygen metabolites (dROMs) and plasma antioxidant capacity (PAC) were quantified using a novel photometric assay validated in our lab. Animals were sampled before feeding and excluded if they met criteria for PPID. Data were compared between breeds with unpaired t-tests and Mann-Whitney tests, and Spearman correlation analysis was performed to determine significant associations between oxidative status and endocrine or obesity parameters ($P < 0.05$). ACTH and leptin concentration were significantly higher in ponies than horses ($P = 0.011$ and $P < 0.001$ respectively), but cortisol and insulin concentrations were comparable ($P \geq 0.197$). dROMs were significantly higher in ponies than horses ($P = 0.026$), but PAC did not differ significantly ($P = 0.677$). dROMS were moderately and significantly positively correlated with insulin concentration ($r = 0.316$, 95% CI 0.01592 to 0.564, $P = 0.034$) and girth circumference ($r = 0.334$, 95% CI 0.03219 to 0.58, $P = 0.027$) in ponies but not in horses ($P = 0.345$, $P = 0.753$ respectively). These data demonstrate differences in endocrine parameters, oxidative markers, and associations between these factors between ponies and horses. The impact of increased dROMs and associations between pro-oxidant markers and insulin in ponies on oxidative damage to tissues – and thus on the risk of PPID development – warrant further mechanistic investigation.

Aurora Kinase A is essential for the maintenance of meiotic spindle stability in ovulated oocytes

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In mammalian oocytes, acentriolar microtubule-organizing centers (aMTOCs) play a critical role in meiotic spindle assembly and stability that is essential for accurate chromosome segregation. These unique aMTOCs are composed of key structural and regulatory proteins, necessary for microtubule nucleation and spindle organization. Pericentrin (PCNT), functions as the major scaffolding protein at aMTOCs. We developed a transgenic oocyte-conditional PCNT knock-down mouse model and established that PCNT depletion leads to the loss of key aMTOC-associated proteins, disrupted spindle stability, and high rates of chromosome errors. In the current study, we used this unique mouse model to assess the role of a key aMTOC-associated kinase, Aurora Kinase A (AURKA), on assembled spindle stability in ovulated (MII-stage) oocytes. Immunoflorescence analysis revealed the absence of AURKA at spindle poles in PCNT-depleted oocytes. Yet, total protein levels of AURKA, its active form (pAURKA) and a key regulator (TPX2) were similar to the control group. These data establish that the localization of AURKA to spindle pole aMTOCs, but not its overall protein levels, is dependent on PCNT. To test AURKA function, control and PCNT-depleted oocytes were briefly (4h) incubated with a selective AURKA inhibitor, MLN8237. In control oocytes, AURKA inhibition promoted: (i) increased chromosome misalignment as well as (ii) altered spindle structures, characterized by significantly reduced spindle length and pole width. These short spindles with highly focused poles showed changes in TPX2 distribution along microtubules, with very bright TPX2 labeling concentrated at MT minus-ends. Interestingly, SR-SIM high resolution microscopy revealed that AURKA inhibition also disrupts the normal organization of aMTOC-associated proteins, PCNT, γ-tubulin and NEDD1, from well-defined 'O' or 'C'-shaped configurations into amorphous small foci at the spindle poles. Notably, AURKA inhibition in PCNT-depleted oocytes also leads to short spindles with tightly focused poles. This indicates that AURKA action to regulate spindle length and pole focusing is, therefore, likely-mediated through microtubule and/or MT-associated protein interactions, instead of aMTOCs. Further studies are needed to address the underlying mechanisms. In summary, these data support that AURKA activity plays an important role in maintaining assembled spindle organization and stability. Funding support provided by NIH (HD0713330 & HD086528) to MMV.

Regulation of early innate immunity to influenza virus within the respiratory mucosa by the serine-threonine kinase, Tumor Progression Locus 2

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Host-encoded interferons (IFNs) are critical factors that mediate innate protection as well as modulate the adaptive immune response to viruses. Type III IFNs (IFNλs) are now appreciated to be the predominant IFNs produced during influenza virus infection, however there is limited information about the host pathways that regulate IFNλ expression. We recently demonstrated that the host-encoded serine-threonine kinase, Tumor Progression Locus 2 (Tpl2), enhances IFNλ production and host protection against influenza virus infection. In our current study, we seek to determine the role of Tpl2 in viral sensing and early induction of antiviral responses within pulmonary epithelial cells. Our hypothesis is that viral pathogen-associated molecular patterns (PAMPs) transduce Tpl2-dependent signals within epithelial cells that lead to early production of Type III IFNs to control viral spread, inflammation, morbidity and mortality. In order to characterize Tpl2 functionality within pulmonary epithelial cells, we have generated two conditional knockout mouse models using *Nkx2.1-Cre* and *Sftpc-Cre^{ER}* to drive deletion within lung epithelial cells types known to be replicative niches for influenza. Herein, we demonstrate that Tpl2 is expressed in alveolar epithelial cells, induced upon influenza infection and is required within lung epithelial cells to limit morbidity and mortality. Tpl2 ablation across multiple lung epithelial cell types enhanced susceptibility to influenza A virus, whereas Tpl2 ablation specifically within type II epithelial cells did not. Ongoing studies aim to delineate the Tpl2-dependent cell types and pathways required for IFN-λ induction and induction of interferon-stimulated genes (ISGs) requisite for efficient recovery from viral infections.

Pharmacokinetics of pergolide mesylate in donkeys (*Equus africanus asinus*)

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Donkeys exhibit the same clinical signs of pituitary pars intermedia dysfunction (PPID) as horses, and are typically treated with pergolide mesylate as recommended for horses. However, drug dosages and dosing intervals recommended in horses cannot be extrapolated to donkeys due to differences in drug distribution and clearance between species. The objective of this study was to determine the pharmacokinetics of pergolide mesylate in adult donkeys. We hypothesized that based on pharmacokinetic variables in donkeys, pergolide will be suitable for once daily oral administration at a comparable dose of 2-4 mcg/kg in this species. On day 1, six healthy donkeys received pergolide (2 mcg/kg) intragastrically. Blood was collected for quantification of pergolide plasma concentrations at 0, 10, 20, 30, 45 min, and 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, and 48 h after this dose. Five additional doses of pergolide (2 mcg/kg) were then administered orally for 5 days at 48, 72, 96, 120, and 144 h after the first dose, and blood again collected at the time points above with the final dose. Plasma pergolide concentrations were measured using UPLC and tandem mass spectrophotometry. Pergolide concentration versus time data after the first and last doses were analyzed based on noncompartmental pharmacokinetics using commercial software, and paired t tests used to compare parameters between time points ($P < 0.05$). C_{max}, T_{max}, AUC, and $t_{½λz}$ differed significantly ($P<0.03$) between the first and last dose. Specifically, C_{max} after intragastric dosing on day 1 (0.16±0.16ng/mL) was more than 6-fold lower than C_{max} after 5 days or oral dosing (3.74±2.26ng/mL). $t_{½λz}$ was 1.7-fold longer after 5 days of oral dosing than on day 1 after one intragastric dose. These data demonstrate differences in pergolide pharmacokinetics between single and multiple doses in donkeys, which could be explained due to differences in drug metabolism resulting in drug accumulation, sublingual absorption of the drug with oral dosing, or both. Maximal plasma concentration reached in donkeys after repeated dosing was substantially higher in donkeys than previously described in horses. Thus, pergolide pharmacokinetics differ between donkeys and horses, impacting species-specific dosing recommendations for this drug.

Bisphenol compounds exert cytoskeletal disrupting activity that perturbs early embryonic development

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The developmental potential of an embryo is critically dependent on the timing and stability of key early stages including, initial cleavage division post fertilization, cell polarization, compaction and cavitation. Hence, compounds that disrupt these important transitions can severely impair embryo development. The endocrine disrupting activity of bisphenol A (BPA), widely used in plastics and epoxy resins, is well documented. However, the potential effects of bisphenol exposure during pre-implantation embryo development are less understood.

In this study, we tested whether exposure to BPA or its common replacement analogue, bisphenol F (BPF), is detrimental to (i) the first mitotic division, (ii) actomyosin dynamics during compaction and/or cavitation, and (iii) cell lineage specification in pre-implantation mouse embryos. In-vitro fertilized zygotes were cultured with increasing concentrations (0, 5, 25µg/ml) of either BPA or BPF at key time points for short periods (8-25h). The embryos were assessed by live cell imaging or fixed for immunofluorescence to evaluate the expression of specific markers such as NANOG and CDX2, which label the pluripotent inner cell mass (ICM) and trophectoderm (TE) cells, respectively. Our analyses revealed dose-dependent disruptive effects on the early embryo. Surprisingly, BPF exposure was more harmful than BPA, evidenced by further delayed mitotic division and lower blastomeres number at all stages. Impaired division was attributed to microtubule (MT) disruption. Embryo development was only partially rescued upon compound removal, resulting in poor blastocysts with disproportionate cell lineage ratios and disorganized actomyosin meshwork. Immunofluorescence analysis also showed disruption of cell polarization and cell zippering at the 8-cell and morula stages, respectively. Consistent with previous studies, embryo hatching was reduced following BPA or BPF exposure during cavitation. Additionally, ICM restriction and CDX2 expression were impaired at this stage.

In sum, these findings demonstrate that a brief exposure to either BPA or BPF is highly detrimental to the first mitotic division post fertilization as well as subsequent pre-implantation embryo development. Notably, exposure to BPF, which is currently utilized as BPA substitute, is more damaging to the early embryo –raising safety concerns regard to its use. Funding support provided by NIH (HD 0713330 and HD086528) to MMV.

Poster Presentation Abstracts

Prenatal exposure to Bisphenol A, S and F increases blood pressure in female rats. #7

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Cardiovascular diseases are the leading causes of mortality among men and women. With the new blood pressure guidelines from the American Heart Association, almost half of the United States population has hypertension (45.6%). The reasons for this high prevalence of hypertension in our population could be several, but the effect of emerging contaminants are overlooked and understudied. Bisphenol-A (BPA) is a widely used plasticizing agent that contaminates the environment. Most humans are exposed to BPA on a daily basis and urine levels of this endocrine disrupting chemical (EDC) are positively correlated with hypertension. The FDA banned the use of BPA in baby bottles in 2012, however, it is still being used in food containers and plastics. Currently, several BPA analogs such as bisphenol-S (BPS) and bisphenol-F (BPF) are used to replace BPA in the plastic industry. But their physiological effects are not clear. In order to study the effects of these EDCs on the development of hypertension, we exposed pregnant Sprague Dawley (SD) rats to saline, 5 µg/Kg BW of BPA, BPS or 1µg/kg BW of BPF. The offspring were allowed to reach adulthood before implantation with a radiotelemeter (Data Sciences International; HD-S10) in the femoral artery for undisturbed monitoring of systolic, diastolic and mean arterial blood pressure and heart rate. Recordings were measured once a week for 11 weeks over 24 hours to establish day and night readings. Night-time systolic BP was significantly elevated in BPA, BPF and BPS exposed rats compared to control. During the day, systolic BP was significantly higher in the BPA group compared to control. Diastolic BP was elevated in the BPS and BPF groups. Heart rate was elevated the most in the BPS group. These results indicate that prenatal exposure to low levels of BPA analogs has a profound effect on hypertension.

New Avian Pathogenic *Escherichia coli* O Serogroup Epidemiology for Poultry Health in Georgia. #3

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Colibacillosis caused by Avian Pathogenic *E. coli* (APEC) is a costly disease for the poultry industry worldwide, significantly affecting one of the world's cheapest sources of high-quality protein, resulting in mortality at the farm and carcass condemnation at slaughter. For years, geographic variation in APEC distribution has been known, with the most common *E. coli* serogroups representing >90% of characterized APEC which includes serogroups O1, O2, and O78. Currently, commercial vaccines are available specifically targeting these serogroups. Here, we propose to fill the gap in knowledge of APEC and perform an epidemiological study to investigate emerging O serogroups that may be contributing to poultry disease in Georgia.

Our research group has developed a PCR-based-assay that identifies the 13 most frequently found O serogroups (including O1, O2, O8, O18, O24, O25, O44, O78, O86, O88, O103, O117 O119 and O186). We found that 72.2 % of isolates from diagnostic APEC, and 29.4 % of the fecal isolates were positive for one of these 13 serogroups tested. The most prevalent serogroups identified among strains of APEC implicated in disease in Georgia include O78, O24, O2 and O25 with a prevalence of 22.8, 14.3, 10, and 8.6% respectively. While serogroups O44, O8, O186, and O18 (6.5, 6.2, 5.1 and 4.5% prevalence) were most common among fecal isolates collected.

Epidemiological analysis of our data has allowed us to identify new emerging clones, such as O25 and O24 that were not previously associated with APEC before, and defining traits of the environment of the host that can potentially lead to *E. coli*-associated disease in production broilers in Georgia. Interestingly we found O8 and O18 serogroups in fecal chicken isolates that are common serogroups found in *E. coli* associated with human urinary tract disease. With better knowledge of the populations of microbiota present, we can better predict vulnerable flocks and identify strategies for collibacilosis control at the farm level. Having a better knowledge of the current APEC causing disease we can identify new potential targets for vaccine development which could potentially reduce losses for producers in Georgia and elsewhere.

Anti-oxidant effects of vitamin C, thiamine, and hydrocortisone on equine leukocyte function in an *ex vivo* sepsis model #4

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Bacterial sepsis and the related systemic inflammatory response syndrome (SIRS) are the leading causes of morbidity and mortality in horses and foals. Immunomodulatory factors such as cortisol, thiamine, and vitamin C are known to alter endocrine and immune function. Administration of these factors in combination has shown to improve outcome in septic people, and may also be beneficial in equine sepsis. However, effects of these compounds alone and in combination on leukocyte function are not well characterized and ideal dosing protocols are not known in people or animals. The objectives of this study were to evaluate dose-dependent effects of hydrocortisone, vitamin C, and thiamine individually and in combination on plasma reactive oxygen metabolites (dROM) and anti-oxidant capacity (PAC) during *ex vivo* exposure to bacteria, and to compare responses between foals and adult horses. We hypothesized that hydrocortisone, vitamin C, and thiamine would decrease bacteria-induced oxidative stress in a dose-dependent and synergistic fashion, and that effects would be similar between adult horses and foals. Whole blood was collected from 7 healthy horses and 7 healthy 1-day-old foals, and exposed to either killed whole-cell *Escherichia coli* or *Staphylococcus aureus* in the presence or absence of biologically relevant concentrations of hydrocortisone, thiamine, and vitamin C individually and in concert at 37°C for 6 hours. Plasma dROM and PAC were quantified using validated photometric assays with the FRAS 5 system (Free Radical Analytical System, Innovatics Labs). Data were analyzed with repeated measures ANOVA ($P<0.05$). Vitamin C significantly suppressed dROM and significantly increased PAC in a dose dependent fashion in both foal and adult horse samples. Hydrocortisone and thiamine alone had no effect on dROMS or PAC in foal or adult horse samples, but potentiated vitamin C's effects on dROM and PAC when exposed in combination. dROM production was significantly lower in foals than adult horses, despite comparable PAC, but the degree of vitamin-C induced suppression of dROM did not differ significantly between horses and foals. In sum, these compounds appear to have immune-modulating effects on equine oxidative stress in a dose-dependent and synergistic fashion *ex vivo*, and warrant further investigation in clinical sepsis.

In vivo effects of pimobendan on platelet function in healthy adult cats #5

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For cats with cardiomyopathy, thromboembolic complications account for 9.5 – 38% of deaths, justifying the use of preventive antithrombotic drugs in high-risk patients. Cats with non-obstructive forms of cardiomyopathy and congestive heart failure are frequently prescribed pimobendan, a positive inotropic and vasodilating agent, alongside standard heart failure medications. In addition to its calcium-sensitizing effects, pimobendan also inhibits phosphodiesterase-3, which may confer a clinically relevant anti-thrombotic effect. There are no published reports characterizing pimobendan's *in vivo* platelet effects in animals. Only one investigation of pimobendan's effects on feline platelets has been reported; in that *in vitro* study, concentration-dependent inhibition of platelet aggregation was demonstrated using feline platelet rich plasma.

The aim of the present study was to evaluate the effect of orally administered pimobendan on platelet function in healthy adult cats. We hypothesized that compared to no treatment, administration of twice-daily oral pimobendan would not be associated with statistically significant changes in platelet function parameters. Six healthy purpose-bred, adult domestic shorthair cats received pimobendan orally at a dosage of 0.625mg/cat (low-dose) BID for 1 week, immediately followed by 1.25mg/cat (high-dose) BID for 1 week. Peripheral venous blood sampling for platelet testing and plasma drug measurement was performed prior to initiation of pimobendan treatment (pre-treatment baseline), 60 minutes post-dose on the morning of the 8th day of treatment with low-dose pimobendan, 60 minutes post-dose on the morning of the 8th day of treatment with high-dose pimobendan, and after a final 1-week washout period (post-treatment baseline). Platelet function was assessed by whole blood aggregometry using collagen and adenosine diphosphate (ADP) agonists, and by use of a platelet function analyzer (PFA-100®). Repeated measures-ANOVA was used to compare various platelet function variables among the four sampling timepoints; a p-value of 0.05 was considered statistically significant.

No significant difference in PFA closure time or any evaluated aggregometry variable was found among treatments. These findings suggest that oral pimobendan produces no measurable antithrombotic effects when administered to healthy adult cats at two clinically relevant dosage rates. Plasma concentrations of pimobendan and ODMP (the drug's active metabolite), measured using high-performance liquid chromatography and mass spectrometry, are pending.

The comparison of skin lesion transcriptomes between human and animal models of chronic cutaneous lupus erythematosus

#6

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Chronic cutaneous lupus erythematosus (CCLE) lesions consist of severe inflammatory processes leading to decreased quality of life and potential skin scarring with disfiguration. The treatment of CCLE needs novel effective therapeutics first testable on animals. To determine the best model for the biological pathways of human CCLE, we compared a meta-analysis of the human CCLE skin transcriptomes with those of their spontaneous canine and murine (MRL/lpr) homologues. Utilizing microarray data of three human CCLE studies, we determined a consensus shared gene list of 245 differentially expressed genes (DEGs) (>2-fold enhanced, $P < 0.05$). The Th1 and interferon (IFN)-related genes (*STAT1, OASL, MX1, IFNG, GZMB, ISG15*) and those encoding T-cell trafficking chemokines (CXCL9, CXCL10 and CCL11) were among the strongest upregulated genes. Using Metacore overlap analysis, the top enriched process networks of human CCLE were upregulations of type I interferon and IFNG signaling, innate immune response to RNA viral infection, NK-cell cytotoxicity and the JAK-STAT pathway. The comparative analysis between species revealed that canine and murine CCLE lesions contained 56% (139/245) and 24% (59/245) of the DEGs of human CCLE skin lesions, respectively. There was a moderate-to-strong positive significant correlation between canine and human CCLE DEGs (Pearson r = 0.52-0.67). The shared canine and human CCLE lesional transcriptome signature reflected a strongly activated pathway of type I IFN signaling involving JAK-STAT, the antiviral actions of type I interferon and IFNG signaling. In conclusion, the skin lesions of canine CCLE appear to reproduce the main immunologic signature of human CCLE.

Independent and combined effects of low-dose Bisphenol A and Diethylhexyl Phthalate on pregnancy outcomes and offspring development in Sprague-Dawley rats #2

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Bisphenol A (BPA) and Diethylhexyl Phthalate (DEHP) are common environmental endocrine disrupting chemicals (EDCs) that are widely used in the plastic industry to increase flexibility and durability. The release of BPA and DEHP into the environment, via manufacturing and leaching from plastic products is well documented, posing an environmental risk for expecting mothers. BPA and DEHP exposures are of concern because of their non-monotonic dose-responses and their biological adverse effects in animals. Reports addressing the mixture effect of these EDCs are limited. Trying to approach a relatively realistic scenario of multi-exposure to EDCs, we considered exposing pregnant Sprague-Dawley rats to individual as well as combinations of BPA and DEHP at environmentally relevant low doses. From gestational day 6 till 21, dams were orally administered either saline (control; n=15), BPA (5µg/Kg BW/day; n=14), LD DEHP (5µg/Kg BW/day; n=6), high-dose (HD) DEHP (7.5 mg/Kg BW/day; n=10), a combination of BPA and LD DEHP (LD B+D, n=6), and a combination of BPA and HD DEHP (HD B+D, n=11). Gestational weights and number of abortions were tracked. Immediately following parturition, number of live births and stillbirths were counted. Pup birth morphometric parameters (chest circumference, abdominal circumference, nose-to-anus length) and anogenital distance were measured. Litter size and weights were monitored on postnatal days (PND) 1, 7,14, and 21. Our data showed that LD individual BPA or DEHP abortion rates were comparable to control dams. Combinations of LD BPA and DEHP increased the abortion rate (14%) making it comparable to the individual HD DEHP (10%) and high dose B+D group (23%). Gestational index (# of dams with live litters/# of pregnant dams) was reduced in HD DEHP (90%), LD B+D (86%), and HD B+D (66%) compared to control (100%) and LD BPA (100%). No differences were detected in gestational weight gains. Our study suggests that EDC mixture, even at low doses, induced a more pronounced effect on pregnancy and postnatal outcomes than individual EDCs. This suggests a great need to (1) reconsider the possible additive, antagonistic or synergistic activities of the resulting EDC mixture to which pregnant individuals are exposed to, and (2) reassess the current threshold model used in EDC risk assessment which does not take into consideration the potential effects of real-life exposures to mixtures containing EDCs at environmental doses.

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Molecular Confirmation of Ranavirus Infection in Amphibians from Chad, Africa. #8

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Ranaviruses are DNA viruses (Family *Iridoviridae*), and are considered an emerging infectious disease of amphibians. Ranavirus infection can have varying pathological effects on infected amphibians, most notably causing significant mortality events and population declines. Despite having a broad global range with reports from 6 continents, only a single incidental finding in *Xenopus longipes* from Africa (Cameroon) is known, and lacks molecular confirmation. Thus, there is a considerable knowledge gap concerning ranaviruses in Africa. We opportunistically obtained tissue samples from 160 amphibians representing 5 genera (*Hoplobatrachus, Hylarana, Ptychadena, Pyxicephalus,* and *Xenopus*) from Chad, Africa. Samples were tested for ranavirus infection using a real-time quantitative PCR assay targeting the major capsid protein (MCP). A total of 25/160 (16%) frogs tested positive including 15/87 (17%) *Hoplobatrachus* spp., 10/58 (17%) *Ptychadena* spp., 0/3 *Pyxicephalus* spp., 0/9 *Xenopus* spp., and 0/3 *Hylarana* spp. Sanger sequencing confirmed all samples were >98% identical to Rana nigromaculata ranavirus (RNRV) and Frog Virus 3 (FV3)-like sequences. Additional gene targets (DNA polymerase [DNApol], ribonucleotide reductase alpha [RNR- α], ribonucleotide reductase beta subunit [RNR- β]) were sequenced to provide further detailed classification of the virus. Sequences of individual gene targets indicate that the ranavirus detected in frogs in Chad is most similar to tiger frog virus (TFV), a FV3-like virus previously isolated from diseased amphibians cultured in China and Thailand. Full genome sequencing of one sample indicates that the Chad frog virus (CFV) is a well-supported sister group to the TFVs previously determined from Asia. This work represents the first molecular confirmation of ranaviruses from Africa, and is a first step in comparing ranavirus phylogeography on a local and global scale.

Rates of Copepod Consumption by Frogs and Fish with Implications for Transmission of *Dracunculus* spp. #9

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Guinea worm (*Dracunculus medinensis*), is a parasitic nematode and the causative agent of Dracunculiasis. Generally transmitted by the consumption of a copepod intermediate host, control efforts by international and national Guinea Worm Eradication Programs (GWEPs) have decreased the number of cases from ~3.5 million human cases in 21 countries throughout Africa and Asia (1986) to 25 human cases in 4 countries (2019). Despite this success, the first domestic dog case in Chad was reported in 2012, and the number of infected dogs in Chad has continued to increase annually. Previous research indicates that dogs consume few copepods when drinking directly from a water source, suggesting alternative routes of infection might be occurring. Previous work has shown that paratenic hosts (amphibians) and transport hosts (fish) may be involved in Guinea worm transmission. Therefore, we compared copepod ingestion among different species of fish, frogs (tadpoles and adults), and newts to estimate their possible role in transmission of Guinea worm as it is unknown whether these aquatic animals ingest copepods at different rates. We hypothesized that fish would consume more copepods than amphibians. We found that fish consumed significantly more copepods (68%, 34/50) compared to tadpoles (16%, 8/50), adult frogs (36%, 18/50), and newts (16%, 8/50) ($p<0.001$). Consumption rates between fish species differed, but not between amphibian species. There was no association of consumption rate with Gosner stage (age) of tadpoles, but there was a positive correlation between copepod ingestion and fish size ($p=0.06$). These data confirm that both amphibians and fish consume copepods, but that fish ingest significantly higher numbers. These results support current intervention strategies aimed at interrupting *D. medinensis* transmission (burying and burning fish entrails), and also suggest additional research is needed for investigating the role of fish in transmission of Guinea worm to dogs in Chad.

Factor XIII expression in normal canine tissues and perivascular wall tumors #10

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Factor XIII (FXIII) primarily functions in the final step of the coagulation cascade, cross-linking fibrin monomers to stabilize blood clots. Its role in cardiovascular and thrombotic disorders is an area of interest in human medicine, as well as its role in neoplasia and pregnancy. In humans, normal pericytes and hemangiopericytomas express FXIII. The role of FXIII, however, in canine medicine is poorly studied. The objective of this study is to use immunohistochemistry to determine FXIII expression in normal canine tissues and perivascular wall tumors, which are thought to arise from pericytes. Using two commercially available anti-human FXIII antibodies, we developed immunohistochemical protocols for the detection of FXIIIa and b subunits in formalin-fixed paraffin-embedded samples of canine blood clots, buffy coat, and bone marrow; these samples were selected because they contain platelets, which are known to express FXIII. For immunohistochemistry, normal tissues were collected in formalin from a freshly euthanized dog, while paraffin-embedded and 8mm punches from paraffin blocks of archival samples of canine tumors (hemangiopericytoma, n=7; hemangiosarcoma, n=10; peripheral nerve sheath tumor, n=11; fibrosarcoma, n=6) were assembled into tissue microarrays. Our protocols have so far proved that FXIIIb is expressed in both canine platelets and megakaryocytes, as well as in liver (hepatocytes), ovary, and testes. Perivascular wall tumors did not uniquely express FXIIIb immunohistochemically, but expression of FXIIIa in normal and neoplastic canine tissue requires further investigation, which is currently ongoing. The immunohistochemical protocol developed in this study can be applied in further FXIII research.

In vitro serial passage to evaluate selection required for the adaptation of human Influenza A viruses to pigs #11

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Interspecies transmission of influenza A viruses (IAV) from humans to pigs is relatively common. These spillover events allow for reassortment between human and swine viruses and provide a platform for genetically diverse viruses to adapt to swine. This had past implications in the epidemiology of IAV in swine and led to the generation of pandemic viruses, a potential risk that still exists. More information is needed on the progression of the genome as novel viruses are transmitted throughout swine populations. Identifying mutations in the different IAV segments during the adaptation of human IAV in swine may help predict evolution and identify viruses with increased risk of becoming endemic in pigs. Madin-Darby Canine Kidney (MDCK) cells are the established cell line for studying IAV replication due to their permissibility to infection with IAV. However, IAV naturally replicates in the respiratory epithelium of its hosts. The objective of this study is to evaluate the adaptations and mutational changes of human IAV that allow for replication in swine. We use immortalized Swine Tracheal Epithelial Cells (STEC) to look at the adaptation and evolution of a human IAV *in vitro* in a substrate that provides a more natural platform than MDCK cells. A protocol was established for the culture of this cell line. Three different IAV's were propagated in MDCK cells and titrated for this study (VIC11: seasonal human IAV; OH04: swine-origin IAV; and VIC11 HA-NA: reassortant containing HA/NA from VIC11 and internal genes of OH04). Serial passages will be performed in the STEC line followed by deep sequencing. We expect that this model will mimic the characteristics of virus evolution during the adaptation of IAV from humans to pigs.

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Construction of recombinant HA for the design of a broadly-reactive H1 influenza antigen #12

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The CDC estimates that influenza has resulted in between 9.3 million - 49.0 million illnesses, between 140,000 - 960,000 hospitalizations and between 12,000 - 79,000 deaths annually since 2010. Although these are very broad ranges, these statistics highlight the devastating effects influenza can have on an individual's health. More broadly effective vaccines are an absolute necessity in order to improve public health. In response to the influenza virus, antibodies of the human body's immune system target the surface glycoprotein antigens of hemagglutinin (HA) and neuraminidase (NA), which are utilized by the virus to attach to a host, through HA, and detach through NA. The effectiveness of the influenza vaccine is heavily dependent on the antigenic similarities between the vaccine strains and the in-season influenza strains. My work involves creating and purifying plasmids that express hemagglutinin, within the H1 strain, and that will be used for the construction of a computationally optimized broadly reactive antigens, or COBRA. These plasmids are designed and based off the sequences and amino acids of the prominent HA's, A/Michigan/45/2015 and A/California/07/2009 and are utilized to be able to recognize both HA's. The constructed plasmids have been named MCs; MC-2 and MC-4 are the primary focus of this project.

Evaluation of endogenous immunomodulatory genes from stimulated and immunosuppressed adMSCs from cats with and without CKD #13

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There is limited understanding of the endogenous activity of feline adipose-derived mesenchymal stem cells (adMSCs) derived from cats with systemic disease; therefore, the immunomodulatory gene expression among adMSCs from healthy cats and cats with chronic kidney disease (CKD) may be dissimilar. To make progress towards using autologous adMSCs as part of the therapeutic regimen in cats receiving renal transplantation, it is essential to understand endogenous gene expression of key immunomodulatory genes in both healthy and CKD cats, as variability in gene expression may have significant implications in their *in vivo* application. We assessed the phenotype and gene expression of six lines of adMSCs each from healthy cats and cats with CKD. To demonstrate that these cell lines were adMSCs, we required that they have a phenotype of CD90, CD105 and CD44 (+) and MHCII (-). RT-qPCR was used to determine gene expression of key immune modulatory genes (IL-6, IL-10, IL-12p40, IL-18, TNF-α TGF-β with both pro-inflammatory and anti-inflammatory function and stimulated them with both exogenous immunosuppressive drugs and pro- inflammatory stimuli. In unstimulated cells, a 4.3-fold increase in the expression of IL-10 was demonstrated in cats with CKD and a trend towards a higher expression of IL-18 in healthy cats was also observed. IL-6, IL-12p40, TNF- measurements are incomplete as well as the pro-inflammatory and immunosuppressive stimulation of cells. Important differences may exist in immunomodulatory capabilities of adMSCs from cats with CKD, and even with limited sample size, this data will help shape their clinical use.

A rapid, parasite-dependent cellular response to Dirofilaria immitis in the jird (*Meriones unguiculatus*) #14

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The Mongolian jird (*Meriones unguiculatus*) is recognized as a permissive host for the filarial parasite *Brugia malayi*. This is believed to result from the immunological characteristics of the species, as particular immunodeficient mouse models exhibit this same permissivity. Jirds are nonpermissive to the filarial parasite, canine heartworm (*Dirofilaria immitis*), so by elucidating differences in early response to infection, we hope to identify mechanisms involved in the species-specific clearance of the parasites. We assessed the species-dependency of cell attachment in vitro. Cultures were prepared as groups: live *D. immitis* L3, live *B. malayi* L3, live co-culture of both parasites, heat-killed *D. immitis*, and heat-killed *B. malayi*. Each group was cultured with peritoneal exudate cells (PECs) of naïve jirds and paired with a media-only control. Host cell attachment and parasite survival was assessed microscopically after 20h incubation. In live conditions, cell attachment to *D. immitis* was 100%, while *B. malayi* was lower (mean = 5.6%), suggesting a strongly species-dependent response from which *B. malayi* could not confer immediate protection in co-culture. When we replicated these experiments with PECs derived from jirds subcutaneously infected with *B. malayi*, results were similar (99.4% and 4.7% of *D. immitis* and *B. malayi*, respectively, exhibited cell attachment). In heat-killed conditions, cell attachment to *D. immitis* was 71.8%, while *B. malayi* was reduced (mean = 16.7%), which may suggest that actively released/secreted products are involved with early immune recognition. Applying Wright's stain, the attached cells were morphologically most consistent with lymphocytes, and the specific nature of this attachment is ongoing.

Prevalence, pathological aspects and genome heterogeneity of feline morbilliviruses #15

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Feline morbillivirus (FeMV) is an emerging member of morbillivirus family. Although association between FeMV and tubulo-interstitial nephritis (TIN) has been suggested in cats, the pathogenicity of the virus is still not clear. In this study, we aimed at: i) determining FeMV prevalence in central Italy, ii) analyzing the genome of circulating FeMVs; iii) studying the association between FeMV and TIN. Urine, blood and serum samples collected from live cats (Group A, n=196) were screened by a $qPCR_{FeMV}$. Internal organs of carcasses (Group B, n=35) were also tested by $qPCR_{FeMV}$. Cats of groups A and B were classified in housed cats (subgroups RC and RCC, respectively) and cats from three colonies (subgroups C and CC, respectively). Serological screening was performed on all cats of group A by IIF. qPCR-positive RNAs were used for next generation sequencing. All tissues which resulted RNA positive and all kidney sections (regardless of the $qPCR_{FeMV}$ results) were evaluated by H&E and immunohistochemistry (IHC). To analyse the association between FeMV and renal damage, two statistical analyses were performed. To investigate the tissue tropism of FeMV, virus histochemistry (VHC) was performed. The prevalence of FeMV infection in subgroups RC and RCC was in line with the infection rates of other Countries (P= 8.66%-10.71%). FeMV RNA was constantly detected in kidney and urinary bladder. A seroprevalence of 21.73% and 17.32% was demonstrated for subgroups C and RC, respectively. Sequences of this study did cluster into two clades within FeMV genotype 1. No statistically relevant relationship between FeMV and renal damage was demonstrated (p=0.0695). IHC analysis of RNA+ carcasses, revealed immunoreactivity within epithelial cells of renal tubuli, inflammatory cells and lymphocytes. Indeed, as demonstrated by VHC, FeMV exhibits a distinct tropism toward different cellular types such as inflammatory cells residing in blood vessels of kidney and brain, airway epithelial cells, alveolar macrophages and, to a lesser extent, the central nervous system. Related morbilliviruses such as canine distemper virus (CDV), have been shown to cause severe immunosuppression and neurological disease in dogs. More efforts and larger scale studies are needed to disentangle FeMV pathogenesis and replication in other tissues.

Sea turtles, worms, and blood flukes: an experimental infection study #16

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There are 29 known species of blood flukes that infect sea turtles; however, more are expected to exist. These parasites infect the cardiovascular, pulmonary, neurological, and gastrointestinal systems and can greatly impact the health and longevity of sea turtles. Despite the pathology caused, there remain large gaps in knowledge concerning the biology of sea turtle blood flukes (TBFs), including the life cycle and transmission of infections. It is important to further elucidate the life cycle of these parasites in order to improve husbandry of captive sea turtles and prevent accidental infection. The aim of this study was to better understand the role of polychaetes as intermediate hosts for TBFs. Annelids collected in South Carolina were examined by microscopy for parasitic infection. Non-infected organisms were maintained in the lab for experimental infections. Infected and deceased annelids were dissected to retrieve any larval TBFs for morphological and genetic analysis. Sea turtle feces, collected from patients at the South Carolina Aquarium and the Loggerhead Marinelife Center, were filtered and examined for parasite eggs to be used in experimental infection studies. We found 25% of the *E. sanguines* collected were infected with larval blood flukes, one of which was confirmed via genetic analysis to be a *Neospirorchis* sp. known to infect sea turtles. Seven different morphological types of parasite ova were filtered out of the sea turtle feces and used for experimental infection studies with captive annelids. Outcomes from this work could aid aquarists and veterinarians in better management of captive sea turtles thus helping to improve the overall health of sea turtle patients and residents of aquariums.

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Effects of trace minerals on the immune response to bovine coronavirus and rotavirus vaccination in dairy cows #17

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Neonatal calf diarrhea (NCD) is a polymicrobial disease responsible for 25% of calf deaths in the United States. Bovine coronavirus (BCoV) and rotavirus (BRV) have been identified as the primary pathogens responsible for NCD. Current NCD vaccines have been shown to elicit variable immune responses to BCoV and BRV. Injectable trace minerals (ITM) concurrent with vaccination has shown beneficial effects on the immune response against viral respiratory pathogens in cattle. The objective is to evaluate the effects of ITM concurrent with a commercial NCD vaccine on the immune response to BCoV and BRV in dairy cows, and on passive immunity in calves. Forty dairy cows were randomly assigned to be administered ITM (Se, Cu, Zn, & Mn; ITM; n=20) or sterile saline (SAL; n=20) concurrent with a commercial NCD vaccine. Cows were vaccinated and given ITM or SAL 7 weeks before calving and then again 3 weeks later. Blood and fecal samples were collected weekly following vaccination, and colostrum collection began after parturition. Blood and fecal samples were also collected from calves. BCoV and BRV specific antibodies will be determined in serum and colostrum via neutralization assays. Total IgG will be measured in calf serum as an indicator for immune passive transfer. PCR will be performed on fecal samples to evaluate viral shedding. We expect that cows treated with ITM will show increased levels of BCoV and BRV specific antibody titers, and that calves consuming colostrum from ITM treated cows will show increased levels of maternal antibodies, reduced viral shedding, and fewer clinical signs compared to SAL calves. If effective, this strategy to enhance protection against BCoV and BRV may help to control NCD in dairy calves.

Multimin USA, Inc
Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Hatching optimization and culture media evaluation for *Ascaridia dissimilis* #18

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Ascaridia dissimilis is the most important nematode parasite of turkeys. Anthelminthic resistance (AR) has been demonstrated in *A. dissimilis*, however, there does not exist an *in vitro* test to detect AR. Efficient hatching and culturing is necessary to carry out *in vitro* assays and investigate AR in the parasite. The goal of this research is to optimize a hatching protocol as well as evaluate culture media and their ability to maintain and develop larvae from L_2 to L_3 and L_4. There exists hatching protocols and culture media evaluations for *Ascaris suum*, a nematode parasite of pigs, and *Ascaridia galli*, a nematode parasite in chickens, but not for *A. dissimilis*. The methods of the investigation will be compiling the most successful hatching techniques and culture media for *A. suum* and *A. galli* and evaluating their success with *A. dissimilis*. *A. dissimilis* and *A. galli* are very closely related in their life cycles, which lends optimism to the success of both the *A. galli* hatching and culturing protocols with *A. dissimilis*. Deshelling of the eggs followed by either centrifugation or carbon dioxide bubbling will be evaluated as possible hatching techniques. DMEM with and without fetal bovine serum, NCTC 135, and RPMI 1640 will be the culture media evaluated. If a hatching protocol is optimized along with a viable culture media to develop the parasite, investigation of an *in vitro* test to detect the genotypic and phenotypic characteristics of the nematodes demonstrating AR will be possible.

Immunogenicity of the canine influenza A virus (CIV) vaccine in parasitized dogs #19

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Canine influenza virus (CIV) causes mild respiratory disease in healthy dogs and potentially severe disease in immunocompromised dogs. Infected dogs may shed CIV for up to 24 days leading to possible outbreaks, particularly in animal shelters, resulting in high costs from quarantines and decontamination, as well as halts to animal intake. Moreover, animal shelters house dog populations at elevated risk of CIV infection and increased likelihood of severe disease. While CIV vaccines are commercially available and shown to be efficacious in healthy dogs, they are not considered a core vaccine per American Animal Hospital Association (AAHA) guidelines. Protective immunization by vaccination has been shown, using other animal models, to be diminished in parasitized animals. Since 1/3 of shelter dogs are reported to be infected by parasites, response to vaccinations may also be inferior. Thus, we sought to evaluate the magnitude and durability of immune responses elicited by CIV vaccination in parasitized and healthy animals. This blinded study included 14 dogs divided into three groups: non-parasitized, parasitized with *Brugia pahangi*, and parasitized with *Dirofilaria immitis*. All groups were vaccinated with Zoetis Vanguard CIV H3N2/H3N8 Killed Vaccine and then boosted. Blood samples were collected pre- and post-vaccinations. Vaccine immunogenicity was measured as serum antibody titers via isotype-specific ELISA and hemagglutination inhibition assays (HAI). We hypothesize that the parasitized dogs will have a reduced magnitude of serum antibody responses after vaccination, suggesting reduced vaccine efficacy. Understanding the impact of a parasitized state on CIV vaccine efficacy may aid in optimizing all vaccination protocols.

Quantification of Pain from Facial Expression Characteristics Assessed from Photos of Horses and Donkeys #20

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In the past decade more attention has been given to the evaluation of pain in animals. Assessment of pain has become more important to horse and donkey welfare alike. This study describes the assessment of objective pain scoring on photos of equines measured with two newly developed pain scales: the Horse Pain Face (HPF) and the Donkey Pain Face (DPF) to determine if prior training on how to use the pain scales lead to consistent scores between observers. Exactly 1654 horse photos and 534 donkey photos were assessed and these scores were used to determine the inter-observer reliability between the two observers. The intra-observer reliability was calculated by rescoring 20% of the photos from both the horse and donkey photo sets (n = 331 and 107, respectively). Both the HPF and DPF scored acceptable inter-observer reliability (Cronbach's alpha = 0.92 for the HPF; Cronbach's alpha = 0.79 for the DPF; $P<0.001$), while good intra-observer reliabilities were found for both scales (Observer 1: Cronbach's alpha = 0.81 for HPF; Cronbach's alpha = 0.84 for DPF; $P<0.00$ and Observer 2: Cronbach's alpha = 0.88 for the HPF; Cronbach's alpha = 0.97 for the DPF; $P<0.001$). This study shows that the inter- and intra-observer reliability for total HPF and DPF scores are acceptable and significant, indicating that the new scoring systems are valid tools for assessment of pain that can be used by any trained individual.

Prenatal EDC Exposure Followed by Chronic Treatment with Estradiol Affects Behavior and Brain Dopamine Levels in Female Rats #21

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Prenatal exposure to low doses of endocrine disruptors (EDs), in particular the widely prevalent plasticizers bisphenol A (BPA) and di(2-ethylhexyl) phthalate (DEHP), has been shown to produce long-lasting behavioral effects in rats. Additionally, changes in estrogen are associated with the development of affective disorders in women; however, the underlying neurobiological mechanisms are unclear. A previous study from our lab found that chronic estradiol treatment led to decreased dopamine (DA) levels within the central amygdala (CeA). This study was conducted to further examine the cumulative effects of prenatal exposure to EDs followed by chronic estradiol exposure in adult female rats on DA levels in stress-related brain areas. Dams were orally administered saline (control; 10 μL/kg), BPA (B; 5 μg/kg), DEHP (D; 7.5 mg/kg) or a combination of BPA+DEHP (B+D) during days 6 through 21 of pregnancy. Adult female offspring were sham-implanted or implanted with pellets that release 17β-estradiol (E2) for 90 days (20 ng/day; Innovative Research America). The offspring then underwent a battery of behavioral tests at the end of treatment. Brains collected from the offspring were sectioned and the CeA, hippocampus (HC), and prefrontal cortex (PFC) were microdissected and analyzed for levels of DA using High-Performance Liquid Chromatography (HPLC). Prenatal exposure to DEHP and/or B+D, but not B, was significantly associated with reductions in DA levels in the CeA and PFC. Within the CeA, only B+D-E2 treated offspring showed a significant decrease in DA ($p<0.05$) in comparison with control offspring. Levels of DA were found to be lower in the PFC as well, particularly in D-sham ($p<0.05$), D-E2 ($p<0.01$), and B+D-E2 ($p<0.05$) offspring compared to controls. No differences were observed in hippocampal DA levels in any of the treatment groups. These results indicate that prenatal exposure to DEHP or a mixture of EDCs in females may lead to alterations in monoamine levels in a region-specific manner, and E2 treatment in adulthood may exacerbate these effects.

Suppression of microglia activation reduces body fat accumulation in rats consuming high fat diet #22

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Obesity is the most common health issue in the United States and can lead to countless conditions including heart, liver, and joint disease. One prominent concern is the prolonged stimulation of microglia in the central nervous system, causing chronic inflammation of the vagus nerve and decreased signaling to the gastrointestinal tract. This slows metabolism and triggers increased body fat accumulation resulting in obesity. The purpose of this study was to determine the efficacy of Ibudilast, an anti-inflammatory drug, in reducing microglia activation and body fat accumulation. Male and female Sprague-Dawley rats were fed standard rodent chow for two weeks and then given either Ibudilast or a placebo and switched to high fat diet for three weeks. Body weight and body fat were monitored throughout the experiment. At the end of the experiment, rats were euthanized, perfused with paraformaldehyde, and hindbrains were collected. The nucleus of the solitary tract (NTS, an access point for the vagus nerve entering the brain) was sectioned and stained with IBA-1, an immunofluorescent stain used as a marker of activated microglia. The results of the study showed that Ibudilast reduces body fat and body weight in both male and female rats. We expect that placebo-treated rats will exhibit more microglia immunofluorescence than will Ibudilast-treated rats. This part of the study is still in process. If immunostaining will support the fat accumulation results, these findings would suggest the potential for Ibudilast to aid in treatment of obesity.

Full-thickness modeling of inflammation in the synovial membrane *ex vivo* #23

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Synovitis is an acute inflammatory response of the synovial membrane to joint trauma or infection and is thought to be a major role player in progressive joint deterioration as seen in osteoarthritis. To study synovitis, researchers have relied on synoviocyte cell culture to mimic and modify the inflammatory events postulated to underpin synovial inflammation. Unfortunately, synoviocytes de-differentiate and lose their phenotypic features when grown in monolayer, limiting our ability to appropriately interrogate the synovial environment *ex vivo*. It is, therefore, the overall goal of this project to develop an *ex vivo* technique to study the role of the synovium in joint inflammation. Our objectives are to establish a culture technique that maintains porcine full thickness synovial explant for up to 14 days and to determine the responses of the synovial explant to an inflammatory stimulus such as Interleukin-1 (IL-1). The fibrous joint capsule and synovial membrane will be removed as full thickness plugs using aseptic technique in block from the joint, and placed in complete medium in a transwell system. Synoviocytes will be monitored for viability, composition, and secretory function through histology and cell function assays. Supernatant will be evaluated for the production of pro-inflammatory markers such as TNFα. Our hypothesis is that the biological and mechanical composition of a synovial plug containing fibrous capsule and intact synovial villi will produce a useful model for researching acute arthritis, and render us able to explore possible applications of biological therapies in synovitis.

Neural circuitry for maternal behavior and recognizing infant distress in the mouse primary auditory cortex #24

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Maternal behavior is an evolutionarily conserved trait that ensures survival of the young and passage of genes to the next generation. A mother's response to infant cries is a key feature of maternal behavior, but the neural circuitry behind this behavior is not fully understood. In mice, maternal behavior can manifest through pup retrieval. Mouse pups emit ultrasonic vocalizations when they are separated from the nest, and mothers ('dams') respond to this auditory cue by retrieving the pups back to the nest. Virgin females co-housed with dams and litters are not initially responsive to pup calls, but start behaving maternally usually after hours to days. Previous work from our lab and others identified left auditory cortex as an important locus for maternal plasticity related to infant distress and recognition of pup call sounds (Marlin et al. Nature 2015). However, little is known about how this learning occurs. Here we aim to identify a method to study observational learning. Dams with pups were placed into one side of a learning arena. On the other side, separated by a barrier, was a virgin OXTR-tdTmouse. After running behavior experiments, the brains of the virgin mice were harvested and stained with c-fos. Results confirmed virgins are able to learn solely through observation and that the lateralization of oxytocin receptors to the left auditory cortex correlated with activity. Further analysis will be conducted to determine the relationship of oxytocin receptors and activities in other sensory cortices.

Comparison of pregnancy outcomes in dairy heifers artificially inseminated with sexed semen deposited in the uterine horns versus the uterine body #25

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Using sexed semen (SS) for artificial insemination (AI) has resulted in lower pregnancy per timed AI (P/TAI) compared to conventional semen in cattle. Horn AI has improved pregnancy rates in other species when using low sperm doses. The objective was to compare P/TAI and pregnancy loss (PL) in dairy heifers inseminated with SS deposited in the uterine horn (UH) ipsilateral to the pre-ovulatory follicle (PF) versus TAI in the uterine body (UB). This study was performed in two dairy farms in Georgia (A and B). In A, 74 Holstein heifers (12mo), received a 5-day Cosynch+CIDR protocol including an intravaginal Eazi-Breed CIDR® for 5d and a dose of GnRH IM (100µg, 2 mL). Immediately after CIDR removal, heifers received a dose of PGF2α IM (25mg, 5 mL) and again 24h later; 72h after CIDR removal heifers received 100µg of GnRH IM and TAI with frozen-thawed SS. In B, 237 Holstein x Jersey heifers (12mo) received a modified 5-day Cosynch+CIDR similar to A but not including GnRH at the time of CIDR insertion nor a second dose of PGF2α after CIDR removal. Before TAI, heifers were examined using transrectal ultrasonography (TRUS) to determine which ovary contained the PF. Each heifer was randomly assigned to TAI either in the UH ipsilateral to the PF (UH, n= 150; A, n= 32; B, n=118) or the UB regardless of the PF location (UB, n=161; A, n= 42; B, n=119). Pregnancy was diagnosed 32d after TAI by TRUS to determine P/TAI. Heifers diagnosed pregnant were re-examined at 60d of gestation to assess PL. Heifers in UH had adequate P/TAI [50.6% (76/150); A: 50.0% (16/32); B: 50.8% (60/118)], which was numerically greater (P=0.12) than that observed in UB [43.4% (70/161); A: 40.5% (17/42); B: 44.5% (53/119)]. Additionally, PL was within normal ranges for dairy heifers (10.0 and 9.4% for UH and UB, respectively). In conclusion, TAI of dairy heifers with SS deposited in the UH ipsilateral to the ovary containing the PF resulted in adequate P/TAI (7.2% greater than UB). Horn insemination with SS might become a valuable tool for optimizing reproductive efficiency in dairy heifers.

Serosurvey for West Nile virus in Ruffed Grouse (*Bonasa umbellus*) across the Midwest and eastern United States #27

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Since its introduction to the US in 1999, West Nile virus (WNV; Family *Flaviviridae*, Genus *Flavivirus*) has spread rapidly and is now endemic throughout much of North America. This virus is estimated to have had devastating impacts on many North American bird species, including suspected population-level declines. However, the potential impacts of WNV on some declining upland game bird species remain largely unknown, despite conservation concerns in many of these species. The ruffed grouse (*Bonasa umbellus*) is an important gamebird and conservation symbol. This species has been experiencing marked population-level declines in some parts of its range since the arrival of WNV. In addition, ruffed grouse have proven susceptible to severe, WNV-associated disease in experimental trials. These findings have elevated concern for this species even further and highlight the need to better understand the potential impacts of WNV on wild ruffed grouse populations. In this multi-year study, we aim to determine the seroprevalence and distribution of wild ruffed grouse with and without anti-WNV antibodies in populations across the eastern ruffed grouse range in the US over a three-year period. During the first year of the study (2018-2019), blood-soaked nobuto filter paper strips from 1,278 hunter-harvested ruffed grouse were collected from 13 states in the eastern US. Nobuto strip eluates were tested for WNV-neutralizing antibodies by plaque reduction neutralization test (PRNT). Samples with ≥90% neutralization were further tested to distinguish between WNV and St. Louis encephalitis virus, another Flavivirus that can serologically cross react with WNV, by a ≥4-fold PRNT90 titer. The overall seroprevalence across the eastern US was 13.7% and ranged from 0% (n=5) to 28.9% (n=235) among states. These data will be analyzed in conjunction with habitat and climate data to help provide a more complete understanding of the complex dynamics between ruffed grouse populations and WNV, and the potential contribution of this virus to wild ruffed grouse population declines. Additionally, this information provides valuable baseline data for future flavivirus serosurveys. This study will aid in the management strategies of ruffed grouse populations by state wildlife agencies and can assist in monitoring WNV activity across the eastern US.

Tumor Progression Locus 2 (Tpl2) prevents immunopathology during Influenza infections #28

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Tpl2 (Tumor Progression Locus 2) is a serine/threonine kinase that transmits signals via several receptor families, including Toll Like Receptors (TLRs), cytokine receptors (TNF-alpha and IL-1 receptors), and Fc receptors, leading to the expression of inflammatory mediators. Our lab has previously shown that the *Tpl2*$^{-/-}$ mice are more susceptible to influenza infection, and the purpose of this study is to delineate the mechanisms responsible. *Tpl2*$^{-/-}$ mice ultimately clear the virus albeit with delayed kinetics. Despite eventual viral control, *Tpl2*$^{-/-}$ mice begin to exhibit severe clinical signs and require euthanasia. Therefore, we hypothesize that an overexuberant immune response rather than impaired viral control leads to severe pathology in *Tpl2*$^{-/-}$ mice in response to influenza infection. Histological examination of the lungs of influenza-infected *Tpl2*$^{-/-}$ mice showed increased alveolar septal necrosis, pleuritis, and more widespread lesions, which are signs of epithelial-endothelial barrier damage and inflammation. In order to assess potential cellular mediators of inflammation, the cellular profile of the lung was analyzed at 7 days post infection (dpi), when significant morbidity was observed in *Tpl2*$^{-/-}$ mice. An excess influx of inflammatory monocytes was observed in the lungs of *Tpl2*$^{-/-}$ mice, along with a corresponding increase in serum levels of MCP-1, the chemokine required for inflammatory monocyte recruitment. Because type I interferons promote inflammatory monocyte recruitment via the induction of MCP-1, ongoing studies are examining whether Tpl2 paradoxically limits inflammation during influenza infection by constraining the production of type I IFNs. This information will enhance our understanding of Tpl2 signaling and interferon regulation, which could provide insights into host-targeted therapies for treating highly pathogenic influenza which is typically associated with excess type I IFNs and robust inflammatory monocyte recruitment.

Effects of intravenous vitamin C on oxidative status in adult horses

#29

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Bacterial sepsis and related systemic inflammatory response syndrome (SIRS) cause morbidity and mortality in horses and foals. Recent studies of human sepsis suggest that intravenous administration of vitamin C, in combination with hydrocortisone and thiamine, might improve survival. However, mechanisms of immunomodulation and optimal dosing strategies for vitamin C in human and equine sepsis are not known. The objective of this study was to evaluate the effects of various doses of intravenous vitamin C to healthy adult horses on vitamin C plasma concentration, plasma antioxidant capacity (PAC), plasma reactive oxygen metabolites (dROM), and neutrophil reactive oxygen species (ROS) responses for future use in septic horses and foals. We hypothesized that intravenous administration of vitamin C to healthy adult horses increases plasma vitamin C concentration and PAC, decreases dROM, and modulates neutrophil ROS responses in a dose-dependent fashion. Healthy adult horses (n=8) received 25, 50, or 100 mg/kg vitamin C or saline intravenously in a randomized cross-over time with a 7-day washout between treatments. Blood was collected at baseline, 2, and 6 hours for assessment of vitamin C concentrations with high performance liquid chromatography (HPLC), and quantification of PAC and dROMs with a validated photometric assay. Neutrophils were isolated and stimulated with killed whole-cell Staphylococcus aureus (SAA) or phorbol myristate acetate (PMA), and ROS production quantified using a validated fluorometric assay. Data were analyzed with repeated measures ANOVA ($P<0.05$). Plasma vitamin C concentration increased significantly from baseline at 2 and 6 hours after drug administration with all three doses, obtaining significantly higher concentrations with higher doses ($P < 0.0001$). PAC did not differ significantly among treatments or time points, but dROMs significantly decreased in response to 100 mg/kg vitamin C. There was not a significant effect of vitamin C on induced ROS responses in isolated neutrophils. The results of this study suggest: 1) plasma vitamin C concentrations increase in a dose-dependent manner after IV dosing; and 2) plasma dROMs decrease in response to higher concentrations of vitamin C. Thus, vitamin C may have dose-dependent effects on oxidative stress in horses and may have therapeutic utility in equine sepsis.

Cataract prevalence in the Large Japanese Field Mouse (Apodemus speciosus) following the Fukushima nuclear disaster #30

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As a result of the Fukushima Dai-ichi nuclear power plant disaster, wildlife within the Fukushima Exclusion Zone (FEZ) are chronically exposed to low levels of ionizing radiation. This study aimed to determine cataract prevalence in mice within the FEZ, as cataracts are a late deterministic effect and easily measurable sequela of radiation exposure. Forty-three mice were sampled: 28 outside and 15 within the FEZ. *In vivo* examinations included assessment for ocular abnormalities, slit lamp biomicroscopic lens evaluation, and cataract grading using the Lens Opacity Classification System III. *Ex vivo* retroilluminated and slit lamp images were re-evaluated by a masked observer. Lifetime radiation dose was estimated individually while accounting for age from cesium-134-137 body burdens and external gamma dose rates at the capture site. Odds ratio showed no increased risk between exposed and control groups for cortical, posterior subcapsular, or nuclear scores. A Spearman rank test showed no correlation between lifetime radiation dose (ranging 0.1-2.3 mGy in control and 24.5-525.4 mGy in exposed populations) and cataract score. A nonparametric T-test showed a difference between *in vivo* exposed (10/30 eyes, 30%) and control (11/56 eyes, 19.9%) cortical cataract scores ($p<0.05$), and no difference between *in vivo* exposed (1/30 eyes, 0.03%) and control (0/56 eyes, 0%) posterior subcapsular cataract scores. Nuclear sclerosis was observed in 100% of the population. Cortical cataract prevalence increased in exposed mice. A dose-dependent relationship for cataract development was not observed and there was no increase in relative risk for cataract development in exposed mice.

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Effect of a Probiotic on Dysbiosis Index in a Hospitalized, Post-Operative Dog Population #31

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Diarrhea can be a complicating factor in critically ill post-operative patients. Dogs undergoing anesthesia, surgery and peri-operative antibiotic use are prone to developing diarrhea due to changes in intestinal flora or motility. This study aims to evaluate the dysbiosis index (DI) of hospitalized dogs following surgery, and to investigate the influence of probiotics on the development of diarrhea. Dogs undergoing hemilaminectomy were prospectively enrolled to evaluate pre-operative and 48-hour post-operative DI. Feces was collected at each time point, scored, and frozen at -80 C until analysis. Dogs were randomized to receive placebo or probiotic (Visbiome®) within 12 hours of recovery from anesthesia. Following hemilaminectomy, dogs were hospitalized at least 2 days post-operatively. All dogs received intraoperative cefazolin and had not received NSAIDs prior to anesthesia. DNA was extracted from the fecal sample, and 7 bacterial taxa were amplified using quantitative PCR. logDNA was expressed for each bacterial group, and then combined using a mathematical algorithm to generate the DI. Of 20 dogs studied, 9 received a probiotic and 11 placebo. Pre-anesthetic DI was not significantly different between groups ($P=0.37$). There was no change in DI before and after administration of a placebo ($p=0.06$). A statistically significant change in DI was seen following administration of a probiotic ($p=0.02$), however DI failed to improve. In this small group of dogs, the use of a probiotic supplement after anesthesia did not improve the DI, although the power of the analysis is low. Further investigation is needed to evaluate the effect of probiotics on DI in dogs following anesthesia and surgery, and specifically with regards to the development of postoperative diarrhea.

Oral administration of dexamethasone sodium phosphate in feline hypersensitivity dermatitis: an open-label study #32

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Feline hypersensitivity dermatitis (HD) is a multifactorial, pruritic condition for which glucocorticoids are considered a mainstay of therapy. However, it can be difficult to administer oral tablets to cats and there are potentially severe side effects associated with parenteral glucocorticoid administration. To date, no studies have reported the efficacy of oral administration of an intravenous glucocorticoid dexamethasone sodium phosphate (DSP; DexaJectSP 4 mg/mL; Henry Schein, USA) solution in cats with hypersensitivity reactions. This open-label pilot study aimed to evaluate the clinical efficacy and tolerability of oral DSP solution for feline HD. Fourteen cats with clinical signs and dermatological lesions compatible with a diagnosis of feline HD were orally administered 0.2 mg/kg/day of DSP for 28 days. One cat was withdrawn by the owner for perceived lack of response after 48 hours. After 28 days of DSP administration, dermatological lesions evaluated through Scoring Feline Allergic Dermatitis (SCORFAD) were significantly reduced from an average score of 7.85 to 3.08 (59.2% reduction, Wilcoxon's ranked sum test $P<0.0001$). In addition, the owner-assessed pruritus visual analogue score (PVAS) was reduced from an initial average score of 7.85 to 2.66 (66.3% reduction, $P<0.0001$). Adverse effects reported were mild (lethargy), transient (polydipsia, sneezing, urinary accidents, hypersalivation), or not observed. There was no reported difficulty administrating the liquid glucocorticoid. Oral administration of DSP significantly reduced clinical lesions and pruritus associated with feline HD after 28 days of treatment.

Characterization of Avian Pathogenic *Escherichia coli* (APEC) from poultry lesions in Georgia #34

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Colibacillosis is a disease of poultry caused by the organism avian pathogenic *E. coli* (APEC) that results in multi-million-dollar losses annually to the poultry industry. Here, we assessed the relationship between strains of *E. coli* recovered from different tissues of diseased chickens submitted to the poultry diagnostic research center (PDRC) and their ability to form biofilm as well as their relationships with virulence traits, antimicrobial resistance, and plasmid replicons they harbor. A total of 61 isolates were assessed for the presence of 86 genes (virulence, antimicrobial resistance, and replicons) using multiplex PCR. Each isolate was also screened for its antimicrobial resistance against 14 antimicrobials using the NARMS panel and ability to form biofilm, which was categorized as negligible, weak, moderate, or a strong biofilm former. Tissue site of isolation was also correlated to biofilm formation and the number of pathogenic genes detected. Overall, 58 strains were able to form biofilms and three strains formed negligible biofilms. A total of 10 isolates were strong biofilm formers; 25 isolates were moderate biofilm formers, and 23 isolates were identified as weak biofilm formers. The data found that almost all strains showed the presence of genes associated with the ColV plasmid with over 90% of isolates harboring *iroN*, *ompTp*, and *hlyF*. In assessing antimicrobial resistance, 20 isolates did not show resistance to any of the 14 antimicrobials screened. One isolate showed resistance to 9 out of the 14 antimicrobials, and the remaining isolates showed resistance to between 1 and 5 antimicrobials. This study suggests that current APEC causing disease in poultry harbor high numbers of virulence genes and some isolates harbor many antimicrobial resistance genes, which can potentially lead to complications in treatment or control of disease outbreaks. The use of a multiplex panel to screen for APEC has shown good correlation with pathogenesis, and tissue source and correlates well with invasive strains. This path panel provides significant value to APEC pathogenesis screening.

Influenza A virus sequencing directly from swab material using Nanopore technology #35

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Influenza A virus (IAV) affects a wide range of hosts. Surveillance efforts to understand virus spread and evolution have focused mainly in wild birds and swine due to their role as natural reservoirs and potential source of zoonoses. Obtaining full genome IAV sequences for such surveillance is costly and requires specialized equipment. Oxford Nanopore Technology has the potential to increase the speed and quantity of sequence data in remote locations where sequencing core facilities are limited like in developing countries. Using this technology in these sites allows to produce real-time sequencing data to rapidly identify the emergence of new variants, including those with the potential to infect poultry and humans. For this purpose, we used combined tracheal/cloacal swabs from wild birds and nasal swabs from pigs collected in Guatemala during 2014-2018 in order to compare this new technology to the current NGS protocols performed by Illumina. IAV genomes from swabs were amplified using by barcoded MS-RTPCR primers currently used for NGS sequencing of IAV genomes to generate 8 full-length viral cDNA. Up to 10 samples were pooled for library preparation using the SQK-LSK109 ligation sequencing kit with a standard FLO-MIN106 flow cell. Runs were performed using the MinION portable device controlled by other device (MinIT), which contains a pre-configured software with concurrent basecalling and built-in data collection. Data analysis to generate IAV consensus sequences were performed using open-source software. Using this protocol, we were able to collect more than 400,000 total reads per run (the total barcoded reads per sample ranges around 4,000 to 110,000) for 10 barcoded samples in less 3 hours. Even though we used around 10 times less starting material than those recommended by the manufacturer, our results are comparable with those obtained with Illumina; suggesting this method can be used feasibly in field samples to produce good quality sequences at lower cost and faster than the current sequencing protocols.

Modulation of microbiome, glucose homeostasis, behavior and cognition in non-obese diabetic mice following daily dosage of LemonGlycerol dietary supplement #33

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The LemonGlycerol dietary supplement (HGG Research LLC; https://www.devertigo.com) has been claimed to support gut and brain health by relieving vertigo, dizziness, headaches, dry throats, and nausea. However, no preclinical studies have been conducted to confirm its efficacy. We have hypothesized that LemonGlyerol can beneficially regulate brain function by maintaining healthy gut-brain axis and gut microbiota (GMB). To test this hypothesis, non-obese diabetic (NOD) mice were orally dosed with vehicle (water), lemon extract, food grade glycerol solution, and LemonGlycerol (0.05 mL) daily for 6 months. In addition to monitoring the body weight and glucose levels, behavioral tests that assess depression and cognitive behaviors were conducted. Our findings suggested that LemonGlycerol treatment resulted in a better glucose tolerance. Moreover, the most prominent difference in the immobilization time of the tail suspension test was observed in the LemonGlycerol group ($P > 0.05$), suggesting a potential decrease in depression. In addition, GMB analysis of the week-4 feces indicated that the GMB between vehicle and LemonGlyerol treatments could be well separated using weighted Unifrac with increases of Bacillales, Desulfovibrionales and YS2, and decreases of Lactobacillales and Streptophyta at the order level. This anti-inflammatory response was also supported by increases in the abundance of the genera Prevotella, Ruminococcus and Staphylococcus. Further analysis of the pathways and functional metagenomics will shed more light on this new prebiotic that might help different patients suffering from the various symptoms listed above and reduce the intake of medications with side effects (Supported in part by NIH R41AT009523).

Impact of deforestation on tick-borne infections of dogs in communities East and West of the Panama Canal. #37

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Anthropogenic land use change, such as deforestation and agricultural development, is often associated with the emergence of vector-borne pathogens. In the Americas, tick-borne diseases, such as ehrlichiosis and anaplasmosis are significant canine health problems. Identifying spatial locations of these tick-borne pathogens would then allow for detection of "high-risk" sites in low-income communities where public health recommendations would have a much-needed, positive impact on preventing the spread of tick-borne disease. The aim of this project is to study relationships between forest cover and tick-borne pathogens of dogs, primarily *Ehrlichia* spp. in Panama. We extracted DNA from dog blood collected from 120 dogs sampled across 6 communities differing in forest cover in rural Panama. *Ehrlichia* diagnosis was performed through broad range genus amplification of the 152bp fragment of the 16-sRNA gene followed by Ehrlichia species (*E. canis* and *E. equii*) diagnosis by amplification of ~395bp fragment of the 16-sRNA gene. (Breitschwerdt et al. 1998). Additional data collected included complete blood counts (CBC), signalment, and body condition. Preliminary results show that 48/72 (70%) of dogs are infected with *Ehrlichia* and infection rates in dogs increase as a function of deforestation. Among *Ehrlichia* positive dogs, 5/35 (14%) were PCR-positive dogs for *E. canis* with the greatest numbers from deforested cattle pasture. For *E. equii*, 5/35 (14%) were PCR-positives with greater numbers in forest matrix. Molecular survey for detection and control of *Ehrlichia* and *Anamplasma* is critical in Panama because these pathogens cause significant canine health problems and pose a public health concern as some are zoonotic.

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Eosinophils are required for the generation of protective adaptive immunity in *Bordetella bronchiseptica* infections #38

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Eosinophils are myeloid immune cells involved in innate immunity against multicellular helminthes and allergic responses. However, recent data has suggested that eosinophils may also play an important role in the immune response to respiratory pathogens. Eosinophils increase in both the spleen and lungs of mice during the peak of *Bordetella bronchiseptica* infection. Furthermore, mice deficient in eosinophils are unable to clear *B. bronchiseptica* from the respiratory tract, experience more severe bronchoalveolar inflammation, and generate lower levels of IgG antibodies. However, the mechanism by which eosinophils affect the generation of adaptive immunity to promote the clearance of respiratory pathogens is still unclear. Here we show that eosinophils can act as effective antigen presenting cells to stimulate both cellular and humoral immune responses. We used flow cytometry to analyze the immune cell populations in the lungs of mice infected with a mutant strain of *B. bronchiseptica* that is unable to modulate the host immune response. Compared to eosinophil-deficient or naïve mice, infected wild-type mice demonstrate higher levels of inflammatory eosinophils in the lungs on day 3, and these cells are capable of antigen presentation. This contributes to a more robust adaptive immune response, and on day 14, wild-type mice have significantly higher levels of B cells in the lungs. Our results suggest that the role of eosinophils is much more dynamic than traditionally believed, and that they are a fundamental part of adaptive immunity. Characterizing this novel function of eosinophils can help us better understand the complexity of the adaptive immune system and how we can use this system to more effectively treat and prevent disease.

Pharmacokinetic evaluation of sotalol hydrochloride in cats #39

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There are few options available to veterinarians for the chronic treatment of ventricular tachyarrhythmias in cats, a potential cause of sudden cardiac death. One option that has been used successfully in humans, dogs, and horses, but not previously evaluated in cats, is sotalol hydrochloride, an oral potassium channel blocker and beta-adrenergic antagonist. The objective of this study was to describe the single-dose plasma pharmacokinetics of sotalol in healthy cats. Our hypothesis was that oral administration of sotalol would lead to measurable plasma concentrations. Each of 6 adult, purpose-bred, domestic shorthair cats received single doses of sotalol, administered IV or PO at 2 mg/kg, separated by a 1-week washout period. Blood samples were obtained at various time points for 48 hours post-dose (PO and IV). Ultra-high pressure liquid chromatography with mass spectrometry was used to quantify sotalol in feline plasma. Compartmental analysis was used to obtain pharmacokinetic parameters. For all cats, mean ± SD T_{max} was 100.0 ± 79.7 min following oral administration, corresponding to a plasma concentration (C_{max}) of 819.2 ± 291.1 ng/ml. Additionally, a short elimination half-life of 156.1 ± 38.6 min suggests that twice-daily dosing should result in no accumulation of sotalol; however, effective plasma concentrations of sotalol remain unknown at this time. Information regarding plasma half-life, bioavailability, and elimination is pending further analyses. The data collected will inform sotalol dose selection for future studies, including a pharmacodynamic evaluation currently being undertaken by the same lab.

A computationally optimized broadly reactive antigen (COBRA) elicits broadly neutralizing antibodies against a conserved influenza virus hemagglutinin B-cell epitope #40

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The development of broadly protective influenza vaccines will represent the main countermeasure to overcome influenza virus spread and improve the coverage offered by the current standard of care.
A computationally optimized broadly reactive influenza virus hemagglutinin (HA) antigen (COBRA), named P1, elicits a broadly reactive antibody (Ab) response against multiple H1N1 strains.
In order to understand the mechanism of P1-conferred breadth of response, we first characterized the breadth of the Ab response at the B-cell level. Specifically, the reactivity of secreted Abs from mice immunized with P1 or seasonal/pandemic vaccine strains was assessed against a panel of H1N1 recombinant HA. Interestingly, while Abs from P1-immunized mice exhibited a broader recognition, those from seasonal/pandemic immunized animals showed a predominant homologous response.
In order to dissect the antibody response, a panel of P1-specific B-cell hybridomas was generated. Subsequently, the corresponding purified monoclonal Abs (mAbs) were assessed for the breadth of HA binding and hemagglutination inhibition (HAI) activity against different H1N1 viruses. Collectively, P1-specific mAbs exhibited a broad spectrum of binding and functional activities, spanning from strain-specific to broadly reactive and neutralizing mAbs, while seasonal/pandemic-specific mAbs displayed a narrower spectrum of activity. Epitope mapping studies revealed that P1-elicited broadly reactive and neutralizing Abs recognize unique conserved epitopes within the HA receptor binding site. Collectively, these studies shed light on the mechanism of breadth conferred by P1 and leverage its optimization for the development of a more effective influenza vaccine.

Elicitation of protective antibodies against the A/Swine/North Carolina/152702/2015 influenza virus (H1N2) #41

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In the last decade, the Swine Flu has contributed to various health scares across the nation, including the 2009 flu pandemic. My work involves testing developed vaccines against the A/Swine/North Carolina/152702/2015 H1N2 influenza strain (SW/NC/15). Although experimental vaccines were found to provide measured protection against the pathogen (SW-1; X-6), antibody-mediated protection due to binding to the hemagglutinin (HA) surface protein has yet to be confirmed. Therefore, I aimed to confirm that elicited antibodies bind to SW/NC/15's HA glycoprotein.
Preliminary hemagglutination inhibition (HAI) data suggests that the protection afforded by the experimental vaccines was not induced by antibody interaction with the HA receptor binding site. Therefore, a recombinant SW/NC/15 HA plasmid was created, and the resulting protein was used to determine if antibodies bind to other virus-neutralizing HA sites. This report entails the antibody response to the SW/NC/15's HA, and it will further elucidate the adaptive immune response mechanism against swine influenza strains.

Effects of potential confounding variables on accuracy and precision of a commercial veterinary hematocrit meter #42

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Rapid and accurate measurement of hematocrit using small sample volumes is useful in critically ill patients. Interference from substances in the blood (eg. lipemia) may influence the function of machines that rely on optical measurement. We aimed to evaluate precision and accuracy of a veterinary point-of-care (POC) hematocrit meter and to study the effects of potential confounding variables on meter function. We hypothesized that the meter is accurate and precise over a wide range of hematocrits and that the confounders would not affect meter function. Canine and feline venous whole blood EDTA samples (n=73) were run in duplicate on the POC, which reported hemoglobin (POCHb), measured via optical reflectance, and a calculated hematocrit (POCHct). Meter results were compared to results from a laboratory-based analyzer (CBC). Samples with grossly visible lipemia, icterus, hemolysis, and auto-agglutination were noted. CBC hematocrit ranged from 3.5-79.8% (mean 37.0 +/-15.35%) and hemoglobin concentration ranged from 1-24.6g/dL (mean 12.2±4.9g/dL). The average differences between duplicate POCHct and duplicate POCHb values were -0.58±2.96 and -0.31±1.06g/dL, respectively. There was good correlation between the meter and CBC for both hematocrit (R^2=0.92) and hemoglobin (R^2=0.91). Bland Altman analysis yielded a mean bias for Hct of 1.17 (SD: +/- 4.35) % with 95% limits of agreement of 9.693 to -7.361 % (Figure 2) and a mean bias for Hgb of 0.20 (SD: +/- 1.55) g/dL with 95% limits of agreement of -2.84 to 3.24 g/dL. There was no apparent influence of visible lipemia (n=12), icterus (n=9), or hemolysis (n=8) on meter accuracy. The POC failed to read 12 samples; 5 had marked auto-agglutination and 7 had hematocrit ≤11%. Overall, the POC had excellent precision and correlation with CBC. The meter was not influenced by icterus, lipemia or hemolysis but did not provide data for samples with autoagglutination or hematocrit ≤11%.

An online-based survey investigating the experiences of AVMA registered veterinarians with persistent hookworm infection in dogs

#43

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Anthelmintic drug resistance in parasitic nematodes of livestock is a well-known and studied issue in modern veterinary parasitology, but knowledge of drug resistance in canine nematodes is more limited and few recent studies exist. However, multi-drug resistance (MDR) to all three major drug classes in the canine hookworm, *Ancylostoma caninum*, has been recently diagnosed in numerous dogs. Thus, this may be an emerging problem in the U.S. To gain a better understanding of the epidemiology of the phenomenon, we surveyed U.S. veterinarians regarding their experience with the treatment of persistent canine hookworm infections, and to determine if there were breed and/or age associations. We sent survey links via email to 3892 AVMA veterinarians from 10 states covering four regions of the U.S., (two each from the West, Midwest and Northeast, and four from the Southeast) using an online survey provided by Qualtrics, a survey management platform free for UGA users. The overall response rate was approximately 10% with 383 complete responses to the survey recorded, with Texas accounting for 21% of the complete responses. Hookworm infections that did not resolve after a single course of treatment were common, with 50% of respondents having seen one case in the last 6 months and 57% of those respondents reporting 2-5 cases in the last 12 months. From the respondents who reported seeing a case within the last 6 months, 84% reported that these patients lived in a private residence at the time of initial diagnosis. Only 23% of respondents reported noticing a breed association with cases of persistent hookworm infection. Of these respondents, around 74% reported an association with greyhounds. These data suggest that persistent canine hookworm infections are fairly common. Given the evidence suggesting that anthelmintic resistance is an emerging problem, especially in populations of greyhounds, many of these persistent hookworm cases may be associated with drug resistance. Research investigating the molecular epidemiology of drug resistance in canine hookworm is planned to determine the prevalence and distribution of this problem.

Measuring pyrantel resistance in the canine hookworm, *Ancylostoma caninum*, using the Larval Arrested Morphology Assay (LAMA)

#44

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Ancylostoma caninum, the canine hookworm, is the most common and important intestinal nematode of dogs. It is also one of the most pathogenic parasites of dogs, clinical signs of infection include anemia, enteritis, and, even causes death in heavy infections. It is also a serious zoonosis causing cutaneous larva migrans in humans.. Resistance in *A. caninum* has evolved to all approved anthelmintics in the US used for its treatment , causing an emerging canine and public health crisis. Pyrantel is one of the most commonly used anthelmintics that treats canine hookworms and it does so by causing paralysis, as a result, the larvae takes on various morphologies *in vitro*. The morphologies are straight, hooked, and curved; and these were determined by conducting a motility assay experiment. The results from the assay were used to classify the larvae as unaffected or affected by the pyrantel drug in the larval arrested morphology assay (LAMA). Resistant and susceptible isolates from lab-infected dogs were used to perform these experiments. Fecal egg counts (FEC) were performed to determine the approximate number of eggs that were present in a sample. After the FEC, the eggs were placed in a charcoal coproculture in order to allow them to develop into the infective third stage. The larvae were then harvested after seven days and placed into the assays for analysis. The LAMA was analyzed by counting the number of affected and unaffected larvae based on their morphology and then taking the percentage of unaffected versus affected morphologies at each concentration. A nonlinear logistic regression analysis was conducted using the program GraphPad Prism. IC_{50} values - which is half the value at which there is a 100% inhibition by the drug - and dose responses were also calculated. The LAMA in its current state gave inconclusive results with regards to evaluating pyrantel resistance in *A. caninum* due to the subjective nature of the classification of the morphologies. Additionally, some larvae did not arrest at the highest concentrations after 48 hours. Further optimization is necessary for the success of the assay and the understanding of pyrantel resistance in this parasite.

Bisphenol S (BPS) disrupts meiotic spindle organization in ovulated oocytes #45

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Bisphenol A (BPA) is a widely-used chemical found in plastics and many common consumer products. It is recognized as a potent endocrine disruptor with estrogen-like activity, and has been associated with significant detrimental effects on human and animal health. Studies demonstrate that BPA adversely affects various aspects of the reproductive system, including germ cell development and function. In females, maternal exposure to environmentally-relevant doses of BPA has been shown to disrupt ovarian follicle development and promote increased rates of meiotic abnormalities in oocytes, including disrupted spindle organization and aneuploidy. Safety concerns regarding BPA has led to the use of alternative compounds such as bisphenol S (BPS). However, the effects of these replacement analogues on oocytes and meiotic division are poorly understood. Therefore, in this preliminary study we tested whether a brief *in vitro* exposure to BPS disrupts meiotic spindle organization in oocytes. To evaluate the effects of BPS, ovulated metaphase II (MII)-arrested oocytes were exposed to increasing concentrations of BPS (5, 25, 50μg/mL) for 4h. Following culture, the oocytes were fixed for immunofluorescence analysis and specific antibodies were used to detect spindle microtubules and pericentrin (PCNT), a key centrosome-associated protein at the spindle poles, while the chromosomes were labeled with DAPI. Our data show that BPS exposure leads to altered spindle structures, characterized by decreased meiotic spindle length and increased spindle pole width. These effects were statistically significant and occurred in a dose-dependent manner. In addition, oocytes exposed to BPS showed some disruption (loosening) of chromosome congression at the metaphase plate, suggestive of a possible effect on chromosome-microtubule attachments. In conclusion, these data indicate that BPS can exert microtubule-disrupting activity that potentially increases the risk for chromosome errors. These finding also provide additional evidence that BPS may not be a safe alternative to BPA. Funding support provided by NIH (HD0713330 & HD086528) to MMV.