THE OHIO STATE UNIVERSITY

COLLEGE OF VETERINARY MEDICINE

COLLEGE OF VETERINARY MEDICINE RESEARCH DAY

2022

BOOK OF ABSTRACTS

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

Office of Research and Graduate Studies

Due to the COVID-19 pandemic, the College of Veterinary Medicine Research Day, the keynote lecture, and PowerPoint presentations, are taking place virtual in 2022. Abstracts and presentations can be found on the College's Research Day website, along with this book of abstracts.

The following pages contain 85 abstracts submitted by 53 graduate students, resident/graduate students and interns, 25 professional DVM students, 2 undergraduate student, 3 post docs, 1 staff member and 1 faculty member in the categories of:

- Immunology and Infectious Diseases
- Molecular and Cellular Biology
- Structure/Function
- Epidemiology and Applied Research
- Clinical Research

We hope you enjoy perusing through the book and learning more about the exciting research performed in our College. We look forward to hosting an in-person Research Day again in April of 2023.

Patrick Green

Patrick L. Green, PhD
Professor and Associate Dean for Research and Graduate Studies
Robert H. Rainier Chair in Industrial Veterinary Medicine and Research
Director, Center for Retrovirus Research
Associate Director for Basic Sciences, Comprehensive Cancer Center

POWERPOINT JUDGES

Thank you to the following faculty for taking time
out of their busy schedules to judge
80 PowerPoint Presentations.

Prosper Boyaka	Teresa Burns
Alex Davies	Sushmitha Durgam
Rebecca Garabed	Alison Gardner
Greg Habing	Jiwoong Her
Brian Husbands	Ryan Jennings
Scott Kenny	Bill Kisseberth
Wendy Lorch	Georgina Newbold
Stefan Niewiesk	Mike Oglesbee
Eric Schroeder	Laura Selmic

A very special **Thank You** to Jeff Workman, Department of Veterinary Preventive Medicine, for his assistance in creating and uploading the webpage for the Abstracts and PowerPoints!

Thank you to Jean Schelhorn for reviewing the abstracts for potential IP disclosures.

2022 Faculty Chair:
Dr. Vanessa Hale
Assistant Professor, Veterinary Preventive Medicine

Organized by:
Michele L. Morscher
CVM Office of Research and Graduate Studies

THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

Research Day Keynote Address

Thursday

April 7, 2022

11:00 am – 12:00 pm

Join Zoom Meeting
https://osu.zoom.us/j/99640890257?pwd=YjdGdmorSWNmTWwxWlJUWnNYUFlNZz09

Meeting ID: 996 4089 0257
Password: 690682

"1999 – West Nile Virus – A perfect Example of the need for a One Health Approach
2020 – COVID-19 - A perfect Example of the need for a One Health Approach"

Presented by:

Dr. Tracey McNamara

Veterinary Pathologist and Professor of Pathology at Western University of Health Sciences College of Veterinary Medicine

Dr. McNamara specializes in the recognition and understanding of the diseases of captive and free-ranging wildlife and is best known for her work on the discovery of the West Nile virus in 1999. In 2004, she worked on DTRA's "Integrated Biosurveillance for Zoonotic Threats" program in Uzbekistan, Kazakhstan and Georgia. She served as lead on a project with Russian colleagues on the "Human-Animal Interface: Improving Biological Threat Detection and Surveillance in Russia" by the Nuclear Threat Initiative's Global Health and Biosecurity program in Wash. DC. Dr McNamara served as a consultant to the National Biosurveillance Advisory Subcommittee (NBAS) from 2008-2009 and continues to be actively involved in the development of the Nation's biosurveillance strategy. Dr. McNamara is a founding member of the Global Health Security Alliance (GloHSA) working with German/US military, the United Nations, medical intelligence and security sectors. She chaired a panel on "Disease X" at the World Health Summit, Berlin, 2018. She helped organize a meeting at the Salzburg Global Seminar on One Health Metrics in November 2019 and is a Salzburg Fellow. She is actively involved in the One Health movement and advocates for a species neutral approach to the detection of pandemic threats. Most recently, she was asked to be a member of the "Red Dawn Breaking Team" on COVID-19, a group of experts advising the Asst Secretary for Preparedness and Response (ASPR) of the United States.

CLINICAL RESEARCH

CR - 1

Title of abstract:	**BONE MARROW MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLE SECRETOME AND TENO-/CHONDROPROTECTIVE PROPERTIES**
Authors:	Z. Belacic, V. Quam, C. Bowlby, S. Long, S. Durgam Dept. of Veterinary Clinical Sciences, The Ohio State University
Abstract:	INTRODUCTION: Mesenchymal stem cells (MSC) are promising therapies for cartilage and tendon/ligament disorders in the horse. MSC exert their benefits largely by paracrine/trophic factors secretion packaged within extracellular vesicles (EV). EV hold a promising 'off-the-shelf' potential while overcoming MSC limitations like processing and cellular content. Our research objectives were to (1) characterize equine bone marrow MSC (BM-MSC)-derived EV (BM-EV) and (2) to evaluate the matrix protective effects of BM-EV on equine intrabursal deep digital flexor tendon (DDFT) and navicular fibrocartilage (NBF) co-cultures exposed to IL-1β inflammation. METHODS: (1) Equine BM-EV were isolated from BM-MSCs (n=5) via ultracentrifugation (30mins@2,000g, 2hrs@100,000g). Subsequent analyses included protein estimation (BCA), cytokine/growth factor ELISA quantification (normalized to 10 ug protein), flow cytometry, and mass spectrometry. BM-EV effect on tenocyte proliferation (MTT assay) and migration (scratch assay) were also performed. (2) Forelimb intrabursal DDFT-NBF transwell co-cultures (n=7) were exposed to 10ng/mL IL-1b and treated with 10ug/mL BM-EV. Culture media MMP-3, MMP-13 and IL-6 were ELISA quantified. Results were analyzed via (1) t-test and (2) one-way ANOVA ($p \leq 0.05$). RESULTS: (1) Mean concentrations of 119.7±56.44ng/mL, 0.009±0.03pg/mL, and 0.02± 0.04ng/mL for IL-1ra, IL-6, and IL-10, and 138.4 ±110.1pg/mL, 5.055±1.664pg/mL, and 2.107±2.261pg/mL for VEGF, TGFβ, and PDGF, respectively were quantified. BM-EV significantly increased tenocyte proliferation compared to control; tenocyte migration was unaffected. (2) BM-EV significantly decreased co-culture media MMP-3 of basal and IL-1β treated DDFT-NBF co-cultures. BM-EV-treated DDFT-NBF IL-6 was significantly higher than basal, IL-1β and IL-1β +BM-EV-treated co-cultures. DISCUSSION: Equine BM-EV are rich sources of anti-inflammatory and tissue anabolic growth factors. BM-EV decreased matrix breakdown enzyme, MMP3 both in the basal and IL-1β-treated explants and highlights the teno-/chondroprotective properties of BM-EV. Delineating the potential roles that BM-EV play in cell proliferation and differentiation, immunomodulation, cell-to-cell interactions, matrix regulation, and protein/peptide delivery is vital for their clinical use aimed at enhancing musculoskeletal tissue healing.
Keywords for abstract:	Bone marrow MSC Extracellular vesicles IL-1Ra IL-6 Teno-/chondroprotection

CR - 2

Title of abstract:	**IDENTIFYING BEHAVIOR CHANGES IN DOGS DURING THE SIX MONTHS FOLLOWING ADOPTION FROM A SHELTER**
Authors:	K. Bohland, M. L. Lilly, M. Herron, A. Arruda, J. O'Quin Department of Veterinary Clinical Sciences (Bohland, Lilly) and Department of Veterinary Preventive Medicine (Arruda, O'Quin), Gigi's Shelter for Dogs (Herron).
Abstract:	Millions of dogs are adopted from shelters in the United States every year, but very little is known about their long-term behavior post-adoption. Our study aimed at identifying the prevalence of behavior problems in dogs after adoption and how those behaviors changed throughout the first six months post-adoption. Ninety-nine dogs adopted from five area shelters were tracked for six months after adoption. Owners were surveyed electronically about their dog's behavior at 10, 30, 90, and 180 days after adoption The Canine Behavioral Assessment and Research Questionnaire (C-BARQ) was used to track behavior over time. A multivariate model was created for each C-BARQ trait, controlling for multiple canine and household factors: use of behavior medications, acquiring other pets, household moves, work schedule changes, shelter origin, age, sex, length of stay, shelter intake reason, and health status in the shelter. At each timepoint owners were also asked about overall satisfaction of their dog as well. The following C-BARQ traits significantly increased during the study period: stranger-directed aggression, attachment and attention-seeking, separation-related behaviors, excitability, touch sensitivity, trainability, and chasing behavior. There were no significant differences in scores for familiar dog aggression, owner-directed aggression, dog-directed aggression, stranger-directed fear, nonsocial fear, dog-directed fear, or energy level. Despite increases in several undesirable C-BARQ traits during the study period there was overall very high owner satisfaction throughout the study. Across all timepoints, 98% of owners indicated their dog adjusted to the new home extremely or moderately well. 93% rated their dog's overall behavior as excellent or good. Additionally, 72% of owners described their dog's behavior as improved throughout the study. This information will provide veterinarians, canine behavior professionals, and shelter staff information to better council owners on the potential behavior changes in dogs after adoption from a shelter.
Keywords for abstract:	Shelter behavior Shelter adoptions Canine behavior C-BARQ

CR - 3

Title of abstract:	**EQUINE MACROPHAGE DIFFERENTIATION, CONTACT-MEDIATED DIFFERENTIAL INTERACTIONS WITH INTRASYNOVIAL FLEXOR TENOCYTES AND CONSEQUENT IMPACT ON TENDON MATRIX GENE EXPRESSION**
Authors:	C. Bowlby, Z. Belacic, S. Long, S. Durgam
Abstract:	INTRODUCTION: Intrasynovial tendon injuries are common debilitating conditions in horses. Macrophages are key immune cells recruited to the injury site, secrete inflammatory mediators, affect tenocyte phenotype, and contribute to dysregulated intrasynovial tendon healing. The research objectives include (1) optimizing equine monocyte-derived macrophage in vitro differentiation, and subsequently determining their inflammatory (M1) and regulatory (M2) cytokines, and (2) investigating intrasynovial flexor tenocyte-macrophage interactions during in-vitro co-culture. METHODS: (1) Peripheral blood CD14+ monocytes (n=5) were cultured with or without GM-CSF (6 days) followed by inflammatory (LPS+IFNγ) and regulatory (IL-4+IL-10) priming (24 hours) and subsequently their morphology, proliferation (MTT) and secretory profiles (ELISA) were assessed. (2) Donor-matched intrasynovial deep digital flexor tenocytes and inflammatory or regulatory macrophage co-cultures (n=4) were established in direct and transwell systems. Following co-culture, mRNA (qPCR) and secretory profiles (ELISA) were assessed. Data were analyzed with one-way ANOVA and significance was set at $p \leq 0.05$. RESULTS: (1) Inflammatory (GM-CSF and LPS+IFNγ priming) M1 macrophages secrete significantly increased levels of IL-1β ($p=0.012$) and TNFα ($P=0.034$). IL-4+IL-10 priming for 24 hours (without GM-CSF pretreatment) yields regulatory (M2-like) macrophages. Control and treated macrophages secrete equivalent TGFβ; and IL-6 is not quantifiable in M1 or M2 media. (2) In contrast, IL-6 mRNA (400-fold; $p<0.01$) and IL-6 (5000-fold; $p<0.001$) secretion significantly increases in tenocyte-LPS+IFNγ-primed macrophage co-culture and co-culture medium. Compared to transwell, direct co-culture significantly down-regulates SOX9, COL1A1 and COL2A1 mRNA in all groups, and significantly up-regulates MMP13 mRNA. DISCUSSION: Equine M1 and M2 macrophages secrete distinct inflammatory cytokines; IL-1β and TNFα are the key cytokines. IL-6 is induced only during macrophage-tenocyte co-culture conditions. Tendon matrix gene alterations occur only during direct co-culture suggesting cell-to-cell contact mediated interactions between macrophages and tenocytes; and are equivalent among M1 and M2 macrophages. Identifying innate immune mechanisms impacting tendon healing is vital for developing immunomodulatory approaches to enhance tendon healing.
Keywords for abstract:	Equine Intrasynovial tenocyte Macrophage Co-culture Matrix gene expression

CR - 4

Title of abstract:	**WATER-BASED MEDIUM-EXPANSION FOAM DEPOPULATION OF ADULT CATTLE**
Authors:	V. Capria, J. Kieffer, T. Cheng, A. Arruda, M. Campler, S. Moeller, and A. Bowman. Departments of Veterinary Preventive Medicine and Animal Sciences
Abstract:	Current options for depopulation of adult cattle outlined in the AVMA Guidelines for the Depopulation of Animals are limited, have logistic constraints, and may not be practical on a large scale. Water-based foam is advantageous because the necessary equipment is readily available, easy to use, and presents minimal personnel risk. Foam has been successfully used to depopulate poultry but no research has been conducted on cattle. With the use of a modified rendering trailer in a field setting, we evaluated the efficacy of aspirated water-based foam for depopulation of adult cattle. Animals were immersed in non-toxic foam to block respiratory exchange of gases and a gated approach with anesthetized cattle was used prior to conscious replicates. A total of 84 head of cattle were used and a subset was implanted with subcutaneous telemetry devices that recorded activity and electrocardiograms. In all trials, cattle were loaded onto the trailer using a single-file ramp and three gasoline-powered pumps delivered foam into the trailer with a 15min foam dwell period. Average (± SD) time to completely fill the trailer was 85 ± 10s. Average (± SD) time from container fill to last movement subjectively heard by observers was 187 ± 70s. No animal vocalizations were heard during foam application or the dwell period, and all animals were confirmed dead upon removal from the container at 15min. Necropsies of the first 12 cattle revealed foam extending to at least the tracheal bifurcation and exceeding this level in 67% (8/12) animals. Average (± SD) time for cessation of movement as determined by activity data was 151.8 ± 76.4s. The results of this study indicate water-based foam is a rapid, effective, and safe method for depopulation of adult cattle with potential operational advantages over current methods.
Keywords for abstract:	Cattle depopulation Water-based foam Foam depopulation

CR - 5

Title of abstract:	**DOES ENDOSCOPY CHANGE CLINICIANS' SUPPORTIVE INTERVENTIONS OR NOT (DECISION)?**
Authors:	M. Chen, J. Howard, A. Rudinky, K. Tolbert, G. Atiee, H.Connell, B. Cunningham, W. Deal, M. DiCicco, C. Fuerst, M. Gonzalez, L. Harjes, T. Hill, A. Jergens, J-S. Palerme, R. Riggs, A. Whitlark, A. Woolcock, J. Steiner, V. Parker, J. Winston
Abstract:	Chronic enteropathy (CE), protein-losing enteropathy (PLE), and intestinal lymphoma (LSA) are common gastrointestinal conditions in dogs. The goal of this study was to determine whether gastrointestinal endoscopic evaluation or histopathologic diagnosis influenced clinician case management of these diseases. We hypothesized that interpretation of endoscopic data and histology results would affect clinician decisions regarding case management in CE, PLE, and LSA in dogs. Ten cases of CE, 10 cases of PLE, and 9 cases of LSA were retrospectively identified by histopathology reports. Each case was curated into 3 case presentations: 1. Total case data including history, physical exam, all diagnostics including endoscopy and biopsy results, 2. Case data without biopsy results, and 3. Case data without both endoscopy and biopsy results. Four academic internists (AI), 4 private practice internists (PI), and 7 general practitioners (GP) were surveyed to evaluate the impact of these scenarios on treatment strategies. Treatment approach in case presentation 1 and case presentation 2 were compared to case presentation 3 for consistency. Frequencies at which treatment decisions were affected or not by additional diagnostic data were compared with chi-squared between diagnoses and practice types. The inclusion of endoscopic data altered treatment decisions in 69.8% of all case scenarios (69.8% CE cases, 64.7% PLE, 75.6% LSA). The frequency at which endoscopic data affected treatment was not significantly different between diagnoses for all practice types. The inclusion of histologic data altered treatment decisions in 81.6% of scenarios (76.2% CE cases, 79.9% PLE, 89.6% LSA). Based on these results, endoscopic and histologic data were used clinically by clinicians of various practice types and influenced the treatment strategies elected. Further evaluation is warranted to investigate the impact of these decisions on clinical outcomes.
Keywords for abstract:	chronic enteropathy inflammatory bowel disease protein-losing enteropathy intestinal lymphoma gastrointestinal endoscopy gastrointestinal histopathology

CR – 7

Title of abstract:	**UTILITY OF CARDIAC MRI TO DIAGNOSE MYOCARDIAL ISCHEMIA AND FIBROSIS IN DOGS WITH CARDIOMEGALY SECONDARY TO MYXOMATOUS MITRAL VALVE DISEASE**
Authors:	W. Clark, R. Winter, T. Aarnes, E. Green, P. Ruz, D. Addison, J. Rhinehart, K. Schober, and H. Friel. Dept of Veterinary Clinical Sciences, Dept of Medicine (Division of Cardiovascular Medicine), Philips Medical Systems.
Abstract:	Background– Some dogs with myxomatous mitral valve disease (MMVD) develop progressive volume overload and cardiomegaly. Histopathologic studies, but not antemortem evaluations, have demonstrated myocardial fibrosis in some of these dogs. Cardiac magnetic resonance imaging (CMR) aids in antemortem diagnosis of myocardial fibrosis in humans. Hypothesis/Objectives– The objectives were to evaluate dogs with MMVD and healthy dogs for myocardial ischemia and fibrosis using serum biomarkers and CMR. We hypothesized that myocardial changes would be evident on both modalities in affected dogs. Animals– Six dogs with MMVD stage B2 and six healthy dogs recruited from a hospital population. Methods– Prospective case-control study. Dogs underwent echocardiography and serum cardiac biomarker measurement (cardiac troponin I (cTnI), galectin-3 (Gal-3)). Dogs were anesthetized for CMR, where T2 weighted images and pre- and post-gadolinium contrast T1 weighted images were acquired and analyzed on dedicated software. Data were analyzed for normality and expressed as mean ± SD or median (interquartile range). Student's t-tests or Wilcoxon rank sums tests were performed. Results– No significant differences were observed for pre-contrast T1 values, post-contrast T1 values, and T2 values (msec) (MMVD 1171.33 ± 52.77, 728 (700-764.75), and 39.2 ± 7.6; controls 1180.83 ± 33.89, 711 (697.5-736.5), and 42.38 ± 12.62; p = 0.72, p = 0.57, p = 0.61). Serum cTnI (ng/mL) was greater in MMVD dogs than controls (0.067 ± 0.019 vs. 0.033 ± 0.021; p = 0.013). Serum Gal-3 (pg/mL) was not different between groups (MMVD 178.07 ± 105.59, controls 305.24 ± 123.29; p = 0.08). Conclusions– Myocardial fibrosis detected by CMR and Gal-3 were not more common in MMVD dogs than controls. Dogs with MMVD stage B2 may not have clinically relevant myocardial fibrosis.
Keywords for abstract:	Cardiac magnetic resonance imaging Canine Myxomatous mitral valve disease Cardiac fibrosis T1 mapping

CR – 8

Title of abstract:	**REDUCTION AND REUSE OF SUTURE: CAN UTILIZING ONE SUTURE PACK FOR FIVE MICE MAINTAIN STERILITY?**
Authors:	J. Copio, M. Walker, S. Meeker. University Laboratory Animal Resources
Abstract:	Efforts to maintain aseptic technique and standard of care while also maximizing efficiency of material use and cost are important goals for research investigators and clinicains to consider, particularly when conducting serial batch surgical procedures. At OSU-University Laboratory Animal Resources, one new, sterile suture pack per mouse is required per institutional guidelines. We hypothesized that, with appropriate aseptic and surgical technique, one pack of sterile suture can be utilized for up to five mice from a single cohort and during the same surgical session. For these studies, mice were randomly assigned to groups in which either one suture pack per mouse was used (N=13) or one suture pack per cohort of five mice was used (N=13). Following euthanasia and aseptic surgical preparation, a dorsal skin incision was created and closed with three simple interrupted 4-0 braided Vicryl suture. Samples were collected immediately following the procedure and incubated in BHI broth at 37°C for 24 hr. 25µ of this broth was then plated on TSA agar media in 1:1 to 1:100,000 dilutions. CFU per plate were quantified after 48 hours of incubation. Positive and negative controls were employed to ensure sterility on fresh suture packs and the capacity for microbial growth on heavily contaminated suture. This study found that, when aseptic technique was followed, reuse of suture on up to five mice did not cause bacterial contamination as evidenced by microbial culture. Our data support the reuse of suture on up to five mice from a single cohort and during the same surgical session providing strict aseptic technique is followed. This procedural refinement offers an efficient option for investigators who commonly conduct rodent batch surgeries, while not compromising animal welfare or aseptic standards.
Keywords for abstract:	Rodent Surgery Asepsis Suture Mouse

CR - 9

Title of abstract:	**RETROSPECTIVE EVALUATION OF THE RESPIRATORY RATE OXYGENATION INDEX IN DOGS TREATED WITH HIGH FLOW NASAL CANNULA OXYGEN THERAPY**
Authors:	L. Eicher[1], A. Young[1], L. Hoover[1], K. Kuo[2], and J. Her[1]. [1]Department of Veterinary Clinical Sciences, The Ohio State University College of Veterinary Medicine, Columbus, Ohio, USA. [2]Department of Clinical Sciences, Auburn University, Auburn, Alabama, USA.
Abstract:	High flow nasal cannula oxygen therapy (HFNC) provides warmed, humidified oxygen via a specialized nasal cannula. This modality reduces the need for intubation and mechanical ventilation for severe hypoxemic patients failing traditional oxygen support. In human respiratory therapy, the respiratory-oxygenation index (ROX) has been validated when evaluating HFNC success or failure. The objective of this study was to evaluate predictors of outcome in dogs treated with HFNC. Eighty-one client owned dogs at two university teaching hospitals were retrospectively enrolled in the study. Medical records were reviewed to identify dogs treated with HFNC and data was collected for statistical analysis. The ROX was defined as the SpO2:FiO2 ratio divided by respiratory rate and the modified ROX index (m-ROX) was defined as the ROX divided by heart rate multiplied by 100. HFNC failure was defined as worsening respiratory status, requirement for mechanical ventilation, or euthanasia due to declining respiratory status. Overall success rate of HFNC was 44.4% (36/81). After 6 hours of HFNC treatment, SF demonstrated the best prediction accuracy (AUC 0.86; $P < 0.0001$), followed by the ROX (AUC 0.85; $P < 0.002$) and the m-ROX (AUC 0.73; $P < 0.122$). Specifically, patients with SF ratio of ≤ 143 at hour 6 of HFNC had a 72% probability of failure with sensitivity and specificity of 79% and 93% respectively. A ROX ≤ 3.68 at hour 6 of HFNC had a 69% probability of failure with sensitivity and specificity of 72% and 92% respectively. The ROX index, m-ROX index, and SF ratio are easily attainable and noninvasive parameters that are useful predictors of HFNC outcome. Prospective studies in these indices are warranted to confirm the findings and to optimize cut-off values to predict outcome in dogs undergoing HFNC.
Keywords for abstract:	Canine Oxygen therapy High flow nasal cannula oxygen therapy Hypoxemic respiratory failure ROX index

CR - 10

Title of abstract:	The Comparison of Micro- and Macrocirculatory Perfusion in Horses with Acute Surgical Colic
Authors:	PW. Foth, E. Cooper, A. Gardner, C. Ricco-Piera, E. Schroeder, MC. Mudge.
Abstract:	Microperfusion is critical to maintain organ function, but may not directly correlate with macroperfusion especially in critically-ill surgical patients. Buccal mucosal dark-field microscopy (DFM) to assess microperfusion was validated in healthy horses, but not yet evaluated in surgical colic patients. We aimed to measure micro- and macroperfusion parameters during colic surgery, to assess changes over time, and describe correlations between micro- and macroperfusion. Buccal mucosal microperfusion was measured yielding values for total vessel density (TVD), perfused vessel density (PVD), and proportion of perfused vessels (PPV). Macroperfusion measurements included direct arterial blood pressure (MAP), HR, and cardiac output (CO) measured by lithium dilution method. Measurements were recorded at 30, 90, and 150 minutes after anesthetic induction. Data were collected for 9 adult horses during emergency colic surgery. Median for PVD, PPV, and HI were 11.74 mm/mm^2 (7.40-16.58), 99.03% (87.58-99.99), and 0.056 (0.036 - 0.120) respectively. Mean for TVD was 12.00 mm/mm^2 ($\pm$3.56). Microvascular parameters (TVD, PVD) highlighted a weak negative correlation (rho = -0.23, -0.23) with CO and weak positive correlation (rho = 0.23, 0.22) with systemic vascular resistance. Poor systemic circulation could have resulted in tissue hypoxic vasodilation. Preferential shunting of blood from the buccal mucosa to other areas of the body could have resulted in even more pronounced local hypoxia and subsequent local vasodilation. The limitations include a small cohort and inherent variation of multiple operators. It is feasible to measure buccal mucosal microperfusion in clinical patients with surgical colic. The results reinforce that macrovascular parameters such as MAP do not adequately assess microperfusion.
Keywords for abstract:	Equine Colic Microcirculation Microscan Anesthesia

CR - 11

Title of abstract:	Physical Exam and Metal Accumulations in Three Wetland Species
Authors:	J. Heinz, M. Flint
Abstract:	Heavy metal toxicity has significant effects on the health of animals and many heavy metals can be found as pollutants after human disturbance of the land. Wetlands help to filter pollutants from the environment and therefore concentrate these heavy metals, which leads to increased heavy metal exposure for natural inhabitants. In this study, two wetland locations with distinct pollution impacts were evaluated. Wetlands at the Wilds are impacted by previous strip-mining, which results in acid mine drainage and metal contamination of the environment; at Winous Point the wetlands are impacted by agricultural pollutants and runoff. Within these wetlands, three wildlife species were evaluated: painted turtles (*Chrysemys picta*), channel catfish (*Ictalurus punctatus*) and bluegill fish (*Lepomis macrochirus*). Ten individuals of each species were collected at each site for a total of 60 individuals. Physical exams were performed and liver samples were collected and tested for eleven metals. Blood was collected on the turtles as well. Hepatic metal accumulations were found to vary based on location and as well as species. Cadmium was only found at Winous Point, with highest prevalence in catfish (1.09± 1.09ug/g). Arsenic was highest in bluegill at Winous Point (1.24 ± 0.63ug/g), while lead was highest in catfish at the Wilds (0.71 ± 0.21ug/g). Iron was higher in at the Wilds than at Winous Point for each species, with catfish having the highest concentrations (8659 ± 4196ug/g). Turtles at the Wilds had significantly higher leucocyte estimates (28.38 ± 6.93K/uL), while female bluegill at Winous Point had a higher rate of being gravid (5/5), which may indicate differences in health of species between sites. Variation in hepatic heavy metal accumulations between species and sites demonstrate the prolonged impact and complex relationship between exposure and health homeostasis in wildlife species, highlighting the need for multifaceted evaluation and monitoring of pollutants in ecosystems.
Keywords for abstract:	Wetlands Heavy Metals Painted Turtle Catfish BLuegill

CR - 12

Title of abstract:	**GLUCOSE AND INSULIN DYNAMICS IN RESPONSE TO MARE'S MILK IN HEALTHY NEONATAL FOALS**
Authors:	J.Horton, H. Kinsella, L. Hostnik, J. Summers, H. Snyder, A. Gerding, T. Burns, R. Toribio
Abstract:	Recently, our laboratory showed healthy neonatal foals exhibit more robust endocrine responses to ingestion of mare's milk compared to equivalent doses of carbohydrates (lactose, dextrose) enterally and parenterally. To further understand the foal's biological response to administered nutrition, this study investigated the glucose and insulin response to mare's milk consumed via direct nursing versus nasogastric intubation (NGT). Twelve healthy Standardbred foals ≤ 4 days of age received: 1) 500 ml mare's milk via NGT (n = 5; MM), 2) nursed directly from the mare's teat for 5 minutes (n = 5; DN), and 3) 500 ml of isotonic crystalloid via NGT (n = 2; CON). Blood samples were drawn frequently for 210 minutes. Foals were allowed to nurse ad libitum at 180 minutes. Blood glucose (BG) concentrations were determined via a point-of-care glucometer and insulin concentrations were measured using a human-specific ELISA. Area under the glucose (AUCglu) and insulin (AUCins) curves were calculated and were compared at all timepoints using two-way ANOVA. All time points were compared to baseline (time 0) using Friedman's test (not normally distributed). AUCgluc and AUCins were significantly higher for MM and DN compared to CON at all timepoints ($P < 0.0001$). AUCgluc was not different between MM and DN at any timepoint. AUCins was significantly higher for DN compared to MM foals at all timepoints ($P < 0.001$). Neither glucose nor insulin concentrations were significantly different compared to baseline at any timepoint. When foals were allowed to nurse, robust elevations in insulin and glucose were observed ($P < 0.003$). Our results suggest that suckling (DN) stimulates more robust insulin responses than MM. In addition, enteral nutrition after prolonged fasting may stimulate exaggerated insulin and glucose responses. This may be a mechanism for foals to compensate for reduced energy stores within the first days of life.
Keywords for abstract:	insulin glucose foal endocrine pancreas energy nutrition

CR - 13

Title of abstract:	**CLINICAL USE OF URSODIOL IN FELINE MEDICINE**
Authors:	E Jachec, A Wood, J Winston. Department of Veterinary Clinical Sciences
Abstract:	Ursodiol is a naturally occurring bile acid prescribed to cats for a wide range of gallbladder and liver conditions. Ursodiol is known for its ability to expand the bile acid pool by increasing bile flow and displacing hepatotoxic bile acids. Additionally, ursodiol has anti-inflammatory and anti-fibrotic properties. Therefore, ursodiol is used in a variety of feline hepatic diseases, including acute/chronic hepatopathies, cholangiohepatitis, and for gallbladder abnormalities. Veterinarians prescribe a wide range of dosages and duration of ursodiol administration. Prescribing trends and clinical characteristics of feline patients receiving ursodiol is unknown. This retrospective study aimed to determine the clinical use, dosage, and duration of therapy of ursodiol administration in feline patients at a teaching hospital over 13 years (2008-2021). Since 2008, 88 feline patients were prescribed ursodiol at the Ohio State University Veterinary Medical Center and were included in this retrospective study. Metadata from these patients, including signalment, history, diagnostics (including laboratory data and diagnostic imaging), ursodiol dosage/duration, and patient outcomes were recorded. Ursodiol was most prescribed as a hepatoprotectant (29/88, 33%), for gallbladder sludge (26/88; 30%), or for cholestasis (17/88; 19%) in feline patients. The most common prescribed dose was 10.89 mg/kg/day (range 5.2 to 33.9 mg/kg/day). An accurate duration of ursodiol administration was unable to be determined. Overall, these findings document ursodiol prescribing trends and clinical characteristics of feline patients presenting to a teaching hospital. These results can help guide novel applications of ursodiol administration in feline patients.
Keywords for abstract:	Bile Acids Ursodiol Feline

CR - 14

Title of abstract:	**CLINICAL OUTCOMES IN CATS WITH RENAL CARCINOMA UNDERGOING NEPHRECTOMY: A RETROSPECTIVE STUDY**
Authors:	S. Kenny, B. Husbands, and L. Selmic. Depts. of Veterinary Clinical Sciences. Outside contributors: M. Cook, K. Mariato, J. Lenz, K. Skorupski, S. Veytsman, B. Janssens, M. Pellen, C. Silveira,
Abstract:	Feline renal carcinomas (RC) are uncommonly encountered. Limited information regarding clinical presentation, post-surgical outcomes, and survival times are available. The purpose of this multi-institutional, retrospective study was to describe the presenting features and clinical outcomes of cats with RC treated with nephrectomy. Thirty-six client-owned cats were included. Medical records from participating institutions were searched to identify cats that underwent nephrectomy and had a histopathologic diagnosis of RC. The most common presenting complaints were weight loss (n = 13) and hyporexia (n = 11). Twenty-eight cats survived to discharge (77.8%). Median progression free interval (PFI) could not be determined, as only 6 cats had suspected recurrence (16.7%) and 7 cats had suspected metastasis (19.4%). The median survival time (MST) for all cats was 203 days (95% CI: 84, 1379 days). However, when cases that died prior to discharge were excluded, MST increased to 1217 days (95% CI: 127, 1641 days). Preoperative erythrocytosis had a protective effect, as these patients had significantly longer MST (p = 0.006). Pre-surgical azotemia, renal tumor size, mitotic index, and tumor histologic subtype were not statistically prognostic. For cats surviving to discharge, prolonged survival times were possible. Due to varied post-operative treatment approaches, conclusions regarding the impact of chemotherapy could not be made. Further studies are necessary to elucidate other potential prognostic factors, the utility of adjuvant treatment, and to identify patients at-risk in the perioperative period.
Keywords for abstract:	Feline Cat Renal Kidney Renal cell carcinoma Transitional cell carcinoma Squamous cell carcinoma Nephrectomy

CR - 15

Title of abstract:	**COST COMPARISON OF HOME-COOKED DIETS VS. COMMERCIAL THERAPEUTIC DIETS FOR DOGS WITH GASTROINTESTINAL DISEASE**
Authors:	G. Kratzer, J. Winston, A. Rudinsky, and V. Parker (Dept. of Vet. Clin. Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH). M. Shepherd (Dept. of LA Clin. Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA). S. Delaney (Balance IT, A DBA of Davis Veterinary Medical Consulting, Inc., Davis, CA)
Abstract:	Nutrition plays a fundamental role in management of canine chronic enteropathies (CCEs). Dog owners may choose to feed home-cooked diets (HCDs) over commercially-prepared diets (CPDs) due to a perceived lower cost; however, there is a paucity of data comparing the cost of these options. We hypothesize that complete and balanced HCDs are more expensive than nutritionally comparable CPDs. Eight HCD recipes (e.g., limited antigen, low fat) were formulated by two board certified veterinary nutritionists to mimic the nutritional and primary ingredient profiles of CPDs designed for the management of CCEs. The total cost [on a per 100 calorie (kcal) basis] of each recipe was determined via collection of raw ingredient prices from the three largest grocery stores in the United States and online supplement prices (e.g., multivitamin/multiminerals, amino acids, omega-3 fatty acids). Prices of dry and canned CPDs were obtained from a national online retailer. Maintenance energy requirements [MER; 1.6 x (70 x $BW_{kg}^{0.75}$)] were calculated for 3 dog sizes (5, 20, 40 kg), and the costs of feeding dogs their MER from HCDs versus CPDs were compared with a Kruskal-Wallis test and post-hoc testing. Feeding HCDs was significantly more expensive than feeding dry CPDs ($P < .0001$) but not canned CPDs ($P > 0.99$). Veterinarians and dog owners should be aware that feeding complete and balanced HCDs is more expensive than feeding dry CPDs for management of CCEs.
Keywords for abstract: Please list your keywords – one per line	Nutrition Chronic Enteropathy Home-cooked diets Commercial diets Therapeutic diets

CR - 16

Title of abstract:	**THE DOG-O-GRAM, A DOGGONE SHAME: QUANTIFICATION OF SCATTER RADIATION PRODUCED IN APPROPRIATELY COLLIMATED VERSUS POORLY COLLIMATED RADIOGRAPHS.**
Authors:	D. Lantz and E. Green. Diagnostic Imaging Department.
Abstract:	The hazards of radiation exposure are well documented. Despite this, poor compliance to safety standards persists in veterinary medicine. The primary methods of protection from excess radiation are: reduce radiation field size with proper collimation, increase personnel distance from the primary beam, decrease exposure time, and wear protective equipment which absorbs scatter radiation. These concepts have been well-known for decades and yet, consistent application of these principles in daily practice is ignored due to perceived inconvenience, improper technique acquiring images, or lack of education about radiation safety. Multiple articles regarding radiation safety in veterinary medicine, spanning from the 1950's to the 2010's, all reveal similarly concerning themes. Namely, the recognition that radiation exposure is dangerous, that protective equipment is available, and that the risks of radiation exposure are known; yet there remains a lack of consistent application of safe practices and use of protective equipment. Collimation is a frequently ignored safety practice in veterinary medicine. The purposes of this study are to compare the average absorbed doses of radiation received between radiographs performed with proper collimation, to those which mimic common ways radiation safety standards are disregarded. We will focus on the following unsafe practices: 1) poor collimation, 2) inclusion of the hand in the primary beam, and 3) use of a lead glove placed on top of the hand rather than properly worn. Furthermore, we will extrapolate risks to the restrainer by comparing these absorbed doses to effective doses which are known to lead to deleterious effects.
Keywords for abstract:	Radiation safety collimation

CRF - 17

Title of abstract:	**ANTI-INFLAMMATORY AND ANABOLIC FACTORS IL-1RA AND IGF-I ARE ENRICHED IN EQUINE BONE MARROW CONCENTRATE COMPARED TO PRP**
Authors:	S. Long[+], G. Maleas[*], H Rice[+], and S. Durgam[+] [+]Dept. of Veterinary Clinical Sciences, The Ohio State University *Tierklinik in Lüsche GmbH, Germany
Abstract:	INTRODUCTION: Orthobiologics bone marrow aspirate concentrate (BMAC) and platelet-rich plasma (PRP) are gaining traction for treating equine articular and tendon/ligament injuries due to their anti-inflammatory properties and autologous nature. Their clinical use is incumbent on clinician preference/experience, diagnosis, clinical signs, and feasibility. Injury-specific treatment protocols are lacking and necessitates delineating their comparative features. This study assesses the cellular, growth factor, and cytokine compositions of paired BMAC and PRP from 27 horses and tests the hypothesis that they will be equivalent in BMAC and PRP. METHODS: BMAC and PRP were prepared via differential centrifugation of bone marrow buffy coat and whole blood, respectively. The cellular composition, growth factors (TGFβ1, IGF-I, PDGF, VEGF), and cytokines (TNFα, IL1β, IL6, IL1 receptor antagonist protein; IL1Ra) were quantified using standard automated hemoanalyzer and ELISA techniques, respectively. Data analyses were conducted with Two-way ANOVA for repeated measures with significance at $p<0.05$. RESULTS: The monocyte (8–fold), lymphocyte (3-fold), neutrophil (14-fold), and leukocyte (6-fold) numbers are significantly increased ($p<0.0001$) in BMAC compared to matched PRP isolates. In contrast, thrombocyte numbers are 5-fold ($p<0.0001$) increased in PRP relative to BMAC. Anti-inflammatory protein, IL1Ra (114-fold), tissue anabolic growth factor, IGF-I (433-fold), and angiogenic factor, VEGF (259-fold) are significantly enriched ($p<0.0001$) in BMAC; and in PRP, PDGF (4-fold) is increased ($p<0.0003$). DISCUSSION: Bone marrow-derived mesenchymal stem cells (MSC) are a promising biologic therapy; however, isolating MSC requires expensive laboratory processing and handling, and as such presents clinical challenges for routine use. In this respect, although BMAC contains fewer MSC, we demonstrate that cellular components of the innate immune system and anabolic growth factors are enriched in 'point of care' equine BMAC. Delineating the distinct cellular, growth factor and cytokine contents of equine BMAC and PRP is foundational to develop injury-specific applications of these orthobiologic therapies, and subsequent use in equine practice.
Keywords for abstract:	Equine bone marrow aspirate concentrate Equine PRP IL-1Ra IGF-I Comparative properties

CR - 18

Title of abstract:	**RETROSPECTIVE EVALUATION OF PaO2:FiO2 RATIO AND SpO2:FiO2 RATIO IN DOGS TREATED WITH HIGH FLOW NASAL CANNULA OXYGEN THERAPY**
Authors:	E. Lu[1], J. Her[1]. [1]Department of Veterinary Clinical Sciences, The Ohio State University College of Veterinary Medicine, Columbus, Ohio, USA.
Abstract:	High-flow nasal cannula (HFNC) oxygen therapy has been gaining popularity in veterinary medicine as an advanced oxygen supplementation for patients that require support beyond conventional oxygen supplementation. For patients receiving HFNC, the ratio of arterial oxygen partial pressure (PaO2) to fraction of inspired oxygen (FiO2) (PF ratio) is used as a clinical indicator of disease severity. Alternatively, oxygen saturation measured via pulse oximetry (SpO2) to FiO2 (SF) ratio has been suggested as a surrogate for the PF ratio. Human studies validated SF ratio to be a reliable surrogate for the PF ratio. However, this correlation has not been investigated in dogs receiving HFNC. The aim of this study is to assess the correlation between PF and SF ratios in dogs treated with HFNC. Medical records from nineteen client-owned dogs underwent HFNC were reviewed. A total of thirty data points with concurrent SpO2 and PaO2 measurement were identified. SpO2, PaO2, and FiO2 were used to calculate the PF and SF ratio. Median PF ratio was 168 (interquartile range; IQR 108-297) and median SF ratio was 186 (IQR 97-243). Correlation coefficients and 95% confidence intervals (CI) were calculated for all the data with Spearman correlation analysis and alternatively with Pearson's weighted correlation analysis of dog averages (weighted by number of measurements per dog) to account for repeated measurements within dogs. PF ratio was positively correlated with SF ratio (rho=0.86 (95%CI 0.73-0.93), weighted r=0.89 (95%CI 0.74-0.96). PF ratio and SF ratio in dogs treated with HFNC have good correlation, suggesting that SF ratio may be a useful, noninvasive surrogate for PF ratio when assessing oxygenation. Further studies are warranted to confirm and validate this relationship in larger number of dogs, and to assess the ability of SF ratio to predict outcome.
Keywords for abstract:	Canine Blood gas analysis Pulse oximetry High flow nasal cannula oxygen therapy Hypoxemic respiratory failure

CR - 19

Title of abstract:	**RADIOMIC HISTOGRAM AND TEXTURAL FEATURES OF POST CONTRAST COMPUTED TOMOGRAPHY IN ORAL MELANOMA ARE NOT PREDICTIVE OF MANDIBULAR AND MEDIAL RETROPHARYNGEAL LYMPH NODE METASTASIS**
Authors:	Lumbrezer-Johnson, S., Hostnik, E., Selmic, L.E., Lapsley, J., Wavreille, V.A.
Abstract:	Oral malignant melanoma frequently metastases to the mandibular and medial retropharyngeal lymph nodes. Lymphadenomegaly and fine needle aspiration with cytological evaluation of regional lymph nodes in melanocytic neoplasms is shown to have a poor correlation with histopathology and survival. Radiomics is a developing field that uses textural analysis of medical imaging to evaluate tumor heterogeneity in an attempt to predict biological characteristics of a tumor. In this retrospective study, 18 patients met the inclusion criteria of a head CT with intravenous contrast and lymph node histopathology within 14 days of one another. This created a population of 40 benign lymph nodes and 8 metastatic lymph nodes. Post-contrast head CT images were uploaded into LIFEx Software where segmentation was used to create a volume of interest around the sampled mandibular and medial retropharyngeal lymph nodes. Radiomic features were analyzed using various voxel sizes, with only the 1 × 1 × 1 mm^3 size sufficient for analysis. No statistical significance (p-value of 0.05) was demonstrated across the various radiomics features analyzed. In cases of oral melanoma, radiomic histogram and textural analyses of the regional mandibular and medial retropharyngeal lymph nodes were not predictive of metastatic disease.
Keywords for abstract:	head and neck cancer machine-based learning computer learning model

CR - 20

Title of abstract:	**APOLIPOPROTEIN B100 IS A POTENTIAL URINE BIOMARKER FOR MPGN IN DOGS WITH PROTEIN-LOSING NEPHROPATHY**
Authors:	C. Ma, R. Cianciolo, J. Hokamp
Abstract:	Membranoproliferative glomerulonephritis (MPGN) is an important cause of glomerular damage and subsequent protein-losing nephropathy in dogs. Renal biopsy is required to definitively diagnosis MPGN and mixed MPGN (both have subendothelial immune deposits) and guide immunosuppressive therapy, which is indicated for immune-mediated causes of glomerular damage only. Therefore, minimally invasive and economical methods of MPGN diagnosis are needed. In a preliminary study, liquid chromatography-tandem mass spectrometry (LC-MS/MS) found that urine Apolipoprotein B100 (ApoB100) is a candidate urine biomarker of canine MPGN. The present study (retrospective, case-control) aims to optimize an antibody for Western blot detection of canine urine ApoB100, confirm LC-MS/MS data, and explore urine ApoB100 as a biomarker of canine MPGN. Human ApoB100 antibody was optimized for Western blot detection of canine urine ApoB100. A 550 kDa band, compatible with ApoB100, was confirmed in urine samples in which ApoB100 had previously been detected by LC-MS/MS. Urine supernatant from 86 dogs with MPGN/mixed MPGN (n=40) and non-MPGN causes of glomerular disease (n=46) (membranous glomerulonephritis (n=12), amyloidosis (n=15), focal segmental glomerulosclerosis (n=12), mesangioproliferative glomerulonephritis (n=6), and podocytopathy (n=1)) were probed with Human ApoB100 antibody. Results were designated positive for ApoB100 if a band was present at 550 kDa and negative if the band was absent. Chi-squared analysis was used to assess for a significant difference in ApoB100 positivity between MPGN and non-MPGN urine samples. **Urine samples were positive for ApoB100 in a significantly greater proportion of dogs with MPGN (68%, n=27) than in dogs with non-MPGN glomerular diseases (26%, n=12) ($p < 0.05$).** In conclusion, Western blot results confirm LC-MS/MS data and further suggests urine ApoB100 is a biomarker of canine MPGN. Future studies aim to quantify ApoB100 in MPGN and non-MPGN urine samples via quantitative Western blot to establish reference intervals, sensitivity, and specificity.
Keywords for abstract:	Glomerular disease Glomerulonephritis Protein-losing nephropathy Dogs Urine biomarkers

CR - 21

Title of abstract:	**A NOVEL TECHNIQUE FOR MEASURING SPASTICITY IN DOGS AFTER ACUTE THORACOLUMBAR INTERVERTEBRAL DISC EXTRUSION (TL-IVDE).**
Authors:	L.M. McAllister, L.G. Bookenberger, A.C. Hechler, R.C. da Costa, S.A. Moore. Dept. of Veterinary Clinical Sciences
Abstract:	Background – Spasticity following spinal cord injury (SCI) occurs commonly, but ways to measure this in dogs are underexplored. Dynamometry is commonly used to quantify spasticity in people. Objectives – Evaluate a handheld dynamometer for quantifying spasticity in healthy dogs and SCI-affected dogs. Animals – Prospective cohort study in 11 healthy dogs and 10 with acute TL-IVDE (SCI-affected). Methods – A handheld dynamometer (The Commander™ console and muscle tester, JTECH Medical) was used to perform measurements at multiple thoracic and pelvic limb joints. Healthy dogs were evaluated at two sessions in a 24-hour period. SCIaffected dogs were evaluated 0, 14, 30, and 60 days after surgical decompression. Measurements were compared between sessions in healthy dogs and between healthy and SCI-affected dogs using a Wilcoxon matched-pairs signed rank test and a Mann Whitney test, respectively. $P < 0.05$ was considered significant. Results – Apart from the carpi, measurements were consistent across sessions in healthy dogs. Measurements were higher in SCI-affected dogs for the carpi at day 0 ($P < 0.0003$); for the carpi and stifles at 14 days ($P < 0.015$); and for the carpi, stifles and tarsi at days 30 and 60 ($P < 0.03$). Conclusions – Dynamometry measurements for most joints are consistent across sessions in healthy dogs, but differ between healthy and SCI-affected dogs for at least 60 days after TL-IVDE. The inconsistency in carpal measurements require further investigation. Dynamometry might represent a helpful quantitative measure of spasticity of pelvic limbs after SCI in dogs and could be used in future clinical studies.
Keywords for abstract:	Dynamometry Spasticity Spinal cord injury

CR - 22

Title of abstract:	**EXAMINING URINE PH, SPECIFIC GRAVITY, AND URINE PROTEIN OVER TIME IN HEALTHY DOGS**
Authors:	A. McGlynn, R. Mrofchak, R. Madan, C. Madden, S. Justice, A. Rudinsky, J. Hokamp, and V. Hale. College of Nursing (Justice), Depts. of Veterinary Preventive Medicine (McGlynn, Madan, Mrofchak, Madden, Hale), Veterinary Clinical Sciences (Rudinsky), and Veterinary Biosciences (Hokamp).
Abstract:	Urine properties such as pH, urine specific gravity (USG), and protein profiles are routinely measured during a urinalysis to assess canine health. Clinicians have well-established reference ranges to assess urine in patients with urinary tract diseases, such as urinary tract infections. Despite this, less is known about the natural fluctuations of urine properties in healthy dogs over time. In this study, mid-stream free catch urine was collected from 14 healthy dogs (7 male, 7 female) over 12 time points that were hours, days, and months apart. We hypothesized that urine pH and USG would vary significantly over time, while protein profiles would be stable due to consistent protein filtration by the kidneys. Urine pH was measured via pH meter; USG was measured via refractometer, and proteins were profiled via 4-12% Bis-Tris gel electrophoresis. Preliminary data showed that USG varied significantly ($p < 0.0001$) between dogs but was consistent over time within dogs ($p = 0.2$) while urine pH did not vary significantly between dogs ($p = 0.588$) because pH was so highly variable within dogs over time. Urine proteins ranged from none to trace amounts. The most commonly detected proteins were at molecular weights consistent with Tamm Horsfall and albumin. Statistics on urine proteins are forthcoming. Our hypothesis for USG was unsupported as it did not vary significantly over time, indicating that a USG measurement at any time should be representative for that dog, while pH was so variable that it should be measured at multiple time points before altering clinical decisions. These findings help define normal variations in urine properties, which can inform clinical decision-making around urine sampling and urinalyses.
Keywords for abstract:	dog urine pH urine specific gravity urine protein

CR - 23

Title of abstract:	**EVALUATION OF OUTCOME IN CATS WITH SQUAMOUS CELL CARCINOMA OF THE EAR CANAL: A REVIEW OF 28 CASES (2010-2021)**
Authors:	A. McGrath[1], C. Chen[1], B. Abrams[2], L. Hixon[3], J. Grimes[3], E. Viani[4], M. McLoughlin[1], G. Tremolada[1], J. Lapsley[1], and L. E. Selmic[1]. [1]Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH, United States [2]Department of Veterinary Clinical Sciences, University of Pennsylvania, Philadelphia, PA, United States [3]Department of Surgery, University of Georgia, Athens, GA, United States [4]Department of Surgery, Angell Animal Medical Center, Jamaica Plain, MA, United States
Abstract:	While ear canal tumors are uncommon in felines, ceruminous gland adenocarcinomas are the most common malignant tumor of the feline ear canal. Squamous cell carcinoma closely follows and is the most frequently diagnosed malignant tumor of the feline middle ear. However, there is limited information on the outcomes of cats diagnosed with squamous cell carcinoma. The objective of this study was to describe the outcome of cats diagnosed with squamous cell carcinoma. Medical records from January 1, 2010 through January 1, 2021 were reviewed to identify cats with a definitive diagnosis of squamous cell carcinoma based on histopathology. Twenty-eight cats were identified. Overall, one cat was euthanized following diagnosis, eight cats were treated with medical management, three cats were treated with coarse fractionated radiation therapy, one cat was treated with systemic chemotherapy, two cats were treated with a combination of coarse fractionated radiation therapy and chemotherapy, eleven cats were treated with surgery, and one cat was treated with a combination of surgery and coarse fractionated radiation therapy. Median survival time of cats treated with surgery was 168 days, compared to 85 days for those treated palliatively with either medical management, radiation therapy, chemotherapy or a combination of radiation therapy and chemotherapy. A poor prognosis was associated with neurologic signs at the time of diagnosis and evidence of vascular invasion on histopathology. While squamous cell carcinoma of the ear canal is a locally aggressive tumor that carries an overall poor prognosis, this case series documented improved outcomes for cats that underwent surgery versus other therapies.
Keywords for abstract:	Ear canal Carcinoma Squamous Cell Cat

CR - 24

Title of abstract:	**TO SAMPLE OR NOT TO SAMPLE: CAPTURING FELINE FECAL MICROBIOME CHANGES WITH HIGH-FREQUENCY SAMPLE COLLECTION**
Authors:	N.J. Nealon[1], H. Klein[1], M. Salerno[1], V.J. Parker[1], J. Quimby[1], A. Rudinsky[1], J. Howard[1], and J.A. Winston[1] [1.] Department of Veterinary Clinical Sciences. The Ohio State University, College of Veterinary Medicine. Columbus, Ohio. 43210.
Abstract:	Microbiome-based fecal evaluations are promising diagnostic tools or assessing feline health. However, given inherent daily changes in the gut microbiome, the ideal sampling frequency is unknown. The objective of this study is to evaluate the impact of different sampling frequencies on the resolution of fecal microbiome data (i.e. changes in species abundance and presence/absence). Our hypothesis is that daily fecal sampling is more effective than weekly or bi/tri-weekly sampling at capturing changes in the feline fecal microbiome in response to a new diet. This clinical trial was performed in six healthy and sterilized purpose-bred cats (3 male, 3 female) group-housed in a research facility. All cats were fed an adult commercial wet food diet, at maintenance needs, to which they had no previous exposure. Feces was collected daily for 36 days. 16s rRNA microbiome analysis (V4 region) was performed using R-Studio. Pairwise PERMANOVA evaluated changes in composition. Subsets of data (i.e., once-weekly samples) was compared to the full (daily) dataset to evaluate daily versus lower frequency (i.e. once-weekly) fecal sampling effects. With daily sampling, twice weekly and three times weekly sampling, the fecal microbiome composition of each cat differed by day for the study duration ($p<0.01$). Despite being co-housed, daily sampling identified distinct microbiome compositions between cats at all time points ($p<0.0001$). When once weekly data was sub-sampled, no compositional differences between cats were detected and there were decreased differences over time (sampling week), ($p<0.05$) for all cats. Daily sampling or a pooled 2-3 day samples may more accurately represent the fecal microbiome versus weekly sampling, which is the sampling norm for many companion animal studies. Ongoing analysis will evaluate taxa contributing to daily microbiome changes and compare sampling frequency schemes to establish appropriate study designs for future fecal microbiome studies.
Keywords for abstract:	Feline Medicine Fecal Microbiome 16S Sequencing

CR - 25

Title of abstract:	**ATTENUATION OF POST-PRANDIAL HYPERGLYCEMIA BY 5'-ADENOSINE MONOPHOSPHATE-ACTIVED PROTEIN KINASE AGONISTS IN EXPERIMENTALLY-INDUCED EQUINE INSULIN DYSREGULATION**
Authors:	E. Pinnell, L. Hostnik, M. Watts, K. Timko, A. Thriffiley, M. Stover, L. Koenig, O. Gorman, R. Toribio, and T. Burns. Depts. Of Veterinary Clinical Sciences
Abstract:	5'-adenosine monophosphate-activated protein kinase (AMPK) agonists, such as metformin (MET) and aspirin (ASP), have been shown to improve experimentally-induced insulin dysregulation (ID) when co-administered. Resveratrol (RES) is an AMPK agonist and also inhibits mammalian target of rapamycin (mTOR) signaling, which is activated in laminitis, making this an attractive therapeutic target for equine metabolic syndrome-associated laminitis (EMSAL). The purpose of this study was to evaluate the effect of combination treatments with RES (10 mg/kg PO q12hr), MET (30 mg/kg PO q12hr), and ASP (20 mg/kg PO q24hr) on experimentally-induced ID. We hypothesized that co-administration of AMPK agonists would improve insulin and glucose dynamics in horses with experimentally-induced ID. ID was induced in 24 healthy adult light-breed horses using dexamethasone (0.08 mg/kg PO q24hr). Horses were assigned to one of 5 treatment groups: RES, MET/ASP, RES/ASP, RES/MET/ASP, and control (CON). Frequently-sampled insulin-modified IV glucose tolerance tests (FSIGTT) and oral sugar tests (OST) were performed at baseline, one week after ID, and after ID plus one week of treatment. Insulin sensitivity (SI) and disposition index (DI) decreased, while basal insulin and glucose concentrations increased following experimentally-induced ID ($p < 0.0001$). Area under the glucose curve (AUC_g) during OST decreased after combination treatment with RES/MET/ASP ($P = 0.0013$). Dexamethasone-induced ID alters glucose and insulin dynamics in horses reflected in minimal model parameters. Additionally, treatment with a combination of RES/MET/ASP significantly attenuates post-prandial hyperglycemia and should be evaluated as a novel treatment for equine ID.
Keywords for abstract:	Equine Metabolic Syndrome Laminitis Insulin Dysregulation AMPK agonists Metformin Aspirin Resveratrol

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Title of abstract:	**COMPUTED TOMOGRAPHY (CT) EVALUATION OF CANINE ANAL SAC APOCRINE GLAND ADENOCARCINOMA**
Authors:	A.B. Repasy, M.E. Schreeg, L.E. Selmic, E.T. Hostnik, W.C. Kisseberth
Abstract:	Canine apocrine gland anal sac adenocarcinoma (AGASAC) is a malignant tumor of the anal gland of the dog and represents approximately 2% of all skin and subcutaneous tumors in this species. The treatment of choice for this tumor type often involves surgery, radiation, or a combination of these modalities with minimal benefit proven with the addition of adjuvant chemotherapy. While more thorough staging of these patients increasingly includes a CT scan of the primary tumor and abdomen, the question remains as to how results of this CT imaging may relate to prognosis, or other features of the disease. This study aims to retrospectively evaluate the effect of primary tumor and metastatic lymph node dimensions using CT on the survival time of patients and the probability of having contra or ipsilateral lymphatic involvement. Given the variable sizes of patients, a secondary goal involves using a standardized ratio comparing these measurements to the diameter of the aorta at L4 in order to better understand the effect of the relative size of tumors and metastatic disease on the above parameters. Between July 2016 and August 2021, 44 abdominal computed tomography (CT) scans were completed on 37 canine patients diagnosed with AGASAC at The Ohio State University Veterinary Medical Center. Patients were included if they had a histopathological diagnosis of AGASAC and were treated at OSU with surgery, with or without the addition of radiation therapy. Data reviewed included histopathological subtype of AGASAC, surgical margins, disease progression, and types of treatments performed. With this information, we hope to provide prognostic information for patients following imaging that can help guide treatment options for both clinicians and owners.
Keywords for abstract:	Canine Anal sac apocrine gland adenocarcinoma Computed tomography (CT)

CR - 27

Title of abstract:	**COLONIZATION AND EFFECT OF NISSLE 1917 E COLI PROBIOTIC ON THE HEALTHY FELINE MICROBIOME**
Authors:	J. Riha, J. Winston, J. Howard, V. Parker, A. Rudinsky
Abstract:	Probiotics are among the most commonly used therapies in veterinary medicine. E. coli Nissle 1917 (EcN) is classified as a non-pathogenic probiotic strain with the ability to produce bacteriocins that exhibit antimicrobial activity against closely related bacteria. We have shown that EcN displays antimicrobial activity against feline uropathogenic E. coli (UPEC) isolates in vitro. UPEC causes of the majority of diagnosed UTIs in felines. The action of EcN on UPEC strains in vitro indicates that UTIs represent another area where EcN could have a positive impact on small animal veterinary medicine. The objectives of this study were to detect short term colonization of EcN in the feline microbiome, to observe strain level diversity of E. Coli following EcN ingestion, and to look at the impact of EcN on the overall healthy cat microbiome. The methods used were to collect fecal samples from five healthy research cats at baseline (day -2 to 0), and throughout the study (days 1-28). Fecal samples were collected, and placed in macconkey broth to isolate E. Coli. The isolated samples were plated onto macconkey agar for 24 hours. Twenty colonies per sample were then plated onto luria agar and incubated for 24 hours before being banked at -80 degrees. DNA extraction was then performed to evaluate how long EcN stays present in the feline microbiome. Although this is an ongoing investigation, there has been E. Coli growth in over 90% of samples, and DNA has been identified and extracted in the first 5 days of collection.
Keywords for abstract:	Probiotics E. Coli Nissle bacteriocins antimicrobial uropathogenic isolates UTI colonization microbiome fecal healthy baseline samples plated extraction ongoing extracted

CR - 28

Title of abstract:	**STANDARDIZED PREPARATION OF CANINE FECAL TRANSPLANT MATERIAL DOES NOT ALTER MICROBIAL COMMUNITY STRUCTURE**
Authors:	N. Randolph*, M. Salerno*, H. Klein, J. Winston. Department of Veterinary Clinical Sciences *Authors contributed equally
Abstract:	Background: Fecal microbiota transplantation (FMT) is the delivery of fecal material from a healthy donor into a diseased recipient to confer a health benefit. FMT is routinely delivered as a slurry via enema or nasogastric tube, but can also be encapsulated for convenient oral administration. The microbial impacts of processing feces for encapsulation are unknown. Hypothesis/Objectives: We aimed to investigate if standardized fecal processing into a 10% glycerol FMT slurry alters the microbial community structure. We hypothesized that microbial community structure would not vary between processing steps. Animals: Feces from four screened healthy canine fecal donors at The Ohio State University Companion Animal Fecal Bank were utilized for this study. Methods: Batches of canine feces underwent a standardized FMT processing protocol. Samples from each donor, across multiple batches, were pulled from specific steps during processing and underwent DNA extraction and Illumina sequencing of the V4 region of the 16S rRNA gene. Amplicon sequence analysis was performed using MOTHUR, DADA2 and R. Results: Analysis revealed significant differences in alpha diversity (Shannon index) and beta diversity (NMDS, Jaccard index) measures between donors. Within an individual donor, no significant differences were observed between processing steps (AMOVA). Sequence analysis using amplicon sequence variants (ASVs) and operational taxonomic units (OTUs) both yielded similar results. Conclusions and clinical importance: Standardized FMT processing for encapsulation does not significantly alter the microbial composition of feces from healthy canine donors, further promoting the practicality of oral FMT administration in canine medicine. Additional studies evaluating microbial viability are required.
Keywords for abstract:	Fecal microbiota transplantation Microbiome Transplantation Canine Fecal capsules

CR - 29

Title of abstract:	**THE APPLICATION OF ALLOSTASIS AND ALLOSTATIC LOAD IN ANIMAL SPECIES**
Authors:	K.E. Seeley, K. Proudfoot
Abstract *(300 word limit)*	Stress is unavoidable and can be an adaptive mechanism for survival. However, chronic stress has been linked to poor health outcomes and increased morbidity. The concept of allostasis describes the physiologic dysregulation that occurs when an organism is exposed to chronic stressors. Allostatic load is estimated in human populations using an allostatic load index (ALI). ALIs have tremendous potential in animal populations and may serve as a tool for evaluating chronic stress across multiple taxa. In order to understand how the concepts of allostasis and allostatic load (AL) have been used in animal species and identify the gaps a scoping review of the literature was conducted. The aims of this review were to 1) describe the extent to which the concepts of allostasis and allostatic load are being applied theoretically to animal populations, with a focus on which taxa and species are represented; 2) identify when direct assessments of allostasis or allostatic load are being made, which species and contexts are represented, and what biomarkers are being used; and 3) describe gaps in the literature and identify areas for future research. A total of 572 papers met inclusion criteria for the review, all taxa were represented with mammals (n=177), fish (n=143) and birds (n=134) being the most commonly studied groups. A subset of these papers (n=63) made direct assessments about allostatic load, however only 6 publications utilized an ALI to do so. Numerous papers made direct conclusions about AL based on a single glucocorticoid biomarker which is problematic, since it is commonly accepted that no single biomarker can serve as a proxy for allostatic load. This review identified significant gaps in the literature and provides directions for future research.
Keywords for abstract:	Allostasis Allostatic load Animals Stress

CR - 30

Title of abstract:	**ESTROGENS AND THEIR ASSOCIATION WITH ILLNESS IN HOSPITALIZED FOALS**
Authors:	H. SNYDER, J. SWINK, J. SUMMERS, J. HORTON, H. KINSELLA, R. TORIBIO
Abstract:	During late pregnancy the equine placenta acquires steroidogenic functions including the synthesis of androgens and estrogens using fetal progestogens as precursors. Previous studies have shown that abnormally high concentrations of progestogens and androgens are linked to sepsis and neonatal maladjustment syndrome. Therefore, endocrine dysregulation contributes to mortality in critically ill foals. We have shown serum estrone sulfate concentrations are higher in septic compared to healthy foals ($P<0.05$). Estrogens (17β-estradiol, estrone, total estrogens) have not been investigated in sick foals. We hypothesize that during hospitalization, critically ill foals will have higher estrogen concentrations that will be proportional to disease severity. In addition, foals with persistent elevation of estrogens will have more severe laboratory abnormalities and increased risk of mortality. This was a prospective, multicenter, and descriptive study in hospitalized ($n = 80$; 50 septic, 30 sick non-septic) and healthy ($n = 20$) foals of < 3 days of age at 4 institutions. Blood samples were collected at 0, 24, 48, and 72 h for estrogen measurement via immunoassays (ELISA, RIA). Data were assessed for normality. Clinical information will be retrieved from the medical records (ongoing). Measurement of estrogen concentrations has been completed. Preliminary results showed that hospitalized (septic, sick non-septic) foals had higher total estrogen concentrations compared to healthy foals ($P<0.01$). Non-surviving foals had reduced clearance of estrone and 17β-estradiol ($P<0.05$). Results from this study will enhance our understanding on the role of estrogens (and other sex hormones) on disease development and progression in sick foals in the immediate post-partum period.
Keywords for abstract:	Neonatal foals Sepsis Estrogens Hormones Endocrinology

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Title of abstract:	**VITAMIN D AND PARATHYROID HORMONE DURING HOSPITALIZATION IN CRITICALLY ILL FOALS**
Authors:	J.Summers, L. Hostnik, H. Kinsella, H. Snyder, J. Horton, R. Toribio
Abstract:	Sepsis is a major cause of foal mortality and is responsible for disorders of mineral homeostasis. Vitamin D is essential for calcium and phosphorus regulation, musculoskeletal health, and immunity. However, little is known about its role in disease progression and mineral dysregulation in critically ill foals. The goal of this project was to determine the dynamics of 25-hydroxyvitamin D and parathyroid hormone (PTH) concentrations over time in hospitalized versus healthy foals while associating metabolite changes with laboratory variables (e.g. calcium and phosphorus), disease severity and mortality. We hypothesized that during hospitalization, critically ill foals will have low $25(OH)D_3$ levels, increased PTH concentrations, and that persistent hypovitaminosis D will be linked to disease severity and increased risk of mortality. Blood samples were collected from healthy, septic and sick non-septic foals of ≤ 72 hours of age (n=42) at 0 (admission), 24, 48, and 72h. Concentrations of $25(OH)D_3$, PTH, calcium, and phosphorus concentrations were measured using immunoassays (ELISA) and chemistry/hematology analyzers. Data was assessed for normality and analyzed by parametric and non-parametric statistical methods. On preliminary analysis, $25(OH)D_3$ concentrations were higher in septic and SNS than healthy foals, which was unexpected. PTH analysis is ongoing (assay discontinued by the company). Septic foals had lower calcium and higher phosphorus concentrations compared to other groups. Non-surviving foals had lower calcium and higher phosphorus concentrations compared to survivors. Our initial findings indicate that septic foals develop hypocalcemia and hyperphosphatemia, which could result in endocrine imbalances. Due to conflicting results, we will be carrying out measurement of $25(OH)D_3$ in a larger population of sick and healthy foals.
Keywords for abstract:	Sepsis Vitamin D Endocrine Foals

CR - 32

Title of abstract:	**A PHASE I STUDY OF UNIVERSAL-DONOR TGFB-IMPRINTED NK CELL THERAPY IN COMBINATION WITH CARBOPLATIN FOR CANINE OSTEOSARCOMA**
Authors:	A. Thakkar [2], W. Dirksen [1], D. Nielsen [1], P. E. Yaxley [1], R.N. Jennings[1], R. Burge[1], B.D. Husbands[1], C. A. Cash [2], E. Troy [2], D. A. Lee [2], W. C. Kisseberth [1] [1] Departments of Veterinary Clinical Sciences and Veterinary Biosciences, Ohio State University, Columbus, Ohio [2] Center for Childhood Cancer and Blood Diseases, The Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus Ohio
Abstract:	Osteosarcoma is one of the most common bone tumors in humans and dogs. Limb amputation with adjuvant chemotherapy is the standard of care but has limited survival benefit. Canine osteosarcoma shares many features with human osteosarcoma, making it an important comparative oncology model. We previously described a method for generating TGFβ-resistant NK cells, which could provide a means to overcome TGFβ-induced immunosuppression in osteosarcoma. We adapted this approach to expand large numbers of allogeneic (A) TGFβ-resistant (T) canine NK cells from healthy blood bank donors (AT-NK). To test the safety, toxicity, and preliminary evidence of efficacy of "off-the-shelf" AT-NK, we are conducting a phase 1 study in dogs with osteosarcoma. Dogs with osteosarcoma undergo limb amputation and four infusions of peri-operative AT-NK, followed by four cycles of carboplatin chemotherapy every three weeks with two infusions of AT-NK weekly after each dose of carboplatin. The dose escalation occurs in a 3+3 design for AT-NK ($1x10^6$/kg, $1x10^7$/kg, and $1x10^8$/kg) to determine the maximum tolerated dose. Monitoring during the study includes physical exam, CBC, serum chemistry profile, and restaging with thoracic radiographs. Samples of tumor and blood samples collected pre- or post-infusion are collected for correlative studies. NK cells were expanded from 7 donors, with a median of $4.9x10^9$ NK cells generated from each canine buffy coat. Three dogs completed AT-NK infusions at dose level-1 ($1x10^6$/kg) and 2 at dose level-2 ($1x10^7$/kg), receiving a total of 54 NK cell infusions. Four out of 5 patients showed no lung metastases, 2 of these developed metastases at other sites. Other than acute infusion reactions that were eliminated by modifying the cryopreservation medium, AT-NK cells have been well tolerated so far in this study and no toxicity related to AT-NK was observed. This study will inform further development of "off-the-shelf" AT-NK therapy.
Keywords for abstract:	Osteosarcoma Immunotherapy Clinical trial Natural killer cells

CR - 33

Title of abstract:	**EFFECT OF HIGH-CARBOHYDRATE FEEDING AND CORITCOSTEROID ADMINISTRATION ON LIPID CONTENT OF EQUINE LIVER**
Authors:	KJ Timko, LD Hostnik, MR Watts, C Chen, A Bercz, RE Toribio, JK Belknap, TA Burns
Abstract:	Nonalcoholic fatty liver disease (NAFLD), a sequela of human metabolic syndrome, is associated with excess lipid accumulation in the liver of patients without alcohol consumption. Equine metabolic syndrome is similarly characterized by insulin dysregulation and dyslipidemias, which could contribute to a form of NAFLD. This study investigated the effect of a high non-structural carbohydrate (NSC) diet and corticosteroid administration on liver lipid content in healthy adult ponies and horses. Mixed-breed ponies were allocated into four groups based on body condition score (BCS) (Lean; [BCS] 4, obese; BCS 7) and diet (Control; NSC ~6%, High NSC; NSC ~42%): Lean Control (n=5), Lean High NSC (n=6), Obese Control (n=4), Obese High NSC (n=5) and fed their respective diet for 7d. Liver samples were collected at the end of the feeding protocol and stained with hematoxylin and eosin (H&E). Liver biopsies were obtained from 14 light-breed horses before and after dexamethasone administration (0.08 mg/kg PO q24) for 7d and stained with H&E. Liver samples were evaluated by light microscopy and scored using a NAFLD scoring system adapted from humans: Steatosis (0-3), microvesicular steatosis (0-1), ballooning injury (0-2), inflammation (0-3), and steatosis location (0-3). The Lean and Obese High NSC diet ponies had higher NAFLD scores compared to control fed obese ponies ($P = 0.01$, 0.04, respectively). The NAFLD scores following dexamethasone treatment were increased compared to baseline ($P < 0.001$). These findings suggest a high NSC diet and dexamethasone administration can induce histopathologic changes consistent with NAFLD in equids within 7 days.
Keywords for abstract:	Equine Metabolic Syndrome Corticosteroid Liver Lipid

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Title of abstract:	**THE IMPACT OF FECAL IDENTIFICATION MARKERS ON THE FELINE MICROBIOME**
Authors:	A.Wood, N.J. Nealon, H. Klein, A. Rudinsky, M. Salerno, J. Quimby, V. Parker, J. Howard, J.A. Winston Department of Veterinary Clinical Sciences
Abstract:	Background: Fecal collection in cats is required for research and clinical purposes but fecal identification is limited by feline elimination behaviors in group-housing and multi-cat households. Crayon shavings and glitter have been used as fecal markers, but their impact on the fecal microbiome is unknown. Objective: To determine the impact of fecal identification markers, specifically crayon and glitter, on the feline microbiome. Animals: This clinical trial was performed in six healthy and sterilized purpose-bred cats (3 male, 3 female) group-housed in a dedicated research facility. Methods: A randomized crossover experiment was performed. Cats received either glitter or crayon shavings mixed in a feline adult maintenance diet for 14 days. A 14-day washout was performed between crossovers. Feces was collected daily from each cat for 16S rRNA gene sequencing (V4 region). Amplicon analysis was performed in R-studio (dada2; phyloseq). Pairwise PERMANOVA analysis compared samples across experimental phases and marker order. Results: Fecal identification markers were readily identified in the feces of all cats. Statistically significant changes to the microbiome were identified with marker order ($P < 0.05$) and across experimental phases ($P < 0.05$) for 5/6 cats. Microbiome changes were cat-dependent ($P < 0.001$), suggesting idiosyncratic microbiome responses to glitter and crayon. Conclusions and Clinical Importance: Both glitter and crayon allow for identification of individual feline fecal samples; however, these markers may impact the microbiome and therefore are not recommended in studies with microbiome endpoints. The difference in response to these markers among cats highlights the individuality of the feline microbiome.
Keywords for abstract:	Feline medicine Microbiome Fecal collection Glitter Crayon shavings

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Title of abstract:	**A COMPARISON OF ADMISSION SERUM PROCALCITONIN, SERUM AMYLOID A, AND PHYSICAL EXAM FINDINGS AS DIAGNOSTIC AND PROGNOSTIC INDICATORS IN THE EQUINE ACUTE ABDOMEN**
Authors:	S. Mordoh, R. Toribio, M. Mudge, A. Gardner
Abstract:	Rapid decision making in an equine acute colic episode is critical, but current diagnostics do not always lead to a clear recommendation of surgical versus medical management. The objectives of this study were to (1) evaluate whether there was a diagnostic and value of the inflammatory biomarker procalcitonin versus the acute phase protein Serum Amyloid A (SAA) and to (2) determine whether there was a correlation between procalcitonin, SAA, and previously reported markers of colic severity. The study included 45 horses with colic admitted to our referral hospital and 20 healthy horses. Blood was obtained via jugular venipuncture at time of arrival. Each colic was categorized according to lesion (medically managed non-inflammatory, medically managed inflammatory, surgical non-strangulating, and surgical strangulating), as well as according to treatment (medical vs. surgical). Data were tested for normality, and analyzed using a t-test, ANOVA, or Pearson's r where appropriate, or each test's non-parametric corollary if indicated. Results were considered significant if $p<0.05$. Admission heart rate was significantly different in horses with medical (non-inflammatory and inflammatory) and surgical (non- strangulating lesions) versus the healthy population. Admission respiratory rate was significantly different in horses with surgical lesions (strangulating and non-strangulating) versus healthy horses. Serum amyloid A and procalcitionin were both significantly different in horses that received surgical intervention versus those medically managed. Heart rate was weakly to moderately positively correlated with admission hematocrit, peripheral lactate, SAA, and respiratory rate. Admission hematocrit was also weakly correlated with admission respiratory rate. Procalcitonin and SAA were successful in predicting surgical versus medical management of colics within the population. Further work on other inflammatory biomarkers such as serum C-reactive protein and soluble CD14 may yield further results in this population. Furthermore, a larger sample size may be needed as several values approached but did not achieve significance ($p<0.05$).
Keywords for abstract:	Acute Phase Protein Colic Horse

EPIDEMILOGY
AND
APPLIED RESEARCH

EAR - 1

Title of abstract:	**ASSOCIATING SWINE WEIGHT AND AGE AT WEANING WITH WEAN-TO-FINISH PIG DEATH**
Authors:	T.-Y. Cheng, W. A. Lopez, and A. G. Arruda. Depts. of Veterinary Preventive Medicine in College of Veterinary Medicine at the Ohio State University and Technical Services Wean to Finish at Pig Improvement Company (PIC)
Abstract:	OBJECTIVE: This study aimed to examine the potential relationship between weaning age/weight and post-weaning mortality in commercial wean-to-finish swine production systems. METHODS: Swine production data of 224 closeouts (4,852,489 pigs in total) were collected from 52 North American wean-to-finish (WTF) farms between December 2016 and October 2020. Average weight (kg), age (day), and the detection of porcine reproductive and respiratory syndrome virus (PRRSV) and porcine epidemic diarrhea virus (PEDV) (yes/no) at weaning were recorded at the closeout level. Weaning weight and age were individually fitted into generalized linear mixed models (M-weight and M-age) along with the count of total WTF pig death (outcome), the count of starting closeout inventory (offset), the detection of PEDV and PRRSV (fixed effects), and interactions among fixed effects under Poisson distribution. Origin sow farms and WTF farm IDs were included as random effects. RESULTS: WTF mortality ranged between 4.3% and 73.4%. Weaning weight and age (median±IQR) were 6.3±1.3 kg and 22.7±4.1 days. Higher WTF mortality (median±IQR) was identified in PEDV-positive closeouts (18.9±12.2%; n=92) compared to negative closeouts (14.0±10.0%; n=132). However, similar mortalities were observed regardless of PRRSV status (16.5±11.9% vs 16.2±12.9%). WTF death counts increased as weaning weight (M-weight) and age (M-age) decreased. There was a significant interaction ($p < 0.001$; F-test) between the PEDV detection and weaning weight/age. By decreasing one kg in weaning weight or one day in weaning age, WTF mortality in PEDV-positive closeouts increased at a higher level; 47.7% (M-weight) and 4.8% (M-age), compared to PEDV-negative closeouts (increased by 21.0% [M-weight] and 1.7% [M-age]). CONCLUSIONS: WTF mortality increased as weaning weight and age decreased, with higher increments in PEDV-positive closeouts. These associations may be used as indices of the quality of piglets at weaning and help producers to predict WTF mortality.
Keywords for abstract:	Swine Mortality modeling Quality of weaning pigs

EAR - 2

Title of abstract:	**CLEANING AND DISINFECTION EFFICACY OF TWO MOP SYSTEMS AND DISINFECTANTS IN A LABORATORY ANIMAL FACILITY**
Authors:	M. Fernandez, V. Capria, V Bergdall. The Ohio State University Laboratory Animal Resources
Abstract:	Sanitation of the animal facility, including the floors, is essential for maintaining biosecurity, and meeting regulatory requirements. However, best practices for method, frequency, and evaluation techniques used to assess floor cleaning and disinfection have not been established in laboratory animal facilities. Research done in human hospital settings suggests that traditional cotton string mop and bucket systems can spread microbial contaminants across floors. Therefore, we evaluated two mopping systems with two different disinfectants: quaternary ammonium compound (QUAT) and cotton string mop, QUAT and microfiber mop, hydrogen peroxide-based disinfectant (HPD) and cotton string mop, and HPD and microfiber mop. Each mop and detergent condition was used to clean two rooms housing mice twice a week for two weeks. The floors were swept then sampled before and after mopping. RODAC plates and ATP swabs were utilized to evaluate disinfection and cleaning. The time to mop each room, time for the floor to dry, and amount of detergent used was recorded. The percent change between the pre-cleaning and post-cleaning samples were compared between each group using the Kruskal Wallis test. The QUAT and cotton string mop condition had a significantly smaller percent reduction in CFU than the other three conditions. Additionally, both QUAT conditions had significantly smaller percent reduction in ATP RLU measurements than the HPD conditions. However, during the first mopping session, only one of the conditions had statistically significant differences: the QUAT + microfiber had a greater CFU reduction compared to the HPD + cotton string. A cost estimate was performed, and the microfiber cleaning process is estimated to be more cost effective due to time saved in personnel hours. The results of this study can be used to select floor sanitation practices in laboratory animal facilities.
Keywords for abstract:	Operations Husbandry Sanitation Biosecurity

EAR - 3

Title of abstract:	**USING A ONE WELFARE-BASED ECOSYSTEM MODEL TO ASSESS HARVESTING OF FISHES FOR PUBLIC AQUARIUMS**
Authors:	B. Fischer, J. Pempek, K. George, J. Flint, T. Wittum, and M. Flint. Department of Veterinary Preventive Medicine, College of Veterinary Medicine and Department of Animal Sciences, College of Food, Agricultural, and Environmental Sciences
Abstract:	Aquatic ecosystems are currently facing a multitude of stressors from anthropogenic impacts, including climate change, pollution, and overfishing. Public aquariums positively contribute to ecosystems through conservation, education, and scientific advancement, but may also negatively detract through wild collection and sourcing from commercial suppliers. Although changes in industry have occurred, evidence-based assessments of how aquariums maintain their populations to determine sustainability are still needed. The objective of this study is to develop and implement a One Welfare-based ecosystem model to assess impacts of harvesting of fishes for public aquariums. Indicators will be selected, nested into physical-chemical, biological, and socio-economic categories, compared to reference parameters, assigned weightings, and included in the model for an overall assessment of ecosystem health of each area. This will be accomplished through 1) assessment of ecosystem health of wild collection sites using physical-chemical, biological, and socio-economic indicators; 2) evaluation of welfare of species at aquariums through semi-annual assessments and behavioral observations; and 3) comparison of ecosystem health of harvesting sites vs. assessments of an aquaculture facility. Data collection is being completed in three parts: 1) holistic assessments of harvesing sites including water and sediment chemistries, population surveying and health assessments of sentinel species, and inclusion of socio-economic impacts of selected aquariums; 2) objective measurements of animal welfare and behavior using scan sampling at aquariums; and 3) identical ecosystem health and welfare assessments at an aquaculture supplier to be compared to wild collection. Strong partnerships with two aquariums and an aquaculture facility have previously been established. Preliminary results show low parasitic loads at wild collection sites and the aquaculture facility (37% and 0.05%). Diversity indexes at aquarium sites ranged from 0.28-1.83 demonstrating potentially stressed aquatic environments. Through this study, we hope to influence use of the developed One Welfare and aquaculture frameworks at additonal aquariums in the future.
Keywords for abstract:	Aquariums Ecosystem Health One Welfare Sustainability

EAR - 4

Title of abstract:	**DEVELOPMENT OF HIGH-FIDELITY NANOPORE SEQUENCING FOR SARS-COV-2 DETECTION AND SURVEILLANCE THROUGH WASTEWATER SAMPLES**
Authors:	S. Golconda, H. Yu, D. Xue, A. Baek, G. Lee, M. Oglesbee, S. Faith and S. Kim. Depts. of Veterinary Biosciences and Microbiology
Abstract:	SARS-CoV-2 shed in the feces of infected individuals has launched wastewater surveillance as a tool to track viral prevalence and evolution. However, challenges include the scarcity of viral RNA and potential mix of SARS-CoV-2 variants. An ideal tool should accurately and sensitively determine variants at the single RNA molecule level and detect unique combinations of variant-specific single nucleotide variants (SNVs). Reverse-transcription (RT) quantitative polymerase chain reaction (PCR) can only provide information on a single signature mutation site. Current short-read SARS-CoV-2 genome sequencing methods, such as the widely used Artic method, also have several technical limitations: (i) the short-read target-specific amplicons of about 400 bases; (ii) the limited read accuracy with Oxford Nanopore sequencing; and (iii) the limited detection sensitivity with random-primer RT and target-specific cDNA amplification. To address these, we are developing a new high-fidelity (HiFi) long-read Nanopore sequencing method to sequence and quantify SARS-CoV-2 variants within wastewater. We use specific RT primers targeting signature SNV sites and a new hairpin ligation method for cDNA PCR. This approach allowed 97% detection of cDNA regardless of length and generated SARS-CoV-2 amplicons up to 6 kb. For HiFi long-read sequencing, we employed rolling circle amplification (RCA) that creates long DNA containing multiple copies of the target. Following Nanopore sequencing, raw subreads within a single RCA read generate consensus sequences with Q30 level (99.9%) accuracy. By developing a new analytic pipeline for SARS-CoV-2 variant calling based on both the global GISAID database for SARS-CoV-2 genome sequences and OSU Infectious Disease Institute's local variant sequences, we could identify circulating variants among wastewater of local communities which were consistent with variants found in patient samples collected from similar areas and times. This study demonstrates a new wastewater pathogen surveillance tool for on-site, high-accuracy detection of viral strains for SARS-CoV-2 and, potentially, other targeted respiratory viruses.
Keywords for abstract:	SARS-CoV-2 Surveillance Oxford Nanopore Wastewater Viral Detection

EAR - 5

Title of abstract:	**BIOFILM FORMING ABILITIES OF *SALMONELLA* SEROVARS ISOLATED FROM CLINICALLY ILL LIVESTOCK AT 48 AND 168 HOURS**
Authors:	S. Locke and G. Habing Department of Veterinary Preventive Medicine
Abstract:	Environmental persistence of microbes can be facilitated by biofilm formation. In Gram-negative pathogens, increased biofilm density has been correlated with decreased biocide efficacy. Although *Salmonella* biofilms have received attention in food processing facilities, little is known regarding the biofilm forming capabilities of a somewhat distinct population of *Salmonellae* responsible for illnesses in livestock and humans. Indeed, routine cleaning and disinfection protocols applied in preharvest environments often fail to eradicate *Salmonella* biofilms. Disrupting the environmental survival of *Salmonella* via biofilm removal, particularly for virulent strains with abundant biofilm growth, will be critical to reduce carriage in livestock reservoirs and the risk of foodborne illness in humans. Therefore, the objective of this study was to characterize the biofilm forming abilities of *Salmonellae* relevant to livestock and human health. Seventy-six isolates from 8 serovars (*S*. 4,[5],12:i:-, Agona, Dublin, Enteriditis, Heidelberg, Montevideo, Newport, Typhimurium) recovered from poultry and clinically ill cattle, swine, and equine were utilized. We hypothesized that biofilm density varies between serovar and increases from 48 to 168 hrs. Isolates were grown in 24-well microplates in tryptone soy broth at ambient temperature. Biofilm density was quantified at each timepoint using crystal violet assays. At 48 hrs., 35.5% (27/76) of isolates were categorized as strong, 42.1% (32/76) moderate, 21.1% (16/76) weak, and 1.3% (1/76) nonadherent biofilm formers. By 168 hrs., 82.3% (63/76) of isolates were considered strong biofilm formers and 17.1% (13/76) were considered moderate. Most weak biofilm formers were *S*. Dublin or Montevideo (15/16). Regardless of serovar, median biofilm density was greater at 168 hrs. relative to 48 hrs. ($p < 0.005$). Results suggest inconsistent (i.e., weekly) cleaning may allow for establishment of mature biofilms in on-farm environments. This study provides baseline data necessary to develop evidence-based cleaning and disinfection protocols effective against the most prolific biofilm forming strains of virulent *Salmonella*.
Keywords for abstract:	Salmonella Biofilms Livestock

EAR - 6

Title of abstract:	**SHORTER SWINE EXHIBITIONS AND DECREASED RISK OF ZOONOTIC INFLUENZA A VIRUS TRANSMISSION**
Authors:	DS McBride1, JM, Nolting1, SW Nelson1, MM Spurck1, NT Bliss1, E Kenah2, SC Trock3, and AS Bowman1 1. Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio, USA 2. Biostatistics Division, College of Public Health, The Ohio State University, Columbus, Ohio, USA. 3. Influenza Division, Centers for Disease Control and Prevention, Atlanta, Georgia, USA
Abstract:	In the United States, diverse lineages of influenza A virus (IAV) are introduced into exhibition swine populations where frequent movement of pigs within networks of shows and fairs facilitates the dissemination and interspecies transmission of IAV. The reduction of zoonotic IAV risk in the US necessitates mitigation of IAV in exhibition swine. Because IAV is likely to be introduced into county fairs via infected, asymptomatic pigs, it is critical to reduce IAV transmission within swine populations at individual fairs. In this study, we evaluate the effectiveness of shortening swine exhibitions to 72-hours or less to reduce zoonotic IAV risk; a measure offered by the Swine Exhibitions Zoonotic Influenza Working Group. We longitudinally sampled every pig daily for the full duration of 16 county fair events during 2014 and 2015 for a total 39,768 nasal wipes from 6,768 individual pigs. Additionally, we estimated IAV prevalence at 195 county fair events during 2018 and 2019 to test the hypothesis that fairs which shortened their swine exhibition to 72-hours or less would have lower IAV prevalence in their swine by the end of the fair. In both longitudinal and cross-sectional studies, we found that shorter duration of swine shows drastically reduces IAV prevalence in exhibition swine at county fairs. Reduction of viral load in the barn within a county fair is critical to reduce the risk of interspecies IAV transmission and ultimately pandemic potential. We find substantial evidence to support the recommendation of limiting swine shows to 72 hours as a measure to reduce zoonotic IAV.
Keywords for abstract:	influenza A virus; swine viral zoonoses prevalence United States

EAR - 7

Title of abstract:	**QUANTIFICATION OF ANTIMICROBIAL USAGE IN DAIRY CATTLE BEFORE, DURING AND AFTER THE IMPLEMENTATION OF FARMWORKERS ANTIMICROBIAL STWARDSHIP TRAINING**
Authors:	R. Portillo-Gonzalez, A. Garzon-Audor, R. V. V. Pereira, G. G. Habing
Abstract:	Antimicrobials are critical to preserve animal health and improve animal welfare. However, their use and abuse represent a public health threat for increasing antimicrobial resistance. Veterinarians prescribed antimicrobials, but farmworkers are responsible for making treatment decisions. Therefore, quantification of antimicrobial usage (AMU) is vital to promote on-farm responsible use. The objective of this study was to evaluate the impact of farmworker antimicrobial stewardship (AMS) training through the quantification of on-farm AMU. We designed a quasi-experimental study with nine conventional dairy farms enrolled in Ohio and Indiana. Six farms received AMS training (training group) and three farms did not (control group). Farmworkers from the training group were invited to participate in a 12-week training program focused on accurate identification of sick cows requiring antimicrobials. We quantified on-farm AMU using the inventory of empty drug containers before, during and after the implementation of the educational training. We located plastic bins per farm at places considered appropriate to collect empty drug containers. Treatment incidence using animal daily-doses (ADD) was calculated based on the active drug ingredient, the on-label recommended dose, and 600 kg of standard-weight per adult cow. Across all farms, a total of 405.7 defined doses (ADD/1000 cow-days) was obtained before, during, and after the intervention. Each farm uses a mean of 15.0 ADD/1000 cow-days with a range of 1.7 to 71.6 ADD/1000 cow-days. A decreased of ADD mean was exhibited for all trained farms from 13.9 pre-intervention to 12.2 ADD/1000 cow-days post-intervention. On the contrary, two of three controls showed an increase in the ADD mean from 7.44 pre-intervention to 9.82 ADD/1000 cow-days post-intervention. Results suggested that AMU among enrolled farms varied significantly and stewardship training influenced AMU on trained farms. Future research on stewardship intervention should include social science approaches to consider ways to overcome barriers and stimulate responsible AMU.
Keywords for abstract:	antimicrobials usage defined daily dose treatment incidence

EAR - 8

Title of abstract:	**MITEBUSTERS: THE SURVIVAL, CONTROL, AND IDENTITY OF RESPIRATORY MITES (*HALARACHNIDAE*) IN SEA OTTER FACILITIES**
Authors:	M. Shields, R. Pesapane. Dept. of Veterinary Preventive Medicine, Ohio State University College of Veterinary Medicine, T. Roth. San Mateo County Mosquito and Vector Control District, and M. Miller. Marine Wildlife Veterinary Care and Research Center, California Department of Fish and Wildlife.
Abstract:	Marine mammals are hosts for several parasitic nasal mites (*Halarachnidae*) which impair respiration by way of mucopurulent exudate, bronchitis, and turbinate lysis. Infestations are common in rehabilitated sea otters (*Enhydra lutris*) and negatively impact survival post-release. Despite disinfection in marine mammal care facilities, infestation is associated with captivity, suggesting that current husbandry practices are inadequate. The risk of environmental transmission and disinfectant efficacy is unknown. To examine whether environmental transmission represents viable risks, and the ability of disinfectants to control mites, this study will analyze mite survival under various environments and disinfectants. Live mites will be exposed to common disinfectants as well as microcosms that mimic the temperature and salinity of enclosures. Mites will be stimulated at regular intervals to evaluate survival and mortality rate survivorship curves will be created. The results will determine whether mites can persist environmentally, potentially infesting naïve animals, as well as the efficacy of disinfectants. Future mite-specific protocols can then be developed to improve animal welfare at marine mammal institutions. Additionally, characterization of mite-host associations has been complicated by repeated revisions to halarachnid mite taxonomy and reclassification of misidentified specimens. Species identification currently requires multiple keys, knowledge of revisions to species classification through time, and training in acarology, which is impractical for marine mammal clinicians. A simple pictorial key to species of halarachnids based on published keys was generated to aid in future identification of mite species.
Keywords for abstract:	*Halarachnidae* *Enhydra lutris* Pictorial key Marine mammal parasites Morphologic identification Parasite control

EAR - 9

Title of abstract:	**DOMESTIC ANIMAL INJURIES NEGATIVELY IMPACT VULNERABLE WILDLIFE SPECIES IN CENTRAL OHIO**
Authors:	C. Souza, M. Markzs, J. Flint
Abstract:	Human-wildlife conflicts have become a daily occurrence in urban areas. Some of these conflicts are linked to high rate of mortality in vulnerable wildlife species. An analysis of the Ohio Wildlife Center's (OWC) admittance data shows an overwhelming number of wildlife presenting to the rehabilitation center with injuries or as orphaned infants due directly to an interaction with a domestic animal, cats and dogs. I reviewed 30,029 representing seventy-six different species admitted to the hospital over a four year period (2016-2020). I identified that domestic animal interactions make up 18% of total wildlife deaths brought to OWC. This conclusion supports the need for further investigation of domestic animal interactions with wildlife to understand and address the need to protect vulnerable wildlife species from domestic animal injuries. This information will aid veterinarians in the effort to both directly protect domestic animals from zoonotic diseases and indirectly protect wildlife populations from increasing rates of mortality due to inappropriate predation.
Keywords for abstract:	Wildlife Domestic Animals Conservation Injuries Mortality

EAR - 10

Title of abstract:	**QUANTIFYING DISEASE TRANSMISSION RISK IN INFANTS USING VIDEO DATA**
Authors:	J. W. Wang, J. Cox, A. Osinuga, H. G. Villasanti, R. Garabed, K. Baker Depts. Of Preventive Medicine, College of Veterinary Medicine, College of Public Health, and Crane Center for Early Childhood Research and Policy, The Ohio State University, Columbus, OH; College of Public Health, University of Iowa, Iowa City, IA
Abstract:	Nowadays, more and more people rely on nonparental care while they're working outside the home. However, children in daycare centers are more likely to get enteric diseases than those who are in home care. Children's unhygienic habits, such as mouthing and touching objects and other people, facilitate the spread of infectious agents. The objective of this study is to quantify enteric disease transmission risks in infants using video data and apply this observation method to the animal population for diseases transmitted through the same route in the future. 18 infants from 8 different rooms at 3 daycare centers were selected to participate. Diarrheal illnesses of infants were reported from parents, facility records, and pathogen detection from fecal samples. However, only 13 infants from 6 rooms were documented on the videos. The caretakers from other two rooms refuse to be filmed on the camera. 3 to 4 on-site cameras were placed in each room to provide structured observations. One-minute videos from the same room with different views were then complied into a ten-minute video for enumerators to view at once. A specialized software, LiveTrack, was used to translate the video into a digital record. After thorough observation of infants' behavior and facility infrastructure, categories of locations, object mouthed, object touched, human interaction and diapering were customized to annotate the videos. Specific protocols were established to reduce inter-observer errors. Annotation testing was used to train enumerators to decrease intra-observer variation, and the output data was then evaluated using Krippendorff's Alpha to measure inter-observer reliability.
Keywords for abstract:	neonatal enteric diseases diseases transmission quantifying video data

EAR - 11

Title of abstract:	**EVALUATION OF WATER-BASED FOAMING AS A MASS DEPOPULATION METHOD FOR SWINE**
Authors:	T.J. Williams[1]; T.-Y. Cheng[1]; M. Campler[1]; S. Moeller[2]; J. Kieffer[2]; A.S. Bowman[1]; A.G. Arruda[1] [1] Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH, 43210 [2] Department of Animal Sciences, College of Food, Agricultural, and Environmental Sciences, The Ohio State University, Columbus, OH, 43210
Abstract:	Swine populations are susceptible to infectious diseases, and urgent responses including large-scale depopulation may be required to control and prevent farm-to-farm transmission during foreign animal disease outbreaks. Several swine depopulation methods suggested by the American Veterinary Medical Association (AVMA) are debatable in their efficiency. In this study, water-based foam (WBF), an AVMA-approved method in poultry, was adapted to swine. Two trials (1 and 2) were conducted for a stepwise evaluation on the use of WBF in swine. Trial 1 assessed time to unconsciousness by submerging 72 feeder pigs (6 pigs each replicate for 12 replicates) in WBF for 6 time periods (2.5-15min post-fill (MPF) in 2.5min intervals). Unconsciousness levels were assessed, and regular gasping and corneal reflex were observed, respectively, in 3 and 12 pigs at 2.5 MPF. Consciousness signs compatible with recoverability were absent at ≥ 5 MPF. In Trial 2, cull sows (3 replicates of 25 sows) were loaded into an adapted rendering dump trailer (40x8.5×6 feet) and submerged in WBF for 5 MPF. Activity bio-loggers were implanted in 6 sows per replicate to measure movements. In Trial 2, the mean durations between end of trailer WBF fill and last animal movement (min:sec) as per bio-loggers were 2:03 (standard deviation [SD]=1:31), 2:00 (SD=1:11), and 1:45 (SD=0:47) for replicates 1, 2, and 3, respectively. All sows were deemed unconscious after unloading from the trailer. Based on our studies, WBF could be an effective method for emergency depopulation in swine. Given our assessment of time to unconsciousness in feeder pigs and the field validation in sows, we recommend that 7.5 minutes of submersion should be used to ensure unconsciousness and subsequent death.
Keywords for abstract:	Swine Depopulation Water-based foam Biosecurity

EAR - 12

Title of abstract:	**RIBOSOMAL PROTEIN S17 IS A HOST DETERMINANT ENHANCING AVIAN HEPATITIS E VIRUS REPLICATION**
Authors:	K.K. Yadav[1], C.M. Lee,.[1], P.A. Boley[1], A. Ghorbani[1], M. Bhandari[1], G. Yadaigiri[1], V. Patil[1], S.P. Kenney[1] [1]Center for Food Animal Health, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster
Abstract:	Hepatitis E virus (HEV), the causative agent of hepatitis E, is an important zoonotic human pathogen. Avian HEV is an important pathogen within the poultry industry and, although it does not infect humans, is used to model human disease. Lack of an efficient cell culture system complicates study of avian HEV. Quasispecies of human HEV can possess natural insertions of ribosomal protein (RP) S17 that bestows growth advantage and expands their host tropism in vitro. RPS17 is part of the 40S ribosomal subunit and is involved in eIF-2 binding. The objective of the study was to construct an avian HEV with the RPS17 insertion to further characterize its replication and pathogenesis. Avian HEV with the RPS17 insertion in the hypervariable region of the non-structural protein was constructed. Capped RNA transcripts of RPS17 were replication-competent after transfection of LMH chicken liver cells. Chickens inoculated intrahepatically with RNA transcripts of avian HEV RPS17 developed active infection as evidenced by fecal virus shedding and viremia. To characterize the pathogenicity, inoculum (10^4 GE/0.5 ml) was prepared from bile, liver homogenate, ileal and cecal contents and given orally to birds. Cloacal swabs and body weights were collected every 3 days. Blood was collected every 7 days. Several birds were necropsied every 8^{th} day until day 40; bile was collected, and organ mass index of liver and spleen were determined. Birds were examined for gross and microscopic liver lesions. RNA was extracted from cloacal swabs, serum, liver, spleen, pancreas, jejunum, bile samples and quantified by RT-qPCR. In conclusion, RPS17 insertion into avian HEV was not only replication competent but enhanced its replication ability in chicken liver cells as well as in SPF chickens when compared to wild type virus by increased capsid protein detected by flow cytometry, indirect immunofluorescence assay and genomes quantified via RTq-PCR.
Keywords for abstract:	Avian hepatitis E RNA Liver replication

EAR - 13

Title of abstract:	**ANALYSIS OF THE EFFECTS OF ANTHROPOGENIC INFLUENCES ON ECOSYSTEM HEALTH IN SOUTHWESTERN LAKE ERIE, USA**
Authors:	E. Vincent, J. Flint, A. Bowman, M. Flint, Dept of Veterinary Preventive Medicine
Abstract:	Multiple areas of Lake Erie are regularly dredged to maintain navigation channel depths needed for safe commercial and recreational boating. Dredged material is dumped into in-water or upland “confined disposal facilities” (CDFs), which can include the creation of dredge spoil islands. Although islands created using dredged materials can provide valuable habitats for wildlife, there remain concerns about the release of industrial contaminants and other pollutants from this material that could have long-term health implications in wildlife species. Our project uses a One Health approach to evaluate the impacts of dredge islands on ecosystem health in southwestern Lake Erie at two sites: a preserved coastal wetland and a CDF. Fish species will be captured to assess biodiversity, parasite burden, immune function, and overall health of fish populations. During a pilot study in November 2021, 2572 fish representing 13 species were captured in a single night. Gizzard shad (*Dorosoma cepedianum*) were the most abundant fish captured (95%), followed by bluegill (*Lepomis macrochirus*, 3%), white crappie (*Pomoxis annularis*, 1%), and black crappie (*Pomoxis nigromaculatus*,1%) No external parasites were noted on skin, fin, or gill cytology. Painted turtles (*Chrysemys picta*) will be captured at each site for physical exams and morphometric data. Turtles will be tested for infectious diseases (Chlamydia, herpesvirus, Ranavirus/FV3-like virus, Mycoplasma, and adenovirus). Blood from painted turtles will be used for estimated white blood cell counts, differentials, lactate levels, and plasma protein electrophoresis testing. In addition, samples from mammal species including muskrats (*Ondatra zibethicus*), American beavers (*Castor candensis*), and American mink (*Neogale vison*) will be collected. Serum, lung tissue, and mucosal swabs from mammals will be analyzed for SARS-CoV-2 PCR and sequencing. Mammals will also be tested for Chlamydia with conjunctival and nasal/oral swabs. Data will be analyzed and used to assess differences in overall ecosystem health between these two sites.
Keywords for abstract:	Ecosystem health Coastal wetland Dredging Wildlife health

IMMUNOLOGY
AND
INFECTIOUS DISEASES

IMID - 1

Title of abstract:	**DEVELOPING AND OPTIMIZING A FLOW CYTOMETRY-BASED ASSAY FOR FELINE INFECTIOUS PERITONITIS VIRUS**
Authors:	B.A. Allen, S.J.M. Evans Department of Veterinary Biosciences
Abstract:	Feline Infectious Peritonitis (FIP) is a deadly viral disease affecting cats that are carriers of the almost ubiquitous feline enteric coronavirus (FECV). Infection with FECV is mostly asymptomatic and transient, but it can mutate into FIP virus (FIPV) causing non-specific, and ultimately fatal clinical signs. Diagnostic testing for FIP is often ambiguous and usually presumptive, with most available tests unable to distinguish between the two different pathotypes (FECV vs. FIPV). A definitive diagnosis is usually made by biopsy and examination of tissue post-mortem. The aim of this study is to develop and optimize a flow cytometric assay for the detection of FIPV within feline macrophages. Felis catus whole fetus (fcwf) cells are grown in standard media. Cells are infected with FIPV, along with sham-infected controls. Then, cells are harvested, fixed, permeabilized, and stained with anti-vimentin and anti-feline coronavirus antibodies and analyzed by flow cytometry. Preliminary results show that flow cytometry detects stained FIPV within fcwf cells, and eventually, we expect this within fluid macrophages from feline patients suspected of having FIP. We anticipate that our flow cytometry-based assay for FIP will be more diagnostically reliable (higher specificity) than currently available assays. A definitive antemortem diagnosis is crucial to veterinarians and clients when it comes to making treatment or euthanasia decisions surrounding FIP. The next steps for this study include testing fluid samples from suspected FIP patients in a clinical diagnostic trial.
Keywords for abstract:	cats coronavirus fip flow cytometry

IMID - 2

Title of abstract:	**SARS-COV-2 PREFUSION SPIKE PROTEIN STABILIZED BY SIX RATHER THAN TWO PROLINES IS MORE POTENT FOR INDUCING ANTIBODIES THAT NEUTRALIZE VIRAL VARIANTS OF CONCERN**
Authors:	Michelle Chamblee1, Mijia Lu1, Yuexiu Zhang1, Chengjin Ye5, Piyush Dravid2, Jun-Gyu Park5, Mahesh KC2, Sheetal Trivedi2, Satyapramod Murthy2, Himanshu Sharma2, Cole Cassady2, Supranee Chaiwatpongsakorn2, Xueya Liang1, Jacob S. Yount3, Prosper N Boyaka1,7, Mark E. Peeples2, 6, 8, Luis Martinez-Sobrido5, Amit Kapoor2,6,8, Jianrong Li1,8* 1Department of Veterinary Biosciences, The Ohio State University, Columbus, OH, USA 2Center for Vaccines and Immunity, Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH, USA 3Department of Microbial Infection and Immunity, College of Medicine, The Ohio State University, Columbus, OH, USA 4Department of Surgery, College of Medicine, The Ohio State University, Columbus, OH 5Texas Biomedical Research Institute, San Antonio, TX, USA 6Department of Pediatrics, College of Medicine, The Ohio State University, Columbus, OH, USA 7Center for Retrovirus Research, The Ohio State University, Columbus, OH, USA 8Infectious Disease Institute, The Ohio State University, Columbus, OH, USA
Abstract:	The spike (S) protein of coronavirus is the main target for neutralizing antibodies (NAbs) and therefore the best target for vaccine development. The S protein trimer is anchored in the virion membrane in its prefusion (preS) but metastable form. The preS protein was previously stabilized by strategically introducing two or six proline substitutions, to generate stabilized, soluble 2P or HexaPro (6P) preS proteins. HexaPro has been shown to be more stable than 2P, but it is not known which form is the most immunogenic. Here, we generated recombinant vesicular stomatitis virus (rVSV) expressing preS-2P, preS-HexaPro, and native full-length S, and compared their immunogenicity in mice and hamsters. The rVSV-preS-HexaPro produced and secreted significantly more preS protein compared to rVSV-preS-2P. Immunization of mice and hamsters with a single dose of each recombinant virus was sufficient to induce high levels of SARS-CoV-2 preS-specific antibodies. Importantly, rVSV-preS-HexaPro triggered significantly more preS-specific serum IgG antibody than rVSV-preS-2P. Antibodies induced by preS-HexaPro neutralized the B.1.1.7, B.1.351, P.1, and B.1.617.2 variants approximately 2-4 times better than those induced by preS-2P. Furthermore, preS-HexaPro induced a more robust Th1-biased cellular immune response than preS-2P. A single, low dose (10^4 PFU) immunization with rVSV-preS-HexaPro and rVSV-preS-2P provided complete protection against challenge with mouse-adapted SARS-CoV-2 and B.1.617.2 delta variant, whereas rVSV-S only conferred partial protection. When the immunization dose was lowered to 10^3 PFU, rVSV-preS-HexaPro induced substantial protection whereas rVSV-preS-2P was not protective. Collectively, our data demonstrate that of the three proteins tested, preS-HexaPro induces the most effective immune response, highlighting the advantages of using preS-HexaPro in the next generation of SARS-CoV-2 vaccines.
Keywords for abstract:	SARS-CoV-2, prefusion spike, variant of concern, immunogenicity

IMID - 3

Title of abstract:	**PATHOGENESIS OF TWO NEWLY DISCOVERED VIRULENCE FACTORS OF *EHRLICHIA JAPONICA*, A MODEL BACTERIUM FOR STUDYING FATAL HUMAN MONOCYTIC EHRLICHIOSIS**
Authors:	R.C. Chien, M. Lin, Y. Rikihisa. Department of Veterinary Biosciences
Abstract:	Human monocytic ehrlichiosis (HME) is an emerging, tick-borne febrile illness that potentially causes life-threatening infections. The causative pathogen, *Ehrlichia chaffeensis*, is a unique, obligate intracellular bacterium, primarily targets monocytes and macrophages. The *in vivo* pathogenesis of severe or fatal HME is not completely understood. Our laboratory developed a fatal HME mouse model by culture isolating *Ehrlichia japonica*, a bacterium closely related to *E. chaffeensis*, which causes severe diseases and death in immunocompetent mice due to cytokine storm and toxic shock-like syndrome. By using Himar1 transposon random mutagenesis system, we recently identified two novel virulence factors of *E. japonica* (EHF_0962 and EHF_RS04100) in the mutants H59 and H43B, respectively. Although these virulence factors are dispensable *in vitro* for infecting macrophage (DH82), endothelial (RF/6A), and tick (ISE6) cell lines, the H59 and H43B mutants cannot rapidly replicate and spread systemically to kill immunocompetent mice. My hypothesis is that the mutants lost ability to overcome *in vivo* innate immune response such as the reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated by phagocytes. To investigate the virulence loss of the mutants, we will establish a time-coursed infection in the mouse model and design specific experiments for testing host innate immune responses. Furthermore, we will generate rescue plasmids to restore the virulence gene in the mutants for the complementation assays. Successful completion of these aims will provide critical knowledge of treating and preventing severe HME.
Keywords for abstract:	*Ehrlichia chaffeensis* Human monocytic ehrlichiosis *Ehrlichia japonica* Mouse model Virulence factors EHF_0962 EHF_RS04100 Monocyte/macrophage

IMID - 4

Title of abstract:	**INVESTIGATING BOVINE ANAPLASMOSIS VIA ANALYSIS OF TICKS FROM OHIO LIVESTOCK**
Authors:	D. L. Cole, J. Kieffer,J. LaKritz, R.R. Pesapane Department of Veterinary Preventative Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio. School of Environment and Natural Resources, College of Food, Agricultural, and Environmental Sciences, The Ohio State University, Columbus, Ohio
Abstract:	Currently no livestock-specific tick studies in Ohio are published in the literature, despite recurring outbreaks of bovine anaplasmosis in the state and the presence of five tick species of veterinary concern. The presence of bovine anaplasmosis in the United States is attributed to an obligate intra-erythrocytic rickettsial bacteria, *Anaplasma marginale*, which can be transmitted in three ways, including: mechanically via biting insects or blood-contaminated instruments (i.e fomites), biologically via ticks, and vertically from mother to calf. Bovine anaplasmosis manifests in cattle as reduced milk production, fever, weight loss, abortion, and many times, death. While there are numerous genotypes of *A. marginale* present in the U.S, it is not currently known which genotypes are circulating in Ohio. In addition to this, Ohio is facing invasion by an exotic tick which targets livestock—the Asian longhorned tick (*Haemaphysalis longicornis)*. Asian longhorned ticks can also transmit Theileria orientalis which can cause bovine theileriosis. The aim of this investigation is to determine if the ticks which infect Ohio livestock carry the pathogens *Anaplasma marginale* or *Theileria orientalis*, and to begin to characterize the species and genotypes of the pathogens present in cattle herds.
Keywords for abstract:	Bovine anaplasmosis ticks livestock

IMID - 5

Title of abstract:	**NEUTRALIZING ANTIBODY RESPONSES ELICITED BY SARS-COV-2 mRNA VACCINATION WANE OVER TIME AND ARE BOOSTED BY BREAKTHROUGH INFECTION**
Authors:	J.P. Evans, C. Zeng, C. Carlin, J.S. Bednash, R. Mallampalli, G. Lozanski, L.J. Saif, E.M. Oltz, R.J. Gumina, and S.L. Liu Depts. Of Veterinary Biosciences, Internal Medicine, Pathology, Animal Sciences, Veterinary Preventive Medicine, and Microbial Infection and Immunity
Abstract:	SARS-CoV-2 is the causative agent of the ongoing COVID-19 pandemic which has resulted in nearly 400 million cases and over 5 million deaths. Unprecedented efforts from the scientific community led to the development of two mRNA vaccines, among others, that have helped to control the worst impacts of the pandemic. However, the durability of protection for these vaccines and how they compare to immunity from natural infection, remain critical concerns. Additionally, the emergence of SARS-CoV-2 variants of concern (VOCs) which exhibit resistance to vaccine-induced neutralizing antibodies (nAbs), continue to threaten the efficacy of the mRNA vaccines. To address these concerns we developed a highly sensitive pseudotyped lentivirus-base virus neutralization assay to examine nAb titers in COVID-19 patients and mRNA vaccine recipients. With this we demonstrate that mRNA vaccinated health care workers (HCWs) reliably exhibit strong nAb response. However, the VOCs exhibit varying degrees of nAb escape with the Omicron variant exhibiting near complete escape from 2-dose mRNA vaccine-induced nAbs, and 2-dose induced nAbs wane substantially 6 months after vaccination against all VOCs. Following booster vaccination 83.3% of HCWs exhibited detectable nAb titers against Omicron, indicating a need for booster vaccine administration for strong protection from Omicron. Additional examination of sera from Delta and Omicron COVID-19 patients demonstrated strong resistance of Omicron to Delta patient sera but sensitivity to Omicron patient sera. Omicron variant sub-lineages were also examined to discern any differences in sensitivity to mRNA-vaccine- or Omicron-infection-induced nAbs. Overall, our results serve to inform the administration of booster doses and any need for a reformulation of mRNA vaccines.
Keywords for abstract:	SARS-CoV-2 COVID-19 Omicron mRNA Vaccine Neutralizing Antibody

IMID - 6

Title of abstract:	**INVESTIGATING THE MECHANISM OF LOW INFECTIVITY OF SARS-COV-2 IN MINK**
Authors:	J. Faraone, J. Evans, K. Xu, S.L. Liu
Abstract:	In April of 2020, a variant of SARS-CoV-2 resulting from a mink to human transmission emerged on a mink farm in the Netherlands, marking the first known animal to human transmission event since the start of the COVID-19 pandemic. This mink cluster five variant (MC5V) carries five mutations in the spike protein including the ancestral D614G mutation, the characteristic mink-adapted receptor binding domain (RBD) mutation Y453F, deletion of H69-V70 in the N-terminal domain (NTD), I692V in S2, and M1229I in the transmembrane domain. MC5V has been reported to have reduced pathogenicity in humans compared to the ancestral Wuhan strain, but the underlying mechanism is unclear. Here we conduct a mutational analysis of ancestral and MC5V spike proteins to characterize the impacts of these key residues. We identify the I692V mutation, which is located immediately after the furin cleavage site in the MC5V spike protein, as the key determinant of the low infectivity of MC5V. Specifically, we observed that the introduction of V692 reduces D614G spike furin processing, reduces spike mediated cell-cell fusion, and diminishes pseudotyped lentivirus infectivity. Critically, we confirm that introducing the I692 reversion partially restored MC5V spike processing and fusogenicity, and increased pseudotyped virus infectivity to levels comparable with D614G. Structural modeling of the SARS-CoV-2 spike containing I692V, which is located immediately after the furin cleavage site in the spike protein, revealed that the mutation disrupts local hydrophobic interactions, likely affecting the conformation of the cleavage site and/or overall spike trimer stability. Therefore, results of this study suggest a critical role for the I692V mutation for impacting furin cleavage site accessibility, emphasizing the importance of non-RBD spike mutations in modulating viral infectivity, transmission, and pathogenesis of SARS-CoV-2.
Keywords for abstract:	SARS-CoV-2 COVID19 MC5V Zoonotic Mink

IMID - 7

Title of abstract:	**IMMUNE PREDICTORS OF ENHANCED CARDIOMETABOLIC RISK IN COVID-19 INDIVIDUALS WITH TYPE 2 DIABETES (T2DM)**
Authors:	Manuja Gunasena1,2, Yasasvi Wijewantha1, Emily Bowman3, Janelle Gabriel3 , Amrendra Kumar3, Aaren Kettelhut3, Krishanthi Weragalaarachchi3, Dhanuja Kasturiratna4, Anna Vilgelm3, Joseph Bednash3, Sonal Pannu3 , Thorsten Demberg5, Nicholas Funderburg3, and Namal Liyanage1 1 Department of Microbial Infection and Immunity, The Ohio state university, 2 Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, 3 The Ohio State University, College of Medicine, 4 Northern Kentucky University, 5 Marker Therapeutics Inc., Houston, TX, United States,
Abstract:	COVID-19, a disease produced by the SARS-CoV-2 virus, has triggered a global p ublic health crisis. SARS-CoV-2 infected patients show a wide range of clinical symptoms, from asymptomatic to severe sequelae and death. People of any age with a history of serious past chronic health problems are more vulnerable to COVID-19, possibly due to a weakened immune system. Such comorbidities include heart and brain problems, kidney impairment, cancer, immunological disorders, obesity and diabetes. The relationship between COVID-19 and diabetes (DM) is complicated and bidirectional. Studies have shown an increased risk of a cardiovascular disease (CVD) in COVID-19 patients with preexisting diabetes. However,the exact mechanism of increased cardiovascular risk in pre-DM, severe COVID-19 patients still unknown. Thus, in this study, we investigated possible immunological markers that predict cardiovascular risk in pre-DM compared to non-DM severe COVID-19 patients. We used fresh whole blood to carry out high dimensional flowcytomtery and plasma biomarker assays.Multi variant analysis was conducted on Pre-T2DM (n=10) and non-DM (n=7) severe COVID-19 patients admitted to the intensive care unit at OSU medical center. We investigated known cardiovascular immune biomarkers and potential new biomarkers that predict increased risk of CVD. Parametric (ANOVA) and non-parametric (Kruskal–Wallis) statistical tests, as well as Dunn's and Tukey's post hoc tests, were used to evaluate multiple group comparisons. We found, significantly higher Neutrophils, Intermediate monocytes and activated NKT-like cells in pre T2DM severe COVID-19 patients. Contrary, NKG2A+CD56DimCD16+ NK cells and activated T cells were significantly lower in the pre-T2DM group. In the severe COVID-19 T2DM patients, we identified higher levels of LBP (Lipopolysaccharide binding protein), FABP4 (Fatty acid-binding protein 4) and sCD14 compared to non-diabetic patients. These data suggest that a dysregulated immune response can exacerbate the viral immune response and CVD risk in pre- DM severe-COVID-19 patients.
Keywords for abstract:	Covid-19 Diabetes Cardiovascular risk Flowcytometry

IMID - 8

Title of abstract:	**LOSS OF PANETH CELLS ALTERS INTESTINAL INNATE LYMPHOID CELLS AND ENHANCES WEIGHT GAIN IN MICE.**
Authors:	M.R. Joldrichsen, E. Kim, H. Steiner, E. Cormet-Boyaka, and P.N. Boyaka. Department of Veterinary Biosciences
Abstract:	Paneth cells are a subset of small intestinal epithelial cells specialized in the production of antimicrobial products and cytokines. Gastrointestinal dysbiosis has been linked to many health concerns and is believed to be a contributing factor in obesity. Despite the role of Paneth cells as a major source of antimicrobial products, the contribution of these cells to health or disease conditions has not been fully explored. We examine the impact loss of Paneth cells would have on the gut microbiota and whether the subsequent dysbiosis could affect metabolic functions of the host and fat accumulation in a mouse model of diet induced obesity. For this purpose, were used wild-type and $Sox9^{\Delta IEC}$ mice, which lack Paneth cells due to a Sox9 gene deletion within the intestinal epithelium. Compared to control wild-type mice, the small intestine of $Sox9^{\Delta IEC}$ exhibited altered profile of ILCs characterized by increase ILC2 and decrease ILC3 numbers. After exposure to a high-fat diet for 13 weeks, the $Sox9^{\Delta IEC}$ mice gained more weight and had higher glucose intolerance. The intestinal homeostasis was also affected, with an increase in intestinal permeability and an increase in ILC1s within the small intestine of $Sox9^{\Delta IEC}$ mice. In the abdominal fat $Sox9^{\Delta IEC}$ mice showed increased numbers of immune cells including inflammatory macrophages, T cells, and B cells. Finally, fecal material transplantation experiments revealed that fecal material from the $Sox9^{\Delta IEC}$ mice transfer the phenotype in recipient germ-free mice as indicated by a trend towards an increase in weight gain and immune cell infiltration of abdominal fat. These results highlight the importance of Paneth cells as regulators of metabolic functions of the host and fat accumulation.
Keywords for abstract:	Obesity Gastrointestinal microbiota Innate lymphoid cells Non-infectious inflammation

IMID - 9

Title of abstract:	**INFLAMMATORY MARKERS OF RESPIRATORY SYNCYTIAL VIRUS INFECTION IN THE COTTON RAT**
Authors	D. Kang, O. Harder, and S. Niewiesk. Depts. Of Veterinary Bioscience
Abstract	Respiratory syncytial virus (RSV) is a common cause of upper and lower respiratory tract infection. We wished to investigate whether RSV infection induces inflammatory proteins as e.g., infection with SARS-COV-2. The inflammatory proteins tested include C reactive protein (CRP), high mobility group box 1 (HMGB1), and prostaglandin D2 (PGD_2). We investigated in the cotton rat (*Sigmodon hispidus*) which inflammatory protein correlated with RSV infection. CRP is often used as a marker of inflammation during bacterial infection but is typically not highly elevated during viral infection. During RSV infection in adult cotton rats, the level of CRP slightly increased during virus replication. The inflammatory protein HMGB1 triggers TLR4 signaling, which induces COX-2 and subsequently PGD_2 expression. Increased PGD_2 production can negatively affect the migration of dendritic cells and the activation of T cells during viral infection. Our results demonstrated that the level of HMGB1 increased during viral growth, peaked during viral clearance, and remained stable for 4 days after complete clearance of virus. For PGD_2, there was an initially peak on day 1 after infection, then a secondary, larger peak during viral clearance. Therefore, in adult cotton rats, CRP may be used as a control for inflammatory protein during infection. In contrast, the increased expression of both HMGB1 and PGD_2 and their role in the pathogenesis of RSV infection need to be investigated further.
Keywords for abstract:	Respiratory syncytial virus Cotton rat (*Sigmodon hispidus*) C reactive protein High mobility group box 1 Prostaglandin D2

IMID - 10

Title of abstract:	**EVALUATION OF A SARS-COV-2 INTRANASAL NANOPARTICLE VACCINE IN FERRETS**
Authors:	CM. Lee[1], PA. Boley[1], KK. Yadav[1], J. Schrock[1], V. Patil[1], R. Gourapura[1], SP. Kenney[1] [1]Center for Food Animal Health, Department of Veterinary Preventative Medicine, Department of Animal Sciences, The Ohio State University, Wooster, OH.
Abstract:	The spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel Betacoronavirus, has resulted in extensive social and economic impacts worldwide. Preclinical studies in suitable animal models are used to study infection mechanisms and in the development of therapeutics against this rapidly mutating virus. Intranasal nanoparticle vaccines have been shown to induce long-lasting mucosal and systemic immune responses. Thus, an intranasal delivered SARS-CoV-2 vaccine will be a noninvasive and promising prophylactic candidate against circulating and emerging viral mutants. Here, we report the effectiveness of a candidate subunit SARS-CoV-2 vaccine in ferrets (*Mustela putorius furo*), an established model of SARS-CoV-2 infection. One-year-old male ferrets (n=6 per group) were inoculated intranasally twice in a 3-week interval with lipid nanoparticle entrapped receptor binding domain (RBD) of the spike protein and nucleocapsid protein (N) of SARS-CoV-2 along with an adjuvant monosodium urate crystal (MSU), and then challenged intranasally with SARS-CoV-2 (1 x10^6 $TCID_{50}$/ml). The control groups were vaccinated with adjuvant or empty liposomes, respectively. Ferrets were sampled at several timepoints throughout the study to examine changes in viral RNA, infectious viral titers, and immune gene expression in the respiratory tract. Three animals in each group were euthanized for necropsy at 7- and 14-days post challenge. We found infectious live virus levels significantly reduced in the lungs, trachea, nasal turbinate, nasal cavity, and oro-pharynx of vaccinated ferrets. Furthermore, RNA viral load was markedly reduced in bronchioalveolar lavage and lung and tracheal tissues of the vaccinates. Immune gene expression profiling in lungs and nasal turbinate of the vaccinates revealed elevated levels of key cytokines and chemokines such as IFNα, IL-17, granzyme A, and MCP1 compared to mock and vaccine controls. These promising results necessitate ongoing development of this intranasal vaccine candidate and provide support for its potential application in humans.
Keywords for abstract:	SARS-CoV-2 Nanoparticle Intranasal vaccine Ferret

IMID - 11

Title of abstract:	**VIRAL EPITRANSCRIPTOMIC DIVERSITY OF THE HIV-1 RNA GENOME AND ITS DYNAMICS IN ADAPTIVE EVOLUTION**
Authors:	G. Lee, A. Baek, S. Golconda, C. Kimmel, D. Xue, and S. Kim. Depts. Of Veterinary Biosciences
Abstract:	Viruses utilize epitranscriptomic regulation as a survival strategy to enhance viral replication and evade host innate immune responses. Here, we introduce a novel concept of viral epitranscriptomics that addresses epitranscriptomic diversity and its dynamic changes as a viral survival strategy. Our group has adopted long-read direct RNA sequencing and bioinformatic tools to analyze the viral epitranscriptomic landscape of full-length HIV-1 RNA and we identified three dominant N6-methyladenosine (m^6A) modifications in the HIV-1 genome. Point-mutations targeting each of the three dominant m^6A significantly reduced corresponding methylation signals, but total viral production and infection rates remained similar to those of wild-type viruses. From single virus level analysis, we found several subgroups of viruses with unique m^6A modification patterns. When any of the dominant m^6A was removed by mutation, the composition of these subgroups significantly changed, suggesting the expansion of more resilient subgroups. In order to further evaluate the compensatory effects and accurately quantify the subgroups, we trained a machine learning model that determines m^6A on a per-read, per-position basis. Combined machine learning and clustering analysis showed that the new compensatory subgroups increased by 1.3-3.3 fold, compared to the wild-type, while the other subgroups without such compensatory effects decreased by 0.34-0.75 fold. Interestingly, a combination of mutations that removed all three major m^6A significantly reduced total viral RNA production, and showed only modest expansions of the compensatory subgroups, indicating the crucial importance of these dominant m^6A and the limitation of compensatory behaviors when all three are missing. Taken together, HIV-1 RNA modification generates various epitranscriptomically distinctive subgroups even among genetically identical viruses, and dynamic changes in the subgroup composition can compensate for major RNA modification deficiencies. This study will provide insights into the viral epitranscriptomic diversity of HIV-1 populations and its dynamics that maximize viral adaptability and survival in diverse host environments.
Keywords for abstract:	Human immunodeficiency virus Viral epitranscriptome RNA modifications Viral survival strategy Direct RNA sequencing

IMID - 12

Title of abstract:	**NOVEL DIAGNOSTICS FOR SEPTIC EFFUSIONS: OPTIMIZING FOUNTAIN FLOW CYTOMETRY AND TESTING THE RAPIDBAC VET IMMUNOASSAY**
Authors:	E. Li, D. Diaz-Vergara, and S. Evans. Depts of Biosciences and Veterinary Clinical Sciences.
Abstract:	Bacterial sepsis is a life-threatening condition with a high case-fatality rate in which rapid diagnosis is critical for survival. The current primary diagnostic tools for identifying septic cavity effusions are bacterial culture, which has slow turnaround time, and fluid cytology, which suffers from low sensitivity. Fountain FlowTM Cytometry (FFC) is a recently developed, compact tool designed to enumerate microbes at very low concentrations in municipal and commercial fluids. A preliminary FFC diagnostic trial conducted by our laboratory on cavity fluids from 60 septic mammalian patients demonstrated a sensitivity and specificity of 60% and 94.4%, respectively. The RapidBacTM Vet (RBV) test is an immunoassay designed for point-of-care detection of bacteria in small animal urine. Both FFC and the RBV test are inexpensive, quick (requiring less than 20 minutes), and relatively simplistic to perform. The aim of this study was to both optimize the use of FFC and to assess the capabilities of the RBV test, for detection of septic effusions. Sterile cavity effusions from mammalian patients were spiked with known quantities of bacteria and then analyzed by FFC. Significant losses of bacteria were observed (32.4%-97.7%) and determined to be due to sample preparation methods. This finding potentially accounts for the previously observed low sensitivity. A case control study (n=18) of the RBV test using 1-2 day-old, refrigerated mammalian cavity effusions yielded a sensitivity and specificity of 100% and 50%, respectively, indicating false positives may be problematic for RBV use to detect septic effusions, and that a more controlled study involving fresh samples is warranted. FFC offers multiple advantages over currently used methods of septic effusion detection and thus warrants further testing.
Keywords for abstract:	sepsis flow cytometry diagnostic tool clinical pathology septic effusions RapidBac Vet Fountain Flow Cytometry

IMID - 13

Title of abstract:	**INTERCALATED CELLS ACTIVATE INNATE IMMUNE DEFENSES IN RESPONSE TO UROPATHOGENIC ESCHERICHIA COLI**
Authors:	S.C. Linn[1,2], L. Schwartz[2], J.D. Spencer[2,3] 1. The Ohio State University, Department of Veterinary Biosciences, Columbus, OH, USA 2. Nationwide Children's Hospital, The Kidney and Urinary Tract Center at The Abigail Wexner Research Institute, Columbus, OH, USA 3. The Ohio State University, Department of Pediatrics, Columbus, OH, USA
Abstract:	Background: Urinary tract infections (UTIs) are common across species. Intercalated cells (ICs), positioned in the kidney collecting duct, combat UTIs by secreting antimicrobial peptides (AMPs) into the urine to kill bacteria. The mechanisms regulating IC AMP production during UTI are unclear. Here, we challenged ICs in vitro with uropathogenic E. coli (UPEC) or bacterial cell membrane components to define the innate immune responses that control AMP production during UTI. Methods: ICs were infected with UPEC or challenged with UPEC cell membrane components including lipopolysaccharide (LPS), muramyl dipeptide (MDP) and γ-D-Glu-mDAP (iE-DAP). Following stimulation, IC lysates were collected, and immune genes were profiled using an antimicrobial response PCR array or targeted qRT-PCR. Western blot was performed to confirm which immune pathways were activated. Results: In response to UPEC, ICs temporally activate immunomodulatory pathways and AMPs. Analysis of PCR array data via STRING and Ingenuity Pathway Analysis identified 15 upregulated genes associated with Toll-like receptor (TLR), NOD-like receptor (NLR), and NF-kB signaling 4 hours post infection. Immunoblotting confirmed downstream targets in these pathways are activated in response to UPEC. qRT-PCR identified that AMPs like Lcn2 are activated while others are suppressed. Upon stimulation with MDP and LPS, qRT-PCR showed upregulation of multiple AMPs – suggesting that NOD2 and TLR4 activation, respectively, may regulate expression. Following stimulation MDP, ICs demonstrate activation of NF-kB and MAPK signaling pathways via immunoblot, correlating to increased AMP expression which is inhibited using drug antagonists. iE-DAP did not affect AMP expression. Conclusion: During UPEC infection, TLR, NLR, and NF-kB responses are activated in ICs. Activation of TLR and NLR signaling may induce downstream targets like AMPs. Confirmation studies are needed to determine how these pathways regulate AMP expression and identify potential regulatory nodes which could serve as future targets to increase AMP production as an additional means to treat UTIs.
Keywords for abstract:	Urinary tract infection Kidney Uropathogenic Escherichia coli Antimicrobial peptides Innate Immunity

IMID - 14

Title of abstract:	***EHRLICHIA* SP. HF OUTER MEMBRANE PROTEINS AS VACCINE CANDIDATES IN A FATAL HUMAN MONOCYTIC EHRLICHIOSIS MOUSE MODEL**
Authors:	M. Mestres-Villanueva, K. Budachetri, T. Zhang, W. Zhang, M. Lin, and Y. Rikihisa. Dept. of Veterinary Biosciences
Abstract:	Ehrlichia chaffeensis is an obligatory intracellular bacterium that causes the emerging tick-borne disease, human monocytic ehrlichiosis (HME), a severe, influenza-like illness. The only treatment for HME is the broad-spectrum antibiotic Doxycycline, and there is no vaccine available. Ehrlichia spp. lack conventional pathogen-associated molecular patterns, but surface proteins and virulence factors have been identified and their roles in transmission and disease have been studied. Entry-triggering protein of Ehrlichia (EtpE) is an invasin required for bacterial entry into human cells; the C-terminal (EtpE-C) binds DNase-X to induce receptor-mediated endocytosis. Outer membrane proteins OMP-1B and P28 are immunodominant major surface proteins and porins expressed by bacteria in ticks and mammals, respectively. Ehrlichia spp. possess Type IV secretion system (T4SS) and VirB2 proteins function as major pilus subunits, thus playing an important role in binding of the host cell membrane and secretion of T4SS effectors into the host cell cytoplasm. The objective of this study is to determine the vaccine potential of four Ehrlichia surface proteins in the mouse model of acute fatal HME, using Ehrlichia sp. HF (EHF). The genes encoding each protein were cloned from the EHF genome into a protein expression vector, followed by expression and purification of the recombinant proteins. Five groups of immunocompetent mice were immunized with rEtpE-C, rOMP-1B, rP28, rVirB2-4 incorporated into immune-stimulating complex (ISCOM) adjuvant, or sham-immunized with ISCOM alone and will be challenged with EHF in tick cells and infected ticks. At euthanasia, blood, spleen, and liver will be collected for analysis of bacterial load and cytokine expression levels via qPCR and RT-qPCR, respectively. The immune cell population in spleen will be analyzed by flow cytometry. This project will lead to a more thorough understanding of the pathogenicity and immunological response in fatal ehrlichiosis patients and on the potential of these antigens for vaccination strategies.
Keywords for abstract:	Ehrlichia Obligatory intracellular bacteria Immunization ISCOM

IMID - 15

Title of abstract:	**PROSTAGLANDIN D_2 CONTRIBUTES TO DELAYED RESPIRATORY SYNCYTIAL VIRAL CLEARANCE IN GERIATRIC COTTON RATS**
Authors:	J. Miller[1], O. Harder[2], S. Niewiesk[1] [1]Department of Veterinary Biosciences [2]Comprehensive Cancer Center
Abstract:	Respiratory syncytial virus (RSV) is a leading cause of respiratory illness worldwide, and poses a serious health risk for the elderly. Among adults >65 years of age, RSV clearance is delayed, contributing to more severe clinical presentation and increased mortality. In the cotton rat model, RSV clearance is similarly delayed in geriatric animals. The delay in clearance is eliminated in geriatric cotton rats treated with the cyclooxygenase (COX) inhibitor ibuprofen, indicating that age-associated inflammation (inflammaging) contributes to delayed RSV clearance. In this study, we further characterized this inflammatory pathway. Prostaglandin D_2 (PGD_2), a product of the arachidonic acid cascade requiring COX activity, was elevated in airways of geriatric cotton rats infected with RSV compared to adults. Administration of PGD_2 to adults induced delayed RSV clearance kinetics similar to that seen in geriatric animals. Induction of low level inflammation through lipopolysaccharide (LPS) also delayed viral clearance in adults. These results provide further evidence that inflammaging is an important and reversible contributor to the prolonged course of RSV-associated disease in the elderly, and indicate that PGD_2 is a key mediator in this pathway. Experimentally, LPS and PGD_2 administration to adult cotton rats offers a model for inducing an inflammaging phenotype for further characterization of the inflammatory cascade leading to delayed RSV clearance.
Keywords for abstract:	Respiratory syncytial virus aging cotton rat prostaglandin D_2

IMID - 16

Title of abstract:	**DIFFERENTIAL EFFECTS OF IFITM PROTEINS ON SARS-COV-2 ENTRY: EXPLORING THE POSSIBLE MECHANISMS OF ACTION**
Authors:	P. Qu, C. Zeng, Y. Zheng, S. Liu. Depts. Of Veterinary Biosciences
Abstract:	Interferon-induced transmembrane proteins (IFITMs) inhibit a number of viruses, including human coronaviruses SARS-CoV-1 and MERS-CoV, particularly at the step of entry. Interestingly, IFITM2 and IFITM3 have been shown to enhance human coronavirus OC43 (HCoV-OC43) infection by unknown mechanisms, and the effect of IFITMs on SARS-CoV-2 infection has been controversial. Here, we provide evidence that the effect of IFITM proteins on SARS-CoV-2 is cell-type as well as IFITM-ortholog dependent. IFITM2 and IFITM3 promote entry of SARS-CoV- 2 into 293T/ACE2 and H1299 cells; however, IFITM1 potently inhibits it. In Calu-3 cells, however, all three IFITM proteins are inhibitory against SARS-CoV-2 similar to SARS-CoV-1. Critically, we find that the differential effects of IFITMs on SARS-CoV-2 is associated with ACE2 receptor expression in different cell types. In particular, overexpression of IFITM3 upregulates the expression level of ACE2 on the cell surface, likely by reducing its internalization, resulting in enhanced viral binding and spike-mediated membrane fusion. Consistent with this notion, deletion of the N-terminal 21 amino acids of IFITM3, which re-localizes the protein from predominantly endolysosomes to the plasma membrane, strongly increases its enhancing effect on SARS-CoV-2 entry. Protein pulldown assay shows that IFITM3 protein are complexed with the SARS-CoV-2 spike, suggesting a possible interaction that is stabilized by ACE2. Altogether, our study revealed some unique mechanisms of IFITM proteins in promoting SARS-CoV-2 entry, especially by engaging with the virus spike protein and ACE2 receptor on the plasma membrane.
Keywords for abstract:	IFITM SARS-CoV-2 Entry ACE2

IMID - 17

Title of abstract:	**ADAPTIVE CHANGES IN VIRAL *ENVELOPE* RESULTING FROM ADAPTATION OF SIMIAN-TROPIC HIV-1 TO MACAQUES CONFERS RESISTANCE TO INTERFERON**
Authors:	A. C. Smith[1], H. Weight[2], J. Overbaugh[2], and A. Sharma[1]. [1]Depts of Veterinary Biosciences and Microbial Infection & Immunity. [2]Divison of Human Biology, Fred Hutchinson Cancer Research Center.
Abstract:	HIV-1 does not persistently infect macaques due to restriction by several type-I interferon (IFN)-induced host-factors. Therefore, chimeric SIV/HIV-1 viruses (SHIVs) encoding the SIV antagonists of restrictive host-factors and HIV-1 Envelope glycoprotein (Env), are used to infect macaques to model HIV-1 infection. A major limitation of the SHIV/macaque model is that SHIVs generated *in vitro* replicate poorly in macaques. A small subset of SHIVs has been successfully adapted for high-level replication through serial passage in macaques. We have previously identified that serial macaque-passage selects for IFN-resistant SHIV variants that have higher replication in macaque lymphocytes. The viral determinant(s) contributing to increased replication and IFN resistance in macaque-passaged SHIVs have not been examined. To identify the viral determinant(s) of macaque-passaged SHIVs that confer resistance to IFN, we generated SHIV infectious molecular clones (IMCs) encoding the parental *env* and representative *env* clones from sequential macaque-passaged viruses. We found that the unpassaged, parental SHIV IMC is potently inhibited by IFN (mean IC_{50} range 27.9 – 49.9 U/ml), whereas the SHIV IMCs encoding macaque-passaged *envs* are resistant to IFN inhibition (mean IC_{50} >5000 U/ml). In addition, we found that SHIV IMCs encoding macaque-passaged *envs* have high replication capacity and most, but not all, have more virion Env content. Next, we took a gain-of-function approach and generated chimeras that introduce portions of *env* gene from IFN-resistant SHIV IMC into the parental IFN-sensitive SHIV IMC. Using this approach, we mapped the determinants of IFN resistance and high replication capacity to two *N*-linked glycosylation sites in the gp120 subunit of HIV-1 Env. In conclusion, the adaptive changes in HIV-1 *env* resulting from serial macaque-passage of SHIVs are sufficient to increase resistance to IFN, replication capacity, and virion Env content. Thus, the host IFN response serves as a strong selective pressure during the process of adaption of SHIV to macaques.
Keywords for abstract:	Human immunodeficiency virus Simian-human immunodeficiency virus Type-I interferon

IMID - 18

Title of abstract:	**BROAD-SPECTRUM AND GRAM-NEGATIVE-TARGETING ANTIBIOTICS DIFFERENTIALLY REGULATE ANTIBODY ISOTYPE RESPONSES TO INJECTED VACCINES**
Authors:	A. Haile, R. Woodfint, E. Kim, M. R Joldrichsen, N. Berhe, W. A Gebreyes, P. Boyaka
Abstract:	Antibiotics are extensively used worldwide for the treatment of common infections by agents such as E. coli and Salmonella. They also represent the most common cause of alteration of the microbiota in people. We addressed whether broad-spectrum and Gram-negative-targeting antibiotics differentially regulate systemic and mucosal immune responses to vaccines. Antibiotics treatment enhances serum IgG1 responses in mice immunized systemically with a model polyvalent vaccine. This increase was not seen for other IgG subclasses and was dependent on the immunogenicity of vaccine antigens. The broad-spectrum antibiotic cocktail also enhanced serum IgA responses. Interestingly, both the broad spectrum and the antibiotic targeting Gram-negative bacteria enhanced the number of IgA antibody secreting cells in the intestinal lamina propria. This effect was unlikely to be due to an increase in cells expressing gut-homing receptors (i.e., CCR9 and α4β7) in peripheral tissues. On the other hand, the microbiome in mice treated with antibiotics was characterized by an overall reduction of the number of firmicutes. Furthermore, Bacteroidetes were increased by either treatment, and Proteobacteria were increased by the broad-spectrum antibiotics cocktail. Thus, immunoglobulin isotype and subclass responses are differentially regulated by oral antibiotics treatment and the gut microbiota shapes mucosal antibody responses after systemic immunization.
Keywords for abstract:	Antibiotics Vaccines IgA Microbiota

IMID - 19

Title of abstract:	**A SAFE AND HIGHLY EFFICACIOUS LIVE ATTENUATED MUMPS VIRUS-BASED SARS-COV-2 VACCINE FOR INFANTS AND CHILDREN**
Authors:	Y. Zhang1, M. Lu1, Mahesh K C2, E. Kim1, M. M. Shamseldin3, C. Ye4, P. Dravid2, M. Chamblee1, J-G Park4, J. M. Hall3, S. Trivedi2, S Chaiwatpongsakorn2, A D. Kenny3, S Srinivasa Murthy2, H Sharma2, X. Liang1, J. S. Yount3,6, A. Kapoor2,5,6, L. Martinez-Sobrido5, P. Dubey3,6, P. N Boyaka1,6, M. E. Peeples2, 5, 6, J. Li1,6* 1Department of Veterinary Biosciences, The Ohio State University, Columbus, OH, USA 2Center for Vaccines and Immunity, Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH, USA 3Department of Microbial Infection and Immunity, College of Medicine, The Ohio State University, Columbus, OH, USA 4Texas Biomedical Research Institute, San Antonio, TX, USA 5Department of Pediatrics, College of Medicine, The Ohio State University, Columbus, OH, USA 6Infectious Disease Institute, The Ohio State University, Columbus, OH, USA
Abstract:	A major drawback of the current COVID-19 vaccines is the lack of sufficient safety and efficacy data in infants and children, precluding their use in infants or children under 5 years of age. With the rapid increase in SARS-CoV-2 cases in children, a safe and effective vaccine for this population is urgently needed. The MMR (measles/mumps/rubella) vaccine has been one of the safest and most effective human vaccines used in infants and children since the 1960s. Here, we developed live attenuated recombinant mumps virus (rMuV)-based SARS-CoV-2 vaccine candidates using the MuV Jeryl Lynn (JL2) vaccine strain backbone. The soluble prefusion SARS-CoV-2 spike protein (preS) gene, with 2 prolines (preS-2P) or 6 prolines (preS-6P) was inserted into the MuV genome at the P-M or F-SH gene junctions in the MuV genome. preS-6P was more efficiently expressed than preS-2P, and preS-6P expression from the P-M gene junction was more efficient than from the F-SH gene junction. In mice, the rMuV-preS-6P vaccine was more immunogenic than the rMuV-preS-2P vaccine, eliciting stronger neutralizing antibodies and mucosal immunity. Sera raised in response to the rMuV-preS-6P vaccine neutralized SARS-CoV-2 variants of concerns, including the delta variant equivalently. IFNAR1-/- mice and golden Syrian hamsters immunized with the rMuV-preS-6P vaccine induced high levels of neutralizing antibodies, mucosal IgA antibody, and T cell immune responses, and were completely protected from challenge by both SARS-CoV-2 USA-WA1/2020 and delta variants. Therefore, rMuV-preS-6P is a highly promising vaccine candidate, warranting further development as a COVID-19 vaccine for infants and children.
Keywords for abstract:	SARS-CoV-2, mumps virus, prefusion spike, vaccine, immunogenicity

IMID - 20

Title of abstract:	***EHRLICHIA* FACTOR THAT ENABLES MAMMAL-TICK TRANSMISSION**
Authors:	T. Zhang[1], K. Budachetri[1], R. Chien[1], M. Lin[1], and Y. Rikihisa[1] 1 Department of Veterinary Biosciences
Abstract:	*Ehrlichia* spp. are emerging obligatory intracellular bacteria transmitted by particular species of ticks, and can cause subclinical or sometimes fatal diseases called ehrlichiosis in various species of mammals including humans. Our lab recently obtained an *Ehrlichia* sp. mutant that has the gene encoding a tandem repeat protein (TRP) disrupted as a result of Himar1 transposon insertion. The *Ehrlichia* TRP is an abundantly produced type I secretion system effector that has been proposed to have multiple functions for ehrlichial infections in mammalian cells, but the exact role of the TRP *in vivo* remains to be determined. Our studies found that the TRP mutant is as virulent as wild-type (WT) *Ehrlichia* in mice with respect to clinical signs and infection of major organs, but has a significantly lower bacterial load in the bloods at 7 days post-infection. As ticks acquire *Ehrlichia* exclusively by blood feeding on infected mammals, we tested whether naïve ticks allowed to feed on mice infected with the TRP mutant and WT could acquire *Ehrlichia*. Our studies showed that ticks cannot acquire the TRP mutant as effectively as WT. We also found the TRP mutant infects and proliferates in tick cell culture as effectively as WT. Thus, this result suggests that the TRP is a tick transmission factor of *Ehrlichia*. Elucidating microbial factors that are required for transmission between ticks and mammals will help develop new therapeutic interventions and preventative measures by breaking the transmission cycle of the tick-borne diseases.
Keywords for abstract:	Disease transmission Tick-borne disease *Ehrlichia* Blood

MOLECULAR
AND
CELLULAR BIOLOGY

MCB - 1

Title of abstract:	**INVESTIGATING THE EPIGENETIC MODIFICATION LANDSCAPE OF THE HIV-1 RNA GENOME AT THE SINGLE-VIRUS LEVEL**
Authors:	A.Baek*, G.Lee*, S.Golconda+, N.Tirumuru*, W.Lu*, M.Sullivan+, L.Wu*, and S.Kim*
Abstract:	Despite the increasing interest, epigenetic modifications in the HIV RNA genome (gRNA), remains poorly understood largely due to the technical limitations. Even the state-of-the-art tools provide only partial and fragmented information lacking both precise modified nucleotide positions and virus-to-virus variations in epitranscriptomic landscape. Unlike other sequencing platforms, Oxford Nanopore Technology (ONT), a new 3rd generation sequencing, enables extremely long-read sequencing and direct detection of DNA/RNA modifications by sequencing native DNA/RNA molecules. This study demonstrates the first full-length 9kb mapping of HIV-1 RNA modifications at both the nucleotide resolution and single RNA molecule level using ONT. Our optimized Nanopore direct RNA sequencing of near-full-length HIV-1 revealed unexpectedly simple modification patterns, presenting only a few dominant and site-specific modifications near the 3'end of the genome. Of 27 most prominent sites, 15 overlapped with m6A DRACH motifs. These potential m6A sites were evaluated and confirmed by (i) a treatment with m6A erasers (fat mass and obesity-associated protein and AlkB homolog 5), (ii) modeling with synthesized m6A oligo controls and (iii) LC-MS/MS. Point mutagenesis targeting three most dominant m6A sites individually abolished corresponding methylation signals, but interestingly these single mutations did not make any notable impact on viral RNA production, packaging or infection. A combination of mutations that remove all the three dominant m6A sites was needed to render a notable inhibition of viral packaging and infection, suggesting essential but compensatory roles of those dominant modifications on maintaining viral activity. This study thus establishes an innovative assay to study HIV-1 RNA modifications and reveal novel insights into the biology of epigenetic modification in HIV-1 pathogenesis.
Keywords for abstract:	HIV-1 RNA m6A modification Nanopore sequencing Direct RNA sequencing

MCB - 2

Title of abstract:	**BLOCKING TUMORIGENESIS AND DEDIFFERENTIATION THROUGH PAK INHIBITION IN BRAFV600E-INDUCIBLE PAPILLARY THYROID CANCER**
Authors:	A. Cox, L. Bautista, B. Ringel. Division of Endocrinology, Diabetes, and Metabolism, Department of Internal Medicine and OSUCCCC.
Abstract:	Thyroid cancer is the most common form of endocrine cancer worldwide, and papillary thyroid cancer (PTC) is the most common subtype of thyroid cancer, accounting for approximately 90% of all thyroid cancer diagnoses. Up to 83% of aggressive PTCs have BRAFV600E driver mutations that contribute to uncontrollable cell proliferation and tumorigenesis through MAPK signaling. We have identified a previously unknown signaling pathway in which BRAFV600E binds with and activates group I p-21 activated kinases (PAKs). This pathway independently also enhances cell cycle progression, cytoskeletal motility, and apoptosis in vitro. To study the in vivo role of PAK activity in BRAFV600E-induced thyroid cancer, we first treated mice with thyroid-specific BRAFV600E overexpression with a PAK inhibitor (G5555) and showed that it reduces tumor growth and prevents cancer development. The current project builds on that work using mice with individual temporal control of thyroidal BRAFV600E overexpression and a molecular inhibitor of group I PAKs (PID) that is highly specific for the thyroid. We tested both prevention and treatment of BRAFV600-inducible PTC using this model. In preliminary data, we demonstrated that PID activation can not only prevent and reverse BRAFV600E-mediated thyroid cancer, it also blocked dedifferentiation in the prevention model and reversed dedifferentiation in the treatment model. In addition, using a single copy knock-in model of BRAFV600E that closely mimics human PTC, co-expression of PID with BRAFV600E also blocked PTC formation. These data suggest that PAK inhibitors may be an exciting new treatment option for BRAFV600E-derived thyroid cancer as both an anti-neoplastic and/or redifferentiating therapy.
Keywords for abstract:	Thyroid Cancer Carcinoma Braf PAK Oncogenesis Tumorigenesis

MCB - 3

Title of abstract:	**EPZ015666, A PRMT5 INHIBITOR, SELECTIVELY TARGETS HTLV-1-INFECTED T-CELL LINES IN VITRO AND IN VIVO**
Authors:	K. Ernzen, C. Phelps, S. Niewiesk, P. Green, and A. Panfil. Dept. of Veterinary Biosciences
Abstract:	**Background:** HTLV-1 is the infectious cause of adult T-cell leukemia/lymphoma (ATL), an extremely aggressive and chemotherapy-resistant malignancy of CD4+ T-cells. Many current therapies improve ATL patient survival, but the patients consistently relapse. Therefore, a need exists to identify novel targets relevant to the pathophysiology of ATL and innovative approaches to hit these targets. Protein arginine methyltransferase 5 (PRMT5) is a type II PRMT enzyme that has been directly implicated in the pathogenesis of multiple different lymphomas. Recently, our group found that PRMT5 is upregulated in HTLV-1-transformed cell lines, during HTLV-1-mediated immortalization, and in ATL patient samples. Here, we hypothesized that PRMT5 over-expression is relevant to HTLV-1-driven T-cell transformation and a commercially available inhibitor of PRMT5 activity (EPZ015666) would provide a novel approach to treating HTLV-1 malignancies. **Methods:** A variety of HTLV-1-transformed cell lines, ATL-derived cell lines, and HTLV-1-negative T-cell lines were treated with EPZ015666 for 12 days. Cellular viability and apoptosis were measured by trypan blue exclusion and flow cytometry, respectively. Freshly isolated PBMCs were co-cultivated with lethally irradiated producer cells in the presence of titrating amounts of EPZ015666. Cellular viability and immortalization were measured over the course of 12 weeks. NOG mice transplanted with HTLV-1-transformed cells were treated with EPZ015666 and tumor burden, IL-2R□, and survival were measured over the course of 4 weeks. **Results:** Inhibition of PRMT5 with EPZ015666 resulted in 1) a dose-dependent selective toxicity in HTLV-1-infected cell lines compared to HTLV-1-negative cells, 2) inhibition of HTLV-1-mediated T-cell transformation *in vitro*, and 3) increased survival
Keywords for abstract:	HTLV-1 T-cell PRMT5 Transformation EPZ015666

MCB - 4

Title of abstract:	**INVESTIGATIONS INTO THE MECHANISM OF ASIC-MEDIATED NEUROTOXICITY**
Authors:	L.A. Lind1,2, L.E. Bauer2, C.M. Ford2, C.C. Askwith1,2 1Department of Veterinary Biosciences, College of Veterinary Medicine 2Department of Neuroscience, College of Medicine
Abstract:	Acid-sensing ion channels (ASICs) are voltage-insensitive cation channels that open in response to acidic extracellular pH. Physiologically, they are important for normal behavior including learning/memory and fear-conditioning. Pathologically, they contribute to neuronal death following cerebral ischemia and inflammation: two conditions where the extracellular pH in the brain becomes acidic for long periods of time. ASIC-dependent neuronal death has traditionally been attributed to excess calcium influx from prolonged channel activation. However, it has recently been proposed that this ASIC-dependent acidotoxicity is initiated by conformation changes in ASICs which alter protein-protein interactions within the intracellular domain of the channels and activate programmed cell death through necroptosis. Recently, we discovered that activation of certain G protein coupled receptors prevents ASIC-mediated cell death without affecting ASIC ion conduction and calcium influx. We hypothesize that activation of these G-protein coupled receptors interferes with the signaling cascades that link ASIC's conformation changes to cell death. The goal of my project is to determine how ASIC-mediated necroptosis differs from classic necroptotic pathways and how activation of specific G-protein coupled receptors interfere with these cellular signaling pathways to prevent acidotoxicity. To this end, I am identifying the ASIC-induced necroptotic signaling pathway in neuronal-like cell lines, primary neurons and isolated brain slices. Using Western blots, we have found that the levels of the necroptosis inducer RIPK1 are profoundly affected by acid exposure in cell lines and primary neurons. Further, the levels of RIPK1 in the brain are not uniform and sensitive to tissue manipulation. These results suggest that ASIC-mediated necroptosis involves a specific RIPK1-dependent pathway. Further work will elucidate the other partners in the ASIC-necroptosis signaling cascades and the impact of GPCR activation on sensitivity to acidotoxicity.
Keywords for abstract:	Acid-sensing ion channels Acidotoxicity Neuronal death Receptor-interacting kinase 1

MCB - 5

Title of abstract:	**INTRAGENIC VIRAL ENHANCER OF HTLV-1 IS DISPENSABLE FOR IN VITRO IMMORTALIZATION AND IN VIVO PERSISTENCE**
Authors:	V. Maksimova, S. Smith, J. Seth, C. Phelps, S. Niewiesk, Y. Satou, P.L. Green, and A.R. Panfil
Abstract:	Human T-cell leukemia virus type 1 (HTLV-1) is the causative infectious agent of adult T-cell leukemia/lymphoma (ATL) and chronic neurological disease. The disparity between silenced sense transcription versus constitutively active antisense (Hbz) transcription from the integrated provirus is not fully understood. The presence of an internal viral enhancer has recently been discovered in the Tax gene near the 3' long terminal repeat (LTR) of HTLV-1. In vitro, this enhancer has been shown to bind host transcription factors, maintain chromatin openness and viral gene transcription, and induce aberrant host gene transcription near viral integration sites. However, the function of the viral enhancer in the context of early HTLV-1 infection events remains unknown. In this study, we generated a mutant Enhancer virus (mEnhancer) and evaluated the effects of the internal viral enhancer on HTLV-1-mediated in vitro immortalization, establishment of persistent infection in an in vivo rabbit model, and disease development in a humanized immune system (HIS) mouse model. The wild-type (wt) and mEnhancer viruses demonstrated similar capabilities in 5' LTR transactivation, virus production, and immortalization efficiency in vitro. Over a 25-week study, the mEnhancer virus was able to establish persistent infection in rabbits, and there were no significant differences in proviral load or HTLV-1-specific antibody responses in animals infected with the mutant compared to wtHTLV-1. HIS mice infected with wt or mEnhancer virus also showed similar disease progression. These results suggest that alone, the viral enhancer mutant has no defect in immortalization, establishment of infection, or disease development. Further studies should be performed to discern whether the viral enhancer cooperates with other transcriptional regulatory elements to induce viral gene expression and contribute to HTLV-1 persistence and pathogenesis.
Keywords for abstract:	HTLV Enhancer Immortalization Persistence

MCB - 6

Title of abstract:	**DEFINING THE HBZ/YBX1 INTERACTION AND ITS ROLE IN HTLV-1 PATHOGENESIS**
Authors:	A. Midkiff, S. Smith, P. Green, A. Panfil. Depts. of Veterinary Biosciences, Center for Retrovirus Research, and Comprehensive Cancer Center and Solove Research Institute
Abstract:	Human T-cell leukemia virus type 1 (HTLV-1) infects an estimated 5-10 million people worldwide and ~5-10% of infected people develop clinical disease (T-cell leukemia or neurodegenerative disease) after a prolonged clinical latency period of several decades. However, progression of disease is not well understood, and patient prognosis is poor with limited treatment options available. The viral protein Hbz was initially identified as an antagonist to the viral oncoprotein and transcriptional activator, Tax. Based on subsequent studies, Hbz is also thought to play a role in infected cell maintenance and oncogenesis. This viral gene is often the only viral transcript expressed in patient cells and its precise role in cell survival remains poorly understood. Our lab recently identified that Hbz interacts with the cellular protein YBX1 via mass spectrometry. YBX1 is a transcription factor involved in growth-associated gene expression. Thus, we hypothesized that the Hbz/YBX1 interaction may play a key role in oncogenesis. Our studies have found that YBX1 activates viral transcription, and we find a synergistic positive effect on transcription with both Tax and YBX1. Hbz is able to repress Tax-mediated viral transcription, and exogenous YBX1 alleviates this repression. Using affinity pulldowns, we found YBX1 interacts with the central basic region of Hbz. Interestingly, we also found that cellular distribution of YBX1 is both cytoplasmic and nuclear, suggesting possible roles for YBX1 outside of HTLV-1 transcriptional regulation. Our work will help to further define the mechanism of action of Hbz and the proteins it interacts with during HTLV-1 pathogenesis. These studies are critical for defining potential targets in future gene editing therapeutic approaches.
Keywords for abstract:	HTLV-1 Human T-cell leukemia virus type 1 Hbz Tax YBX1 Retrovirus T-cell leukemia

MCB - 7

Title of abstract:	**LUNG METASTATIC ENGRAFTMENT ATTENUATES EGFR-MEDIATED MAPK SIGNALING DYNAMICS IN TRIPLE NEGATIVE BREAST CANCER**
Authors:	V. Murthy, A. Davies. Dept. of Veterinary Biosciences
Abstract:	The majority of breast cancer (BC) mortalities can be attributed to metastasis to distant organs. The overall survival for BCs is about 90% but decreases to less than 30% in cases of metastasis to distant organs. In triple negative BCs (TNBCs), an aggressive subtype of BCs, metastasis to the lung results in poorest clinical outcomes, with overall 5-year survival of about 10%. Genetically, TNBCs lack the classical hormone receptors - estrogen receptor (ER) and progesterone receptor (PR)- and human epidermal growth factor receptor 2 (HER2) expression but over express epidermal growth factor receptor (EGFR). Further, downstream EGFR targets Ras/extracellular signal-regulated kinase (Ras/ERK) proteins are highly activated. However, targeted therapies against EGFR and Ras/ERK are largely ineffective in TNBCs. The largest contributor to acquired resistance in TNBCs is the developing early metastatic niche. Development of the metastatic niche is a crucial step in cancer cell metastasis, aiding cells in the colonization of secondary tissues. However, how the metastatic niche affects cancer cell signaling is not well understood. Cellular signaling fluctuations result in temporal variability in gene expression and consequently affect cell fate, proliferation and survival. Using time-resolved live single cell imaging we show that the lung micro environment suppresses signaling in TNBCs. We identify EGFR as the primary driver of this suppression resulting in a decrease of single cell signaling variability ultimately leading to the inefficacy of EGFR therapies.
Keywords for abstract:	Cancer Breast Cancer ERK metastasis microenvironment

MCB - 8

Title of abstract:	**ESTABLISHMENT AND CHARACTERIZATON OF TWO NOVEL CANINE GLIOMA PATIENT-DERIVED LINES**
Authors:	M.S. Schrock[1], G.E. Pluhar[2], M.R. Olin[3], R.T. Bentley[4], S.A. Moore[5], M. Venere[1], and M.K. Summers[1]. [1]Department of Radiation Oncology, College of Medicine, The Ohio State University; [2]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota; [3]Department of Pediatrics, College of Medicine, University of Minnesota; [4]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Purdue University; [5]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University
Abstract:	The mean overall survival for patients with human glioblastoma (GBM) remains 12-18 months with no improvement since the incorporation of temolozolomide into standard of care 17 years ago. A major barrier to developing safe, effective treatments is that GBM mouse models are poor predictors of drug responses in humans. The NCI-led Comparative Brain Tumor Consortium is a multi-instiutional effort aimed at validating naturally occurring glioma in pet dogs as an improved animal model for human GBM. While this team has made many advances in updating diagnostic criteria and genetic characterization of canine glioma, there remains a scarcity of high grade canine glioma cell lines to use for *in vitro* studies. The goal of this project was to establish novel canine glioma cell lines to better represent the distinct types of glioma (oligodendroglioma and astrocytoma) through methods that avoid the use of fetal bovine serum and transformation *in vitro*, which are known to contribute to gene expression changes and the acquisition of genetic aberrations. Through a multi-institutional collaboration, we acquired two fresh and ten frozen samples of high grade canine glioma. Cells were implanted subcutaneously and intracranially in athymic nude mice then monitored for growth visually (SQ) or with microMRI imaging (IC). Upon identification of successful cell growth in mice, cells were harvested and mouse cells were depleted with magnetic cell sorting. Two lines were established, which exhibit a varied morphology, similar to human GBM lines cultured and established with similar methods. Both lines are sensitive to radiation and an experimental mitotic inhibitor, C38. Future work will focus on engrafting more samples and further characterizing the established lines with whole exome sequencing and array comparative hybridization to to identify driver mutations, aneuploidies, duplications and deletions.
Keywords for abstract:	Canine glioma Human glioblastoma Patient-derived lines

MCB - 9

Title of abstract:	**INVESTIGATING DIFFERENTIALLY ABUNDANT METABOLITES IN DOGS WITH AND WITHOUT UROTHELIAL CARCINOMA**
Authors:	A. Scott[1], M. Bernier[2], C. Madden[1], D. Dhawan[3], W. Kisseberth[1], S. Justice[4], D. Knapp[3], V.L. Hale[1] [1]Ohio State University College of Veterinary Medicine [2]Ohio State University Campus Chemical Instrument Center [3]Purdue University College of Veterinary Medicine [4]Ohio State University College of Nursing
Abstract:	Bladder cancer is the sixth most common form of cancer in the United States. Naturally occurring urothelial carcinoma (UC) in dogs closely mimics characteristics of human bladder cancer. The microbiome has been linked to tumor development and response to treatment in some cancer types; however, it is unknown what role microbes may be playing in UC development and progression in humans or in dogs. Urine from 11 dogs with and 11 dogs without bladder cancer underwent untargeted metabolomics followed by pathway analyses. We also identified differentially abundant metabolites between the groups. Overall, we observed global differences in metabolite profiles including specific pathways related to aromatic compound degradation. Aromatic compounds include petroleum distillates such as toluene, benzene, and xylene, and secondary metabolites linked to toluene metabolism include hippuric acid and o-cresol, which were both increased in the urine of dogs with cancer. These differences indicate differing metabolism in dogs with and without cancer. However, continued investigation is necessary to elucidate the role of host and microbes in metabolizing aromatic compounds and determining what role this metabolism may play in UC.
Keywords for abstract:	Bladder cancer Urothelial carcinoma Microbiome Metabolomics Hippuric acid o-cresol Toluene degradation

MCB - 10

Title of abstract:	**PRMT5 INHIBITION MODULATES E2F1 AND P53 TO RESTORE CELL CYCLE REGULATION AND DRIVE AN APOPTOTIC CELL DEATH RESPONSE IN IBRUTINIB RESISTANT MANTLE CELL LYMPHOMA**
Authors:	S. Sloan, F. Brown, M. Long, J. Chung, J. Helmig-Mason, W. Hanel, E. Brooks, R.A. Baiocchi and L. Alinari.
Abstract:	Ibrutinib is widely used for patients with relapsed/refractory mantle cell lymphoma; however, the majority of patients ultimately develop resistance and face poor overall survival. There is an urgent need for novel therapies targeting pro-survival signaling pathways triggered by ibrutinib resistance. Inhibition of Protein Arginine Methyltransferase 5 (PRMT5i) represents a novel therapeutic approach to overcome ibrutinib resistant MCL (IR-MCL). Genomic deletions of MTAP are commonly detected in IR-MCL and sensitizes cells to further inhibition of PRMT5. Although the published literature defines this therapeutic rationale for targeting PRMT5 in cancers with deletion of MTAP, the literature lacks studies specifically targeting PRMT5 in this context. Comparing MCL cell lines with primary ibrutinib resistance, we found an inverse correlation between PRMT5i and ibrutinib. In MCL cell lines, low expression of MTAP also correlated with lower global Symmetric Di-Methyl Arginine and enhanced sensitivity to PRMT5i. Genomic deletion of MTAP enhanced sensitivity to PRMT5i, whereas MTAP knock-in decreased sensitivity to PRMT5i. The treatment with MTA, further confirmed that genomic deletion of MTAP confers enhanced vulnerability to PRMT5i in MCL cell lines. In a mouse model from an MCL patient with IR-MCL, treatment with PRMT5i decreased tumor infiltration of multiple organs compared to ibrutinib or control. To mechanistically address the nature of the anti-tumor activity, we treated mice and subjected tumor cells to transcriptomic profiling by RNA-sequencing. We identified 1014-down and 1124-up differentially expressed genes. Gene Set Enrichment Analysis identified activation of the p53 pathway, with repression of E2F targets, and cell cycle checkpoints. In MCL, PRMT5i resulted in cell cycle arrest and apoptosis by transcriptional activation of TP53 target genes. Western blot analysis confirmed accumulation of DNA damage and apoptotic markers that are further enhanced with combination of doxorubicin. We are currently exploring novel combinations to maximize the therapeutic potential of PRMT5 inhibition in this disease.
Keywords for abstract:	Lymphoma MCL PRMT5 Cancer Ibrutinib

MCB - 11

Title of abstract:	**GENE TARGETED EDITING TO DISABLE THE ONCOGENIC RETROVIRUS HTLV-1**
Authors:	Susan M. Smith, Tasha Wilkie, Nagaraja Tirumuru, Amanda Panfil, Kristine Yoder, and Patrick Green. Depts. Of Veterinary Biosciences and Cancer Biology & Genetics.
Abstract:	Human T-cell leukemia virus type 1 (HTLV-1) is an oncogenic retrovirus and the etiologic agent of both adult T-cell leukemia/lymphoma (ATL) and a progressive chronic neurodegenerative. HTLV-1-associated malignancies occur after a prolonged clinical latency period of up to several decades, and very few successful treatment options exist. Unlike other oncogenic retroviruses, HTLV-1 does not encode host-derived oncogenes or lead to insertional cis-activation of a cellular oncogene. Instead, the virus encodes two genes that are essential for transformation and proliferation, *Tax* and *Hbz*. *Tax* is essential for *de novo* infection and cellular immortalization while *Hbz* promotes proliferation and survival of infected cells. Additionally, HTLV-1 uniquely persists in infected hosts through mitotic host cell division, and the viral genome is highly conserved. Given this conservation, genomic editing has strong promise. Clustered regularly interspersed short palindromic repeat (CRISPR)/Cas9 genome editing of retroviral proviruses has been limited to HIV-1. We propose HTLV-1 as an excellent model to advance this technology. Our team constructed a library of 163 gRNAs covering the Tax, HBZ, and viral long terminal repeats (LTRs) coding regions. These gRNAs were sub-cloned into a CRISPR lentiviral vector which expressed the *Cas9* gene and a puromycin resistance gene. VSV-G pseudotyped lentivirus was produced and transduced into HTLV-1-infected T-cell lines. Following puromycin selection, the cellular proliferation rate was analyzed by MTS assay. Our preliminary results show 67 gRNAs significantly reduced cellular proliferation. Ongoing studies involve 1) investigation of off-target editing by gRNAs using an integrase-defective lentiviral vector and 2) measurement of viral transcript levels. The top five gRNA candidates per viral gene/LTR will be selected for NSG sequencing and used in our *in vivo* transplantation NOG mouse model. Our experiments will determine the effectiveness of CRISPR genome editing for disabling HTLV-1 and further inform genome editing strategies for HTLV-1 treatment.
Keywords for abstract:	HTLV-1 CRISPR/Cas9 genome editing

MCB - 12

Title of abstract:	**NRP2 SIGNALING IN MALIGNANT PERIPHERAL NERVE SHEATH TUMORS**
Authors:	Tyler, K.A., Shive, H.R.
Abstract:	**Background:** Malignant peripheral nerve sheath tumors (MPNST) are soft tissue sarcomas that have a notoriously poor response to conventional chemotherapy/radiation. Signaling pathways that contribute to MPNST pathogenesis are not yet fully defined. Neuropilins are cell surface receptors that function as co-receptors for various transmembrane receptors and interact with many ligands. One such ligand is semaphorin 3F (SEMA3F), which is an extracellular signaling protein that plays a role in many pathways of growth and development, including tumorigenesis. **Objective:** Our objective was to determine how NRP2 and SEMA3F modify MPNST phenotypes. Using human MPNST cell lines, we investigated the role for the neuropilin receptor NPR2 in MPNST growth, migration, and invasion. **Methods:** MPNST cells underwent a co-culture migration assay with 293TN cells expressing SEMA3F. MPNST cells with NRP2 knockdown were analyzed for rate of cell growth by serial Cyquant assays, migration by transwell migration, and proliferation and apoptosis by caspase 3/7 assay. **Results:** Migration of MPNST cells was increased in the presence of SEMA3F. Cyquant assays revealed NRP2 knockdown cell lines have a lower rate of growth compared to controls. NRP2 knockdown cells lines also exhibited increased apoptosis compared to control lines. **Conclusions:** Our data suggest that NRP2 knockdown cell lines do not exhibit signs of growth and proliferation compared to controls, suggesting that NRP2 is necessary for development of MPNSTs in vitro. Our data also show that MPNST migrated at increased rates in the presence of SEMA3F, suggesting that the relationship between NRP2 and Sema3F is important to MPNST behavior.
Keywords for abstract:	Soft tissue sarcomas Cell signaling Cell migration

MCB - 13

Title of abstract:	**ISOLATION OF PERIOSTEAL-DERIVED CELLS FROM PORCINE AND OVINE MODELS TO OPTIMIZE MIGRATION ON BIOMIMETIC SCAFFOLDS**
Authors:	M. Walker, H. Powell. University Laboratory Animal Resources; Departments of Biomedical Engineering and Materials Science
Abstract:	Traumatic injuries, neoplasia, and osteolysis can result in large segmental bone defects that present particular clinical challenges. Autogenous bone grafts, allografts, and synthetic bone implants have been used to manage large defects; due to poor incorporation with the patient's bone, however, complications such as nonunion, malunion, and infection are common. The current project harnesses the osteogenic properties of periosteum to investigate its potential for segmental defect healing when used in conjunction with bioengineered nanofiber scaffolds. The periosteum provides vasculature, cytokines, proteins, and mesenchymal stem cells that are central to new bone formation. Biomimetic scaffolds act as a foundation on which periosteal cells can migrate to incorporate with host tissue. This investigation specifically aims to demonstrate isolation strategies that will provide an ample source of periosteal-derived cells (PDCs) from translational large animal models. Tissue from human femoral, porcine tibial, and ovine tibial specimens was minced, cultured, and quantified using both migration assay and enzymatic digestion with collagenase. A central objective of cell isolation is to demonstrate that nanofiber scaffolds can promote expansion of periosteal cells. Bovine-extracted collagen nanofiber scaffolds were created in either an aligned or random orientation. Half of the scaffolds were doped with hydroxyapatite (HA). Immunohistochemical staining was used to determine the orientation (random v. aligned) and architecture (collagen v. HA-doped collagen) that resulted in maximum cellularity. Pilot results support the hypothesis that periosteal cell migration is successfully promoted on biomimetic scaffolds. *In vitro* optimization of this project will inform future trials in murine and large animal models with the ultimate goal of clinical implementation of the bioengineered construct in human patients.
Keywords for abstract:	Biomedical engineering Tissue engineering Periosteum Periosteal-derived stem cells Bone/fracture healing Osteogenesis Cell isolation Laboratory animal models

MCB - 14

Title of abstract:	**FUNCTIONAL ANALYSIS OF A NOVEL TYPE IV SECRETION SYSTEM EFFECTOR OF *ANAPLASMA SPECIES***
Authors:	L. Wang, M. Lin, and Y. Rikihisa. Depts. Of Veterinary Biosciences
Abstract:	*Anaplasma phagocytophilum* (*Aph*) is a gram-negative obligatory intracellular bacterium that causes emerging infectious disease, human granulocytic anaplasmosis (HGA), potentially fatal severe influenza-like illness. The type IV secretion system (T4SS) directly inoculates bacterial molecules into human cells. My hypothesis is a putative T4SS effector APH0874 has a significant role in *Aph* infection of human cells. *Aph* mutant expressing FLAG-tagged APH0874 C-terminus (FLAG-APH0874C) containing T4SS secretion signal was constructed by Himar1 transposon mutagenesis system. Immunofluorescence assay shows that FLAG-APH0874C is produced by *Aph* mutant and secreted into the host cytoplasm and localizes to the *Aph* inclusion membrane. APH0874C was cloned and expressed, and the recombinant protein was purified to immunize the rabbit for antibody production. By immunofluorescence assay with rabbit anti-APH0874C serum, native APH0874 is also secreted and localizes to *Aph* inclusions. By transfecting APH0874-GFP, APH0874N (C-terminal half-deletion)-GFP, and APH0874C1 (N-terminal half-deletion)-GFP into uninfected or *Aph*-infected RF/6A cells, APH0874 and APH0874C1 localize to the Golgi apparatus in un-infected cells and to *Aph* inclusions in *Aph*-infected cells, suggesting a part of Golgi membrane is recruited to *Aph* inclusions by APH0874C1. Therefore, current and future studies aim to (1) determine molecular targets of APH0874 in human cells by using APH0874C1 as bait, (2) analyze mechanisms of APH0874 to target Golgi and *Aph* inclusions, (3) elucidate roles of APH0874 in *Aph* infection *in vitro***.** Further studies on functions of *Aph* T4SS effector molecules and signaling pathways will advance our understanding of the complex interplay between obligatory intracellular pathogens and their hosts.
Keywords for abstract:	*A. phagocytophilum*, T4SS effectors, Golgi, molecular targets

MCB - 15

Title of abstract:	**REGULATION OF AKT SIGNALING DYNAMICS BY THE IGF-IGFBP AXIS IN THE LUNG METASTATIC MICROENVIRONMENT**
Authors:	R. Makkawi and A. Davies
Abstract:	The lung is a common metastasis site for various cancers, such as sarcomas and breast cancer. During metastasis, disseminated cancer cells are able to adapt to the signaling cues in the lung microenvironment, resulting in changes in their signaling pathways and their responsiveness to chemotherapeutics. Of particular interest is the PI3K/AKT pathway, which regulates cell growth and proliferation. It is dysregulated in a variety of human cancers, making it a potential target for therapy. AKT signaling is highly sensitive to Insulin-like growth factor (IGF) and its cognate receptor, IGFR. One of the key regulators of IGF signaling are IGF-binding proteins (IGFBP), a family of proteins that interact with IGF and both positively and negatively modulate its function. Although the IGF-IGFBP signaling has been implicated in several cancers, how IGFBP production by cancer cells and cells of the microenvironment influence cancer cell signaling is yet to be studied in fine detail. We hypothesize that AKT signaling is regulated by the IGF-IGFBP axis in a manner that favors persistent activation of pro-oncogenic AKT signaling in cancer cells. Consistent with this hypothesis, our preliminary data show that changes in IGF levels, or drug treatments, initially suppress AKT signaling but is followed by restoration of AKT signaling in cancer cells to basal levels over time. These changes correlate with altered IGFBP expression profiles in both cancer cells, and cells of the lung microenvironment, suggesting an adaptive regulatory mechanism to compensate for low AKT signaling. Our future work aims to understand the regulation of cancer cell signaling more fully through IGF and IGFBPs in the metastatic microenvironment and develop strategies to suppress it.
Keywords for abstract:	AKT Metastatic Microenvironment IGFBP IGF Signaling regulation

STRUCTURE/FUNCTION

SF - 1

Title of abstract:	**NON-COMPARTMENTAL PHARMACOKINETICS OF THREE INTRAVENOUS DOSES OF MYCOPHENOLATE MOFETIL IN HEALTHY STANDARDBRED MARES**
Authors:	D. L. Burroughs, G. Lorch, Y. Guo, K. Hill, E.L. Schroeder, L.K. Cole, and M.A. Phelps. Departments of Veterinary Clinical Sciences and Pharmaceutics and Pharmacology
Abstract:	Mycophenolate mofetil (MMF) is the prodrug of mycophenolic acid (MPA) that acts as an immunosuppressive agent. During biotransformation of MMF to MPA, additional metabolites including MPA phenol glucuronide (MPAG), MPA acyl glucuronide (AcMPAG), and MPA phenol glucoside (MPG) are formed. The objective of this study was to define the non-compartmental pharmacokinetic (PK) parameters of three single doses of intravenous (IV) MMF and its downstream metabolites in healthy horses. Our hypothesis was that plasma concentrations of MPA, MPAG, AcMPAG, and MPG would increase with each incremental IV drug dose. Six healthy Standardbred mares were included. Generic MMF (Par Pharmaceuticals, Chestnut Ridge, NY, USA) was reconstituted and administered as a single IV-bolus at 1.0 mg/kg, 5.0 mg/kg and 10.0 mg/kg with an eight-day washout between treatments. Blood samples were collected immediately prior to MMF administration and over 24 hours. A liquid chromatography-tandem mass spectrometry assay was developed following FDA guidance to determine plasma MMF, MPA, MPAG, AcMPAG, and MPG concentrations. Each horse was analyzed independently, followed by calculation of geometric mean and coefficient of variation. Non-compartmental PK parameters were determined for MMF and all metabolites at all doses. MMF was rapidly biotransformed to MPA within 30 minutes. At the 10.0 mg/kg dose, MPA, MPAG, AcMPAG and MPG C_{max} were 18,055 ng/mL (21.1%), 2,892 ng/mL (37.9%), 4,460 ng/mL (43.8%), and 1,244 ng/mL (22.2%), respectively, and AUC_{inf}_obs were 18,655 hr*ng/mL (26.1%), 10,243 hr*ng/mL (41.0%), 4,533 hr*ng/mL (39.9%), and 2,461 hr*ng/mL (23.3%), respectively. MPA C_{max} and AUC_{inf}_obs were highest at the 10.0 mg/kg dose. Within the 10-fold dose range, the increase in C_{max} and AUC_{inf}_obs for MMF and its metabolites was nonlinear. In conclusion, horses biotransform MMF into MPA, MPAG, AcMPAG, and MPG via the glucuronidation and glucosidation clearance pathways. Equine reference PK profiles for MPA and the metabolites MPAG, AcMPAG, and MPG were established.
Keywords for abstract:	Dermatology (equine) Immune-Mediated Disease Mycophenolate Mofetil Pharmacokinetics Pharmacology (equine)

SF - 2

Title of abstract:	**LYMPHOID B-CELL FOLLICLES PROTECT THEIR RESIDENT T CELLS FROM ANTIBODY-BASED T-CELL-TARGETING AGENTS**
Authors:	S. Kim*, R. Shukla, S. Cressman, A. Kim, A. Tracey, N. Liyanage and S. Kim Depts. Of Veterinary Biosciences and Microbial Infection and Immunity, The Ohio State University, Columbus, OH
Abstract *(300 word limit)*	Antibody and immunotoxin-mediated therapeutic T-cell depletion have become increasingly useful in T cell lymphoma, HIV/AIDS, allograft rejection, and transplant medicine. Despite the tremendous hope generated by these new agents, their treatment outcomes have often been limited and inconsistent for unclear reasons. Here, we demonstrate that B-cell follicles in secondary lymphoid organs protect their resident T cells (CXCR5+ T cells) against anti-CD3e monoclonal antibody (mAb) or CD3e-immunotoxin (CD3e-IT: anti-CD3e mAb conjugated with saporin or diptheria toxin)-mediated T-cell killing in various organs. We found that both anti-CD3e mAb and CD3e-IT are effective and specific in depleting T cells *in vivo*. Remarkably, however, we found that CD3e-IT was inefficient at killing a subset of T-cell populations that express CXCR5, including follicular T-helper cells (T_{FH}), are an important therapeutic target in HIV/AIDS and T-cell lymphoma therapy. Immunohistochemistry analysis showed that CD3e-IT efficiently depleted T cells in the T cell zone outside the B follicles, whereas T cells within the follicles were enriched. When CXCR5(+) and CXCR5(-) T-cell killing efficiency was compared *in vitro*, there was no notable difference between these two cell types, indicating that CXCR5+ T cells are not intrinsically resistant to CD3e-IT. Our short-term *in vivo* CD3e-IT binding assay – where CD3e-IT was retro-orbitally injected 8 minutes prior to euthanasia – also showed that, in spleen, CD3e-IT binds to CXCR5(-) T cells with a significantly better efficiency than to CXCR5(+) T cells. Lastly, using CD4/iDTR transgenic mice that expressed Diphtheria toxin receptors on T cells, we compared *in vivo* T-cell depletion by three different protein reagents, including Diphtheria toxin, CD3e mAb and CD3e-IT, and the results showed that B-follicles protect their resident CXCR5+ T cells from all these protein agents. Our findings, therefore, suggest a potentially a new treatment resistance mechanism mediated by the normal lymphoid B-cell follicles against T-cell depletion protein agents.
Keywords for abstract:	A new treatment resistance mechanism B-cell follicular structure CXCR5+ T cells CD3e-IT CD3e monoclonal Ab Diphtheria toxin T-cell lymphoma HIV/AIDS

SF - 3

Title of abstract:	**EVALUATION OF HISTOPATHOLOGIC SECTIONING OF CANINE SOFT TISSUE SARCOMAS**
Authors:	H. Wittorff, R. Jennings
Abstract:	Soft tissue sarcomas (STS) are a group of mesenchymal neoplasms with the potential for metastasis and recurrence. The current method for determining excision status of soft tissue sarcomas is histological assessment of radial sections. However, recent research argues tangential, or en face, sectioning provides a complete margin evaluation and therefore, is a more sensitive method in some tumors. A more sensitive margin assessment may better inform prognosis. The goal of this study was to compare standard radial sectioning to tangential sectioning in a retrospective study of 21 canine soft tissue sarcomas. For this study, cases of canine STS were evaluated by radial and tangential sectioning. The total percent "dirty" surface areas from tangential margins were measured and calculated using image analysis. The measured histologic tumor-free margins (HTFM) of the radial sections were compared against the "gold standard" tangential results using statistical analysis and a receiver operating characteristic (ROC) curve. Out of a total of 14 negative radial margins, 8 (57.1%) were positive on tangential margin analysis. Histopathologic evaluation of radial sectioning had a low sensitivity (46.7%; 7/15) when compared to tangential sectioning when positive margins were defined as HTFM = 0 mm. Radial margin analysis reached 100% sensitivity when positive margins included HTFMs ≤ 4 mm. The mean HTFM of negative tangential margins (n=6) was 6.79 ± 4.58 mm while the mean HTFM of positive tangential margins (n=15) was 0.367 ± 1.03 mm. These results suggest that radial sections with HTFMs > 0 mm should not always be considered completely excised. For canine STS with HTFMs between 0 mm and 4 mm, tangential margin analysis would be the more sensitive method. Future studies should use methods to increase the accuracy of image analysis and should consider case follow-up to determine the prognostic significance of positive margins detected by tangential sectioning.
Keywords for abstract:	Histology Canine Soft Tissue Sarcomas Margins