THE OHIO STATE UNIVERSITY

COLLEGE OF VETERINARY MEDICINE

COLLEGE OF VETERINARY MEDICINE RESEARCH DAY

5 APRIL 2018

BOOK OF ABSTRACTS

PROGRAM

April 5, 2018

POSTER JUDGING

Graduate Student Posters
8:00 am – 10:30 am
(closed session – only open to those being judged)

AWARDS PRESENTATION

Wexner Auditorium
12:00 pm

GRADUATE STUDENT and POST DOC PLATFORM PRESENTATIONS

Jingyou Yu
Jacob Al-Saleem

KEYNOTE SPEAKER

Wexner Auditorium
immediately following the awards and platform presentations

Dr. H. Morgan Scott
Professor
Texas A&M University

"Use, Overuse and Misuse of Antibiotics in Food Animal Agriculture: Do Bacteria Know the Difference; If so, How do They Let us Know?"

POSTER SESSION

1st and 2nd Floors – Vet Med Academic Building
11:00 am – 5:00 pm

CHAIRED BY

Dr. Greg Habing

ORGANIZED BY

Michele Morscher

Special thanks to Marc Hardman in the College's Technology Services for printing the posters

POSTER JUDGING SESSIONS

Wednesday, April 4, 2018
2:00 – 5:00 pm
Undergraduate and
Veterinary Student Poster Judging

Thursday, April 5, 2018
8:00 – 10:30 am
Graduate Student Poster Judging

Thank you to the following faculty and guests for taking time out of their busy schedules to judge 80 posters.

Andreia Goncalves Arruda

Robert Cartee

Renukaradhya Gourapura

Sanggu Kim

Krista La Perle

Margaret Mudge

Mike Oglesbee

Nidhi Rumpal

Prosper Boyaka

Joelle Fenger

Vanessa Hale

Lindsey Kock

Wendy Lorch

Peter Nara

Gireesh Rajashekara

College of Veterinary Medicine

Research Day

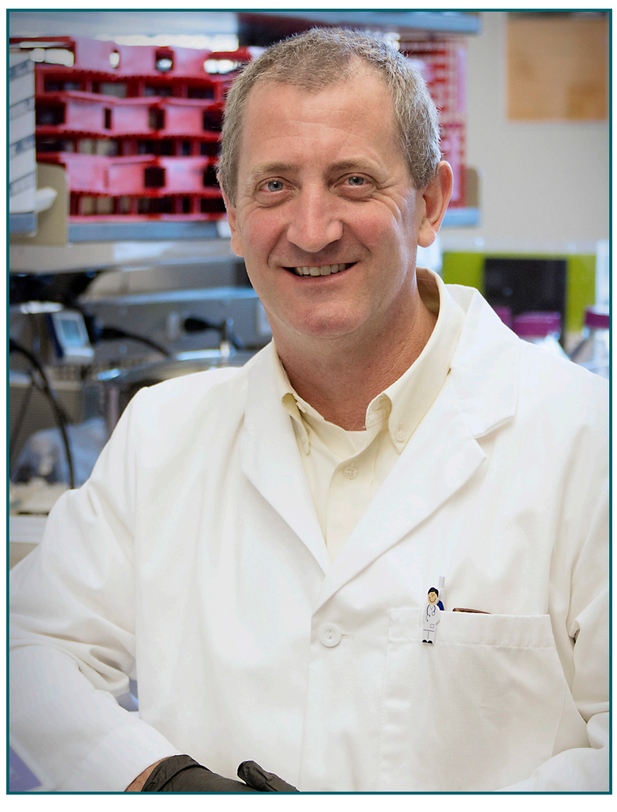
Awards Presentation, Graduate Student and

Post Doc Platforms, and Keynote Address

Thursday, April 5, 2018

Noon – 2:00 pm Wexner Auditorium

"Use, Overuse and Misuse of Antibiotics in Food Animal Agriculture: Do Bacteria Know the Difference; if so, How do They Let us Know?"



H. Morgan Scott, DVM, PhD

Professor, Epidemiology

Texas A&M University

Poster Judging:

April 4th, 2 – 5 pm for professional students

April 5th, 8 – 10:30 am for graduate students

THE OHIO STATE UNIVERSITY

COLLEGE OF VETERINARY MEDICINE

Office of Research
and Graduate Studies

IMID – 25

Platform Presentation

ZIKA VIRUS DOWN-REGULATES AXL AND TIM-1 FOR OPTIMAL SPREAD.

J. Yu, Y.M. Zheng, and S.L. Liu. Depts. Of Veterinary Biosciences

The interplay between the virus and host largely determines the outcome of viral infections. The phosphatidylserine (PS) receptors, AXL and TIM-1, have been recently shown to be candidate entry factors for ZIKV infection in vitro. However, if and how ZIKV infection regulates these entry factors remains unknown. Here we examined AXL and TIM-1 expression following ZIKV infection of human alveolar basal epithelial cell line A549, glioblastoma cell line U87, as well as primary neuron progenitor cell and primary trophoblasts. Our results showed that both the Asian strain ZIKV strain FSS13025 and the African ZIKV strain MR766 down-regulate AXL, and to a lesser extent, TIM-1 expression in viral infected cells. Furthermore, we observed that multiple ZIKV proteins, including E, NS2A, NS3 and NS4B, are responsible for this down-regulation. Additional experiments showed that down-modulation of AXL and TIM-1 can be overcome by treating cells with lysosomal inhibitor NH4Cl and autophagy inhibitor 3-MA. Moreover, we showed that the downregulation of AXL and TIM-1 expression reduces ZIKV superinfection, and diminishes host cell death and innate immune signaling. Our findings reveal that ZIKV down-regulates their candidate entry factors, thus allowing for optimal viral spread and pathogenesis.

Keywords: ZIKV, AXL, Tim1, Down-regulation

IMID – 2

Platform Presentation

IDENTIFICATION AND CHARACTERIZATION OF NOVEL TAX-1 INTERACTING PROTEIN, SNX27, AND ITS ROLE IN HTLV-1 PATHOBIOLOGY

J. Al-Saleem[1,2], M. Kvaratskhelia[3], L. Ratner[4], and P. L. Green[1,2]
[1]Center for Retrovirus Research, The Ohio State University, Columbus, OH, USA; [2]Department of Veterinary Biosciences, The Ohio State University, Columbus, OH, USA; [3]Division of Infectious Diseases, School of Medicine, University of Colorado Denver, Aurora, CO, United States; [4]Division of Oncology, Washington University, St Louis, MO, USA

HTLV-1 and HTLV-2 are highly related viruses, with differential pathogenic outcomes in humans. While HTLV-1 is associated with several diseases, such as adult T cell leukemia, HTLV-2 is not associated with disease. The trans-activator of HTLV-1, Tax-1, has higher transforming potential then its HTLV-2 homolog, Tax-2. It is believed that this difference in transforming capacity plays a pivotal role in HTLV-1 pathogenesis. We propose that Tax-1 interacts with cellular gene products via domains lacking in Tax-2, and that these interactions contribute to pathogenesis. We performed proteomic screens of Tax-1, Tax-2, and Tax-1 mutant binding partners to identify specific interactions and the critical binding domain. Novel interactions were confirmed and mapped by co-immunoprecipitation studies and further characterized by biochemical and biologic assays. We identified a novel cellular interacting partner of Tax-1, Sorting Nexin 27 (SNX27). SNX27 regulates the localization and expression of transmembrane proteins via interactions with its PDZ domain. SNX27 has been demonstrated to regulate glucose transporter 1 (GLUT1), and SNX27 knock down in HeLa cells results in a dramatic redistribution of GLUT1 from the cell surface to the lysosome. GLUT1 serves as one of three receptor molecules for HTLV-1. We proposed that Tax-1 alters GLUT1 localization post-infection via its interaction with SNX27. We confirmed that Tax-1 and SNX27 interact via their PDZ domain binding motif and PDZ domains, respectively. We further show that SNX27 expression levels are inversely related to virus release and that GLUT1 surface localization and glucose transport function is reduced by Tax-1 overexpression. This work demonstrates a novel mechanism by which HTLV-1 regulates a surface receptor molecule post-infection and this interaction could serve as a target to inhibit viral spread.

Keywords: HTLV, Tax, SNX27, GLUT1

CLINICAL RESEARCH

CR – 1

CANINE INTRADERMAL TEST THRESHOLD CONCENTRATION VARIATION BETWEEN LOTS AND MANUFACTURERES FOR SEVERAL SPECIES OF ALLERGEN EXTRACTS. S.B. Abrams*, G.N. Brock†, M. Palettas†, M.L. Bolner‡, T. Moore-Sowers‡, G.A. Plunkett‡, L.K. Cole*, S.F. Diaz* and G. Lorch*

*Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, 601 Vernon L. Tharp St., Columbus, OH, 43210, USA
†Center for Biostatistics, Department of Biomedical Informatics, The Ohio State University, 1800 Cannon Dr., Columbus OH, 43210, USA
‡ ALK-Abelló, 1700 Royston Dr. Round Rock, TX, 78664, USA

One of the challenges in veterinary aeroallergy is the variability of the aqueous allergen extracts used for intradermal testing (IDT). Optimal extract threshold concentrations (TCs) for IDT is defined as the highest concentration of allergen that results in positive reactivity in ≤10% of a normal population to minimise false positive and negative reactions in dogs with atopic dermatiits. Limited information is available on IDT TCs for common allergens from different allergen extract suppliers. The objective of this study was to determine and compare the TCs for IDT allergen extracts in healthy dogs using extracts from two allergen suppliers. We hypothesized that IDT TCs would vary according to allergen extract and manufacturer. Allergen suppliers were ALK-Abelló and Greer. IDT was performed in 35 healthy non-allergic dogs. Eleven allergens from each cmopany were diluted to six protein nitrogen unit concentrations. Reactions were scored subjectively on a scale of 0 to 4+, and objectively as the average of the vertical and horizontal diameter of each reaction. Threshold concentrations were defined as the highest concentration of an allergen where ≤10% of dogs had a positive reaction (≥2+) at 15 minutes. Using generalized estimating equations, the TCs of lamb's quarter, American elm, black walnut, box elder, red cedar, white oak, Johnson grass, Timothy grass and Dermatophagoides farinae were determined for ALK-Abelló extracts. The TCs for ragweed, lamb's quarter, English plantain, American elm, black walnut, box elder, Johnson grass and Timothy grass were identified for Greer extracts. Percent concordance of the objective measurement with the subjective score was 77.3% for ALK and 75% for Greer allergens. As TCs differed between allergens and manufacturers, not all allergens are interchangeable nor should all allergens in an IDT panel be used at the same concentration.

Keywords: Canine atopic dermatitis, allergen threshold concentrations

CR – 2

ESTICULAR HARVEST AS A TOOL TO INCREASE MILT AVAILABILITY IN SAUGER (SANDER CANADENSIS). B. Blawut [a], C. R. Moraes [a], S. A. Ludsin [b], B. Wolfe, S. Hale [c], Rich Zweifel [c], D. Sweet [c], M.A. Coutinho da Silva [a]

[a] Department of Veterinary Clinical Sciences, Ohio State University; [b] Aquatic Ecology Laboratory, Ohio State University; [c] Ohio Department of Natural Resources, Division of Wildlife

The objective of this study was to evaluate testicular harvest as an alternative to strip spawning to maximize milt availability in an economically important sport fish, Sauger (*Sander canadensis*). To accomplish these objectives, we collected milt from 20 male sauger using two techniques: strip spawning (SS) and testicular harvest (TH). On the first day (Day 0), milt was collected from 20 wild-caught male Sauger using the SS technique. On Day 5, fish were randomly divided into two groups: Group TH: fish (n=10) were euthanized and milt was collected by TH; Group SS-TH: milt was collected by SS (n=10). Five days later (Day 10), the same fish were euthanized and milt was collected using TH. We compared total sperm production and motility characteristics among the collection methods. Total (70.11 ± 2.11 % vs. 44.27 ± 5.68 %) and progressive motility (18.49 ± 1.63 % vs. 8.05 ± 1.43 %) were higher in TH than in SS ($p < 0.001$). TH samples contained fewer sperm with normal morphology (76.41 ± 1.28 %) than in SS (92.83 ± 0.96 %, $p < 0.001$). TH increased the total number of sperm collected (55.40 ± 3.55 x 10^9 sperm) compared SS collection (7.71 ± 2.67 x 10^9 sperm, $p < 0.001$). One stripping coupled with TH resulted in a 10-fold increase in the number of potentially fertile sperm (motile, morphologically normal) compared to multiple strippings alone (39.45 ± 4.10 x 10^9 sperm vs. 3.61 ± 4.10 x 10^9, $p < 0.001$). Additional strippings prior to TH did not increase sperm production (31.14 ± 4.90 x $10^{9,}$ $p = 0.53$). These improvements in the methods to maximize milt yield from each male are important first steps to stabilizing yearly hatchery production of this economically important sportfish independent of spring weather patterns.

Keywords: Hatchery, Sauger, Spermatozoa, Testicular, Milt, Production

CR – 3

ASSESSMENT OF FERTILITY IN MALE CATS THROUGH CYTOLOGIC EVALUATION OF TESTICULAR CYTOLOGY. C. Cordero Aponte[1], S. Horvath[2], D. Pontius[2]. C. Premanandan[1] [1]Department of Veterinary Biosciences, [2]Department of Veterinary Clinical Sciences.

Assessment of male feline fertility can play an important role in breeding management and biomedical research. Samples collected to assess fertility in tomcats are evaluated for characteristics such as concentration, progressive motility and morphology. Obtaining semen samples can be a difficult task and are usually low in volume. Testicular biopsies can also be utilized to assess testicular function, but these may result in post-biopsy complications that can affect fertility. It has been proven that testicular fine needle aspiration (FNA) serve as a tool for evaluation of fertility in reproductive settings. Compared to testicular biopsies, FNA has been proven to be a less invasive and quicker approach to obtaining valuable information regarding fertility in multiple species. In this study, testicles were collected post-orchiectomy from fifty cats ranging from 6 months to 6 years of age. Each testicle was aspirated for cytology and sectioned for impressions smears and histological evaluation. Two hundred cells were counted per slide and identified appropriately. The quantified cell populations were used to establish a Sertoli cell index (SEI) and sperm cell index (SI) for each testicle. The testicular cytology counts were grouped into five groups according to age and analyzed statistically. Our results showed slight variations between aspirates and impressions for all post-pubescent testes, but particularly in cats of one year of age. Only Sertoli cells were observed in pre-pubertal samples. The groups consisting of testes from older felines displayed a more consistent range of indices and SEI may be a more valuable index for providing information about potential problems affecting spermatogenesis due to less variation between cats presenting normal testicular pathology. SEI was found to be much higher in cats with testicular degeneration compared to cats with normal testicular pathology. In conclusion, testicular FNA can be an effective adjunct method of assessing fertility in male cats.

Keywords: spermatogenesis, feline, aspirate, testicular, cytology

COMPARISON OF SMALL VOLUME BOLUS ADMINISTRATION OF HYPERTONIC SALINE, COLLOID, AND HYPERTONIC SALINE-COLLOID COMBINATION IN DOGS WITH ISOFLURANE-INDUCED HYPOTENSION. K. Gerken, T. Aarnes, P. Lerche, R. Bednarski, T. Wittum, E. Cooper. Dept. of Veterinary Clinical Sciences.

The objective of this study was to determine the effect of small volume boluses of hypertonic saline, synthetic colloids, or a combination of the two on systolic arterial blood pressure in dogs with isoflurane-induced hypotension. Seven healthy adult purpose bred dogs were anesthetized on three separate occasions in a randomized, crossover design. Hypotension was reversibly induced using increasing isoflurane concentrations with a target systolic blood pressure of 70-80 mm Hg. Heart rate, arterial blood pressures (systolic, diastolic, and mean), end tidal carbon dioxide, end tidal isoflurane concentrations, perfusion index and plethysmographic variability index via pulse oximetry, mean right atrial and mean pulmonary arterial pressures, and cardiac output by thermodilution were measured. Hemodynamic parameters were assessed at baseline then at intervals (5, 10, 15, 30, and 60 minutes) after administration of a 3 ml/kg bolus of hypertonic saline, synthetic colloid (hydroxyethylstarch), or hypertonic saline-colloid combination over 5 minutes. Data were analyzed using a linear mixed model with the dog treated as a random effect. Multiple comparisons between time periods were performed using Tukey's multiple pairwise comparison method. Statistical significance was set at $p<0.05$. Administration of a 3 ml/kg bolus of hypertonic saline or colloid did not significantly improve the systolic arterial blood pressure or other hemodynamic parameters, nor did the hypertonic saline-colloid combination. Small volume administration of hypertonic saline, colloid, or hypertonic saline-colloid combination did not improve hemodynamic function of dogs with sustained isoflurane-induced hypotension.

Keywords: colloids, hypertonic saline, cardiac output, anesthetic hyportension, plethysmography

CR – 5

ATURAL HISTORY AND LONG-TERM CLINICAL OUTCOMES IN DOGS WITH CERVICAL SPONDYLOMYELOPATHY. N. Gosselin and R. da Costa. Veterinary Clinical Sciences.

Cervical spondylomyelopathy (CSM), also known as wobbler syndrome, is the most common disease affecting the cervical spine of large and giant breed dogs. CSM is characterized by static and dynamic compression of the spinal cord, nerve roots, or both in the cervical region. There are two forms of this disease: disc- and osseous-associated CSM. Disc-associated CSM occurs most commonly in mature or geriatric large breed dogs, while osseous-associated CSM occurs most commonly in young giant breed dogs. Current treatment options for CSM include surgery, of which there are over 30 techniques reported, or medical management. Holistic treatments, such as acupuncture, are frequently sought after by owners as well. No consensus currently exists on the best treatment for dogs with CSM. At present, only one study reports long-term clinical outcomes in medically-treated dogs with osseous-associated CSM (n=7)1.

Owners of previous medically-managed CSM patients seen at the Ohio State University Veterinary Medical Center in the last 8 years were telephoned, and interviews were conducted with regard to the dog's current status, overall quality of life, and date and cause of death. The objective of this study is to gain a better understanding of canine osseous-associated CSM patients' long-term welfare across various treatment modalities. Because canine CSM is the natural model for cervical spondylotic myelopathy in people, this study can assist both medicine and veterinary medicine in developing the best methods to manage and treat these diseases.

Keywords: Wobbler, Cervical, Spondylomyelopathy, Spinal, Compression

CR – 6

PERI-ANESTHETIC RISKS IN BRACHYCEPHALIC DOGS.

M. Gruenheid, TK Aarnes, MA McLoughlin, EM Simpson, DA Mathys, DF Mollenkopf, TE Wittum

Anesthesia of brachycephalic dogs is believed to carry greater risk of morbidity and mortality. The aim of our study was to determine if brachycephalic dogs are predisposed to peri-anesthetic complications compared to non-brachycephalic breeds, and to estimate the prevalence and risk factors of peri-anesthetic complications in brachycephalic dogs. Our hypothesis states that brachycephalic dogs would be at a greater risk for peri-anesthetic complications compared to non-brachycephalic dogs. Brachycephalic dogs and non-brachycephalic dogs anesthetized between January and December 2012 were matched for invasiveness of procedure, with lesser priority for age, weight and sex. Ordinary logistic regression models were used to compare the odds of peri-anesthetic complications. A total of 446 dogs showed a 49.1% prevalence of anesthetic complications and an 8.7% prevalence of post-anesthetic complications. Post-anesthetic complications occurred in 13.9% of brachycephalic and 3.6% of non-brachycephalic dogs. Risk factors associated with a greater complication rate included brachycephalic status, ASA status, induction agent, invasiveness of the procedure, and anesthetic duration. The odds for developing anesthetic complications (OR = 1.57, P = 0.03) and post-anesthetic complications (OR = 4.33, P = 0.001) were greater for brachycephalic dogs compared to non-brachycephalic dogs. Odds for developing post-anesthetic complications were greater for dogs undergoing an invasive procedure (OR = 5.21, P = 0.002), for dogs with an ASA score of III (OR = 3.79, P = 0.01), and for dogs receiving ketamine-benzodiazepine for anesthetic induction (OR = 4.45, P = 0.01) compared to propofol ± lidocaine. Study limitations are related to retrospective study design.

Keywords: Brachycephalic dogs, Anesthesia, Complications

CR – 7

ANTHELMINTIC EFFICACY AGAINST PERSISTENT *ANCYLOSTOMA CANINUM*

L. Hess, L. Millward, and A. Marsh. Departments of Veterinary Preventive Medicine and Veterinary Clinical Sciences.

Monitoring the efficacy of anthelmintic treatments is an important aspect of small animal practice to ensure that treatments are effective and to monitor possible drug resistance in small animal nematodes such as *Ancylostoma caninum*. Drug resistance in livestock and equine nematodes is well recognized and is a significant problem. Drug resistant nematodes are not thought to be as common in small animals. It is unknown how frequent or widespread drug treatment failure is in dogs because there is a lack of subsequent routine follow-up fecal analysis after standard deworming procedures. Deworming dogs is an important public health measure as both *A. caninum* and *Toxocara canis* are zoonotic parasites. The study aimed to determine the efficacy of anthelmintic treatments for 8 cases of persistent or refractory hookworm infection in greyhound dogs. Dogs were treated with a combination of Drontal Plus and Advantage Multi and followed to evaluate drug efficiency and clearance of *A. caninum*. Feces were collected pre-treatment and 7-10 days post-treatment. A Modified Stoll test was performed on fecal samples to determine *A. caninum* egg counts and to calculate fecal egg count reduction. PCR-RFLP confirmed *A. caninum* identification. All 8 cases in the study achieved a negative fecal sample after 1 to 5 sequential monthly combination treatments. Of those 8 dogs, 5 continued to remain negative until the end of the study period with the use Advantage Multi monthly. A combination of Drontal Plus and Advantage Multi demonstrated efficacy in resolution of persistent patent *Ancylostoma caninum* shedding. This treatment protocol may prove beneficial for clinicians when presented with dogs shedding canine hookworm ova that appear unaffected by standard protocols. This information is especially significant for greyhounds, which can be more susceptible to resistant hookworm infections and have decreased steady state tissue levels of anthelmintic medications due to their naturally lean body condition.

Keywords: Nematodes, anthelmintic, drug efficacy, public health

CR - 8

CANINE REDUNDANT DORSAL TRACHEAL MEMBRANE OR OVERLYING ESOPHAGUS? K.E. Holland, W.T. Drost, A.E. Schkeeper, and J.D. Bonagura. [1]Veterinary Clinical Sciences, The Ohio State University, Columbus, OH 43210.

Introduction/Purpose

12% of dogs undergoing survey thoracic radiography at our facility have a smoothly margined, broad-based soft tissue opacity (STO) summating with the dorsal aspect of the trachea on lateral projections. This STO may represent either a redundant/invaginated dorsal tracheal membrane (DTM) or superimposition of the esophagus. Our specific aim was to use computed tomography (CT) to determine the source(s) of the STO identified on lateral thoracic radiographs.

Methods

Twenty dogs with no clinical signs of tracheal collapse and no history of coughing were recruited. Ten dogs had STO over the trachea on at least one lateral thoracic radiograph and ten dogs had no STO. Sedated right and left lateral CT scans were performed from the base of the skull through the caudal extent of the lungs. Two blinded observers evaluated the survey radiographs and transverse CT images. Tracheal dimensions were measured by a third unblinded observer. These measurements were used to calculate ratios of minimal tracheal luminal height and area to the normal values.

Results

The tracheal luminal height ratios were significantly smaller in the affected group than the control group with $p<0.05$. The esophagus was positioned along the side of the trachea in at least one site in all dogs. In all but one of the affected dogs, the esophagus or hypaxial musculature indented the dorsal tracheal membrane, deforming the tracheal lumen.

Discussion/Conclusion

We propose that the term “cradling” be used to describe the indentation of the flexible dorsal tracheal membrane by the esophagus or hypaxial musculature—the presumed source of radiographic STO. The clinical significance of STO was not addressed in this study. As the esophagus was positioned lateral to the trachea on CT images in all dogs, including the control dogs, superimposition of the esophagus should no longer be suggested as the cause of this STO.

Keywords: CT, Redundant dorsal tracheal membrane, Cradling

CR – 9

INTEROBSERVER AGREEMENT OF MECHANICAL SENSORY THRESHOLDS IN NORMAL DOGS. A. Kerns, L. Cook, N. Kieves, S. Moore

Electronic von Frey anesthesiometry (VFA) has been previously reported by our laboratory and others as a useful method of mechanical quantitative sensory testing (QST) for evaluating neuropathic pain in dogs. Intraobserver agreement has been previously shown to be good to excellent; however, interobserver agreement has not been previously reported and is vital to the use of this technique in multicenter veterinary clinical trials in neuropathic pain. The goal of this study was to evaluate the interobserver agreement of sensory thresholds obtained using electronic VFA in a group of normal small breed dogs.

Twenty healthy dogs (< 20kg) were recruited from the general practice population at the Ohio State University Veterinary Medical Center. Three novice evaluators used an electronic von Frey device (IITC Systems) to measure mechanical sensory threshold (ST) after a training session conducted by an expert evaluator. Each dog was evaluated by all three investigators on the same day with both evaluator and limb test order randomized and testing sessions separated by 5 minutes.

Mean ST values were averaged for all four limbs to produce a single value per dog for comparison between evaluators. Agreement between evaluators was determined using the intra-class correlation coefficient (ICC; two-way model for consistency, single measures). ICC across all three evaluators was 0.48, indicating moderate agreement. Moderate interobserver agreement is likely not sufficient to support the use of this technique in multi-center clinical trials, and our results underscore the importance of using a single evaluator for this QST technique in canine neuropathic pain studies.

Keywords: Quantitative sensory testing, von Frey anesthesiometry, Sensory threshold, Intraclass correlation coefficient, canine

CR – 10

PLASMA CYTOKERATIN-18 LEVELS AS NON-INVASIVE BIOMARKER OF EARLY GASTROINTESTINAL TOXICITY IN DOGS RECEIVING TOCERANIB. RL Kovac, GA Ballash, JM Fenger, CA London, EE Warry.

Toceranib phosphate is a tyrosine kinase inhibitor (TKI) frequently used in veterinary medicine for treatment of mast cell tumors. Gastrointestinal (GI) toxicity is the most commonly encountered side effect of this drug. There are no markers to identify dogs that would benefit from the use of concomitant medications to prevent GI toxicities. Cytokeratin 18 (CK18) is expressed in epithelial cells, notably gastrointestinal mucosal epithelium. Epithelial cells undergoing apoptosis release CK18 that is detectable in plasma. Data from human lymphoma studies indicate that high plasma CK18 expression correlates with patients experiencing epithelial toxicity, suggesting that circulating CK18 may have utility as predictive biomarker for gastrointestinal toxicity. Therefore, the objective of our study was to determine plasma CK18 levels in dogs with mast cell tumors (MCTs) receiving single-agent toceranib therapy as a possible means for the detection of GI toxicity. Blood was collected from 20 healthy dogs to establish a reference range for plasma CK18 levels. 25 client-owned dogs with a diagnosis of MCT were enrolled. Patients were treated with toceranib (2.75 mg/kg EOD) and plasma was collected at days 0, 7, 14, 21, and 28. Drug-related adverse events (AEs) were defined and graded according to the published VCOG-CTCAE criteria. Plasma CK18 measurements were obtained using a commercially available canine enzyme-linked immunosorbent assay (ELISA) kit to detect CK18 (ABClonal). Determination of plasma VEGF concentrations in treated dogs was performed using a canine VEGF Quantikine ELISA kit (R&D Systems) as a surrogate biomarker of pathway inhibition by toceranib. No changes in plasma CK18 were correlated with toceranib-related adverse effects and/or grade. However, of the dogs experiencing gastrointestinal toxicity, most were mild. These data suggest that plasma CK18 is not a suitable biomarker for the early detection of toceranib-related gastrointestinal toxicity.

Keywords: canine, biomarker, mast cell tumor , cytokeratin 18

CR – 11

EFFECTS OF PERIOPERATIVE BUPIVACAINE AND BUPRENORPHINE IN A RAT THORACIC SPINAL CONTUSION MODEL. DM LeMoine[1] and D McTigue[2]
[1]Department of Veterinary Preventive Medicine, College of Veterinary Medicine and [2]Department of Neuroscience, Wexner Medical Center, The Ohio State University, Columbus, OH 43210

Spinal cord injury (SCI) affects the lives of millions of people worldwide, and research into SCI and repair is ongoing. Rat spinal contusion, the most common SCI animal model, requires surgery which may cause postoperative pain. Many investigators are approved to withhold analgesics based on reported lack of sensation following SCI, anesthetics used, and potential confounding effects on the research. However, there are no reports evaluating the impacts of perioperative analgesics on pain behaviors and limited reports assessing impacts on functional and molecular outcomes in this model. Female Sprague Dawley rats (n=48) underwent thoracic spinal contusion, either with analgesia comprised of bupivacaine (4 mL/kg SC) and varying regimens of buprenorphine (0.05 mg/kg buprenorphine HCl SC q8h for 24h or 72h, or 1.2 mg/kg slow-release buprenorphine) or with matching doses of sterile water. Semi-quantitative cageside assessment scores were elevated postoperatively ($p<0.05$) and returned to baseline at 4-5d, but were unaffected by treatment, perhaps because the scale assesses other components of well-being in addition to pain. Rat Grimace Scale (RGS) scores indicate increased scores relative to baseline in both groups ($p<0.05$), with delayed onset of pain in treated animals. On the day of surgery, treated animals displayed greater movement time than controls ($p<0.05$), suggesting these animals experienced less pain on the day of surgery. Treatment did not significantly impact locomotor recovery over time ($p=0.5$), white matter sparing in the spinal cord ($p>0.05$), or expression of genes involved in pain signaling and Toll-Like Receptor pathways in the spinal cord ($p>0.2$). These data indicate that rats exhibit mild-moderate pain following thoracic spinal contusion procedures that should be treated, and that perioperative analgesia with bupivacaine and buprenorphine is not contraindicated for the studied experimental outcome measures.

Keywords: Spinal cord injury, rat, analgesia, pain management, Rat Grimace Scale, locomotor recovery, RNA

CR – 12

CRYOPRESERVATION OF DIROFILARIA IMMITIS MICROFILARIA-INFECTED BLOOD FOR TEACHING DIAGNOSTIC TECHNIQUES AND PRACTICING PARASITE IDENTIFICATION. S. Long and A. Marsh. Dept. of Veterinary Preventitive Medicine.

Diagnosis of Dirofilaria immitis, the causative agent of Heartworm, is critical as this disease can be fatal, is expensive to treat, and undiagnosed dogs serve as an infection source. Heartworm spread is influenced by factors like climate change, wildlife reservoirs, mosquito populations, and undiagnosed dogs. Diagnostics is confounded by antibody-antigen blocking causing false negative antigen and antibody testing; therefore, microfilaria detection is important for diagnostics. However, obtaining fresh blood containing D. immitis microfilaria when scheduled to teach the technique presents a challenge. To address the issue, this research evaluated using cryopreserved blood in two standard diagnostic tests, Modified Knott's and carbonate filter tests. The specific aims included 1) determine if cryopreserved D. immitis microfilaria could still be detected and 2) compare the morphology of the microfilaria following cryopreservation over time. With this information, teaching laboratories, including the new CVM Clinical Skills Laboratory, could substitute cryopreserved microfilaria for fresh blood. To evaluate the cryopreservation, two different isolates of freshly harvested whole blood containing D. immitis microfilaria were used. One aliquot of blood was removed, designated as a baseline and was processed immediately. The remaining blood was mixed 1:1 with Glycerolyte, divided into aliquots and froze at -20 °C for processing on days 7, 21, and 90. The quantity and morphology of microfilaria were compared with baseline and between aliquots. After freezing, the microfilaria was detected in lower yields compared to baseline. Interestingly, the Glycerolyte preserved red blood cells, making the cells difficult to lyse, resulting in a more difficult slide to read due to the intact cells. These results suggest that using cryopreservation to store blood containing microfilaria for teaching diagnostics will work; the visible microfilaria concentration will be less; and individual microfilaria will be more difficult to see. We plan to evaluate students' ability to perform the assays and identify microfilaria with these less than optimal samples.

Keywords: Parasitology. Education, Heartworm, Clinical Skills Laboratory

CR – 13

A NOVEL STABILIZATION TECHNIQUE FOR CRANIAL CRUCIATE LIGAMENT RUPTURE IN CATTLE. J.W. Lozier, A. J. Niehaus, C. A. Hinds, S. Durgam, S. Jones, J. Lakritz.

Cranial cruciate rupture in cattle is a common cause of lameness referred to the stifle and results in gains, production, and long term use of the animal. Several techniques to stabilize the stifle joint in cattle suffering from cranial cruciate rupture have been developed, but are either not appropriate for mature cattle, or have a high complication rate, often leading to failure. The aim of this study was to develop a novel stifle stabilization technique that would be appropriate for large cattle and mitigate catastrophic complications, particularly septic arthritis, by placing an extracapsular prosthesis through bone tunnels in the femur and tibia. Isometric points in the femur and tibia were determined with lateral radiographs to determine the optimal location for placement of the prosthetic implant. Nylon leader line (800lb test) and stainless steel crimp configurations were distracted to rupture to determine the optimum prosthesis. Bone tunnels were created in the locations deemed optimal by the radiographic study, and the optimum prosthetic/crimp configurations were placed through the bone tunnels in 4 live cattle. Lameness was graded weekly for 3 months. Bone tunnel placement was determined to be distal and caudal on the femoral condyles, and cranial and proximal on the tibial tuberosity. Nylon leader line looped and crimped with 3 stainless steel crimps was found to be the optimum configuration. Lameness of cattle with stabilized stifles was not significantly different than lameness of cattle not stabilized. However, all 8 cattle had medially luxating patellas at the time of slaughter. This technique needs to be implemented in patients with naturally occurring disease to determine its effectiveness for stabilizing cranial cruciate ligament deficient stifles in cattle.

Keywords: Cattle, Bovine, Cranial cruciate ligament, Lameness

CR – 14

BEHAVIORAL VALIDATION OF ANALGESIA OF CARPROFEN IN THREE FORMULATIONS IN A MOUSE CELIOTOMY MODEL. M. McKeever, K. Brannick, C. Freed, University Laboratory Animal Resources, The Office of Research, The Ohio State University, Columbus, Ohio.

Approximately 95% of animal research is based on mouse and rat models, but analgesic practices for rodents are often extrapolated from other species. In laboratory animal medicine, the clinician is guided by the tenets of the “3 R’s,” replacement, reduction, and refinement. Assessing appropriate pain control in rodent models is an area to apply empirically based refinement in both methodology and dosing. Parenteral and oral administration of non-steroidal anti-inflammatory drugs (NSAIDs) is common practice; injectable administration requires repeated restraint and may increase stress, while oral formulations allow for variable consumption levels. These differences may result in inconsistent analgesic efficacy. The purpose of this study was to confirm analgesic efficacy of two oral Carprofen options relative to an injectable formulation post-celiotomy, and to report practical administration differences among the three modalities of pain control. Twelve-week-old C57BL/6Crl male mice (n=27) were housed three per cage and provided Carprofen using one of the following delivery methods: medicated water (0.02mg/mL), medicated gel (0.025mg per gram), or a daily injection (0.22mg). The target dose was 5mg/kg/day for a 25-gram mouse. Carprofen was provided 24 hours prior to the celiotomy procedure through 72 hour post-op, and each animal received a single dose of buprenorphine (0.1 mg/kg) at surgery. A 1-1.5cm-midline incision was created under isoflurane anesthesia, and a retractor placed for 3 minutes prior to closure. Individual animal weights and cage level food, gel, and water intake were measured daily. Analgesic efficacy was evaluated using activity levels in open field testing (OFT), the mouse grimace scale (MGS), and time-to-integrate-to-nest test (TINT). Provision of medicated gel resulted in the lowest average mouse dose per cage, ranging from 1.78-5.72mg/kg relative to medicated water (4.72-9.78mg/kg) and injectable (4.86-5.61mg/kg). Although there was significant variability between dose ranges for each group, no statistical differences were found between treatment groups for all behavioral parameters, suggesting both oral delivery methods of Carprofen provided comparable post-surgical analgesia with daily injections in a mouse celiotomy model. The practical information compiled from dosing in three modalities aims to guide principal investigators in choosing an appropriate analgesic regimen for rodents models.

Keywords: Mouse, Celiotomy, NSAID, Carprofen, Analgesic efficacy, Pain control, Behavioral validation

CR – 15

CRYOTHERAPY IN AN INDUCED LAMENESS MODEL: IS THERE ANY ANALEGESIA? V. Quam, J. Yardley, C. Fahy, C. Paz, J. Belknap. Department of Veterinary Clinical Sciences.

Ice is a common therapeutic used on horses in competition, but the analgesic effect of cryotherapy in horses has not been explored. A cross-over study including 10 horses was conducted to objectively evaluate the change in degree of lameness following one hour of cryotherapy in horses with induced lameness.

A moderate (visible) lameness was induced using a well-described lameness model, in which reversible heel pain is created with set-screws inserted into a custom-made shoe. The degree of lameness was recorded with the Lameness Locator, a non-invasive commercial product using inertial sensors. In the treatment condition, horses were placed in a Soft Ride boot with an ice slurry for 60 minutes following induction of a lameness, whereas the limb was kept in a Soft Ride boot at ambient temperature for 60 minutes in the control condition. After 60 minutes in the treatment or control condition, the degree of lameness was recorded at 5, 10, 15, 20, 30, 45, and 60 minutes post-treatment.

The degree of lameness at each of these time points was compared to the degree of lameness present immediately following placement of the set screw using a Wilcoxon sign test to assess analgesic effect between matched pairs. After 60 minutes in ice, horses had a greater degree of lameness reduction relative to those in the control condition at the first assessment, 5 minutes post-treatment. There was no substantial difference in lameness reduction between groups at any other time point.

The degree to which Soft Ride boots contribute to lameness reduction in both conditions is a proposed area of further inquiry. There is evidence that ice provides some transient analgesia in horses, but additional research is required to determine the ideal method, duration, and temperature for optimizing athletic performance and minimizing lameness.

Keywords: Cryotherapy, Sports Medicine, Lameness locator, Induced Lameness

CR – 16

VALIDATION OF PROTEIN ARGININE METHYLTRANSFERASE 5 (PRMT5) AS A CANDIDATE THERAPEUTIC TARGET IN THE CANINE MODEL OF NON-HODGKIN LYMPHOMA. K.A. Renaldo[1], L.E. Courtney[1], K Shilo[2], R.A. Baiocchi[3], W.C. Kisseberth[1]. [1]Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH; [2]Department of Pathology, The Ohio State University, Columbus, OH; [3]Division of Hematology, Department of Internal Medicine, The Ohio State University, Columbus, OH

Lymphoma is a common, aggressive malignancy in dogs. While generally responsive to treatment with traditional cytotoxic drugs, remission times are short and cures rare. New treatment strategies are therefore needed. Protein arginine methyltransferase 5 (PRMT5) is a type II protein arginine methyltransferase (PRMT) enzyme whose relevance to cancer biology has become increasingly evident. In this study, we characterized patterns of PRMT5 expression in canine lymphoma and correlated these with histologic subtype using tissue microarrays (TMAs). We characterized expression of PRMT5 in canine lymphoma tissues and methylation targets in canine B-cell lymphoma cell lines and treated cells with different PRMT5 inhibitors to determine effects on PRMT5, target proteins and antitumor activity. We also assessed PRMT5 inhibition *ex vivo* in patient B-cell lymphomas via flow cytometry and apoptosis with annexin V/PI staining. Canine diffuse large B cell lymphoma showed cytoplasmic staining for PRMT5 (48.8% strong, 50.0% weak, n = 165) compared to negative or weak staining in normal and hyperplastic lymph nodes (n = 40). Lymphoblastic T cell lymphoma samples showed strong nuclear staining (40%, n= 10). No nuclear staining was appreciated in normal or hyperplastic lymph nodes. Similarly, canine B-cell lines showed high expression of PRMT5. The PRMT5 small molecule inhibitor PRT220 inhibited growth of CLBL-1 with IC 50 ’s of 60.1 nM and 16.6 nM at 48 hours and 96 hours, respectively. Histone (H3R8 and H4R3) methylation status was suppressed at the IC 50’s for CLBL-1. *Ex vivo* patient samples showed suppression of growth at 24 hours at concentrations of 1 uM and 10 uM. In conclusion, we demonstrated that PRMT5 is expressed in canine lymphomas and PRMT5 inhibition can suppress the growth of canine lymphoma cell lines, supporting the continued use of the spontaneous canine lymphoma model for the preclinical development of PRMT5 inhibitors.

Keywords: lymphoma; PRMT5, protein arginine methyltransferase 5, tissue microarray

CR – 17

IMPACT MODEL AND SUBCHONDROPLASTY FOR EQUINE PALMAR OSTEOCHONDRAL DISEASE. H. Rice, A. Bertone, M. Brokken, E. Hostnik

Objective: To create an impact model of palmar osteochondral disease in the horse and evaluate the application of a new injectable bio-absorbable bone substitute material (BSM) into the medial condyle to support the damaged subchondral bone and overlying cartilage. Methods: The distomedial third metacarpal condyle was exposed and a compressive lesion was created using 80 psi (27.6 MPa) in both forelimbs of 6 healthy horses. A single forelimb was randomly selected for injection of BSM into the compressive lesion (treatment limb). MRI of the forelimb metacarpophalangeal joints was performed pre- and post-compression and/or BSM injection. Horses were euthanized under general anesthesia. Samples were obtained for histomorphometric evaluation. MRI slices and 3D reconstructed images were analyzed. Results: A small region of disruption to the cartilage was visible on MR slices in the location of lesion in all limbs. No evidence of bone edema or hemorrhage was visible on the post-procedure MRI images for control limbs, but inflammatory cells, hemorrhage and microfractures were present histologically. BSM was visible in all post-MRI images (treatment limb) and distributed to the area of injury. No increase in inflammatory cells or damage to trabeculae was seen in treatment limbs. There was no significant difference when comparing the pixel density between pre- and post-MRI slices in control and treatment limbs in all quintile ranges evaluated. 3-D iages identified the impact injury and showed the benefit of MRI slice technology. Conclusion: Our model accurately portrays the acute stages of POD histologically; however, the lesions are not obviously evident with MRI. 3D reconstructed images are useful for evaluation of external lesion location. Evaluation of alternative or novel sequences to better assess the early diagnosis and potential alteration of disease progression.

Keywords: Palmar Osteochondral Disease, Impact Model, Subchondroplasty, Magnetic Resonance Imaging, Subchondral bone

CR – 18

RESPONSE OF THE ENTEROINSULAR AXIS IN HEALTHY NEONATAL FOALS TO FASTING AND DEXTROSE ADMINISTRATION. L.Rings, L. Dunbar, T. Burns, S. Vijan, R. Toribio Department of Veterinary Clinical Sciences

The enteroinsular axis (EIA) comprises intestinal factors (incretins) that promote pancreatic insulin release and suppress glucagon secretion. Glucagon-like peptide -1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are the main incretins. Disorders of energy homeostasis are common in sick foals and incretin alterations may contribute to energy dysregulation in critically ill foals. The EIA has been evaluated in horses, but, information in healthy and sick foals is lacking.

The goal of the study was to investigate the GLP-1 (total and active), GIP, and insulin response to fasting and dextrose (oral and intravenous) administration in healthy newborn foals. Healthy Standardbred foals (n=17) <72 hours old underwent oral and intravenous glucose tolerance tests. After 1 hour of fasting, a dextrose bolus (300 or 500 mg/kg) was administered orally or intravenously. These dextrose doses stimulate the EIA in horses. Blood incretin and insulin concentrations were measured at 0, 5, 10, 15, 30, 45, 60, 90, 120, 150, and 180 minutes by immunoassay.

GIP concentrations decreased after dextrose administration regardless of dose or route by 68-75% of baseline values ($P < 0.05$). GLP-1 total and active concentrations decreased to 52-70% of baseline values ($P < 0.05$) at 180 minutes. A mild elevation in GLP-1occurred at 5-15 minutes. There was a minor insulin response to oral but high insulin release from intravenous dextrose.

Incretin concentrations increased above baseline within 15 minutes after foals were allowed to nurse freely. The rapid GIP and GLP-1 release after nursing compared to their minimal response to oral dextrose suggests that these dextrose doses were insufficient to elicit a strong EIA stimulation (higher dextrose doses will be required) or that factors in milk (e.g. lactose, fatty acids) may be necessary to activate the EIA in the equine neonate.

Keywords: Enteroinsular, incretin, insulin, foal, equine

CR – 19

A NOVEL STRATEGY SLIT/ROBO AND CXCL12/CXCR4-MEDIATED SIGNALING MECHANISMS THAT MODULATE SMALL CELL LUNG CANCER PROGRESSION AND METASTASIS. S. Sassi[1], D. Ahirwar[2], L. Elgaddafi[2], and R.K. Ganju[2]. Depts. of Veterinary Biosciences and Pathology

Small cell lung cancer (SCLC) represents 20% of lung cancers and is characterized by early dissemination, development of chemo-resistance and a poor prognosis. SCLC is a highly aggressive malignancy with a limited spectrum of therapeutic options. Therefore, identifying early biomarkers and targets metastasis may lead to the development of innovative therapies that will improve the survival of SCLC patients. Slit2 has recently emerged as an important tumor suppressor gene in breast and other cancers and acts through Roundabout Homolog1 (Robo1) receptor. Furthermore, genome-wide association studies also indicate that Slit2 downregulation by promoter hypermethylation is closely linked with poor prognosis in lung cancer. The CXCR4 receptor is an alpha-chemokine receptor specific for CXCL12. In this study, we sought to characterize the effect of Slit2 on the CXCL12/CXCR4-mediated metastatic properties of SCLC. We demonstrate here the expression of Robo1 and Slit2 in SBC5, a highly metastatic SCLC cell line and SBC3, a low metastasizing SCLC cell line. By western blot analysis and immunofluorescence, we found that Slit2 expression was very low in SBC5 compared to SBC3 and normal epithelial cell line BEAS. We also show that Slit treatment inhibits CXCL12/CXCR4-induced SCLC cell chemotaxis and chemoinvasion, the fundamental components that promote metastasis. We found that soluble Slit2 treatment significantly reduced the number of tightly packed colonies in a colony formation assay using SBC5 cells, suggesting that Slit2 acts as a tumor suppressor in SCLC. Also, we found that Slit2 suppressed local SCLC growth and invasion in vivo. We showed that Slit2 inhibits CXCL12 and CXCR4 stimulated PI3K pathway that subsequently activates the protein kinase AKT. We assessed for the first time, the effect of the Slit2/Robo1 axis in modulating CXCL12/CXCR4-induced SCLC growth and metastasis these results may open new avenues of treating small cell lung cancer, and especially through Slit2 modulate CXCL12/CXCR4.

Keywords: Roundabout (Robo), Slit homolog 2 protein, Small cell lung cancer (SCLC), Fluorescence-activated cell sorting (FACS) , SDF-1 (stromal cell-derived factor-1(CXCL12), C-X-C chemokine receptor type 4 (CXCR-4), Human bronchial epithelium, normal (BEAS-2B)

CR - 20

EQUINE PLASMA IONIZED CALCIUM, MAGNESIUM, AND IONIZED MAGNESIUM TO CALCIUM RATIO IN SAMPLES PROCESSED AND STORED BY REGULATORY METHODS COMPARED TO A CLINICAL LABORATORY STANDARD METHOD

S. A. Schumacher[1] DVM, J. Yardley[1], DVM, C. Uboh[2] PhD, and A. L. Bertone[1] DVM, PhD

Magnesium sulfate ($MgSO_4$) is an abused substance in equine competition as a performance altering calming agent. Administration of $MgSO_4$ increases the active ionized form of magnesium (iMg) which can be measured for clinical and calming significance. Our goal was to evaluate the impact of collection method, sample processing (shipment and analysis) and long term storage on plasma iMg, iCa, pH and the iMg/iCa ratio. Our hypothesis was that there would be no significant difference in the iMg/iCa ratio in samples immediately processed in a clinical pathology laboratory and samples collected, processed and stored, and analyzed as per regulatory process. Four blood samples were collected from each of 51 horses on the same day and assigned to each of four groups; Group (Gp) 1- collection in a heparinized syringe and blood directly processed in a clinical pathology laboratory (standard); Gp 2 – collection into a plastic plasma separator tube (PST) centrifuged just prior to analysis, and plasma processed in the same clinical pathology laboratory; Gp 3- collection into a PST, shipped in a refrigerated state to a regulatory laboratory, centrifuged just prior to analysis, and plasma processed in the regulatory laboratory; and Gp 4- collection into a PST, shipped in a refrigerated state to a regulatory laboratory, centrifuged, and the PST stored frozen at -80˚C for 90 days, thawed, and plasma processed at a regulatory laboratory. Results for iMg/iCa revealed an overall mean of 0.377 SD±0.038 and was highly correlated with iMg ($r=-.933$; $p<0.01$). Samples in Gp 3 had the highest iMg/iCa mean at 0.417 SD±0.028 before decreasing in Gp 4. iMg and iMg/iCa ratio increased with processing and decreased with freezing and storage. These results support the use of the iMg/iCa ratio as the measurement of choice in the development of regulatory thresholds.

Keywords: Magnesium sulfate (MgSO4), iMg, iCa, pH

CR – 21

MEASUREMENT OF THE VENTRICULAR OUTFLOW TRACTS BY ECG-GATED COMPUTED TOMOGRAPHY ANGIOGRAPHY AND TRANSTHORACIC ECHOCARDIOGRAPHY IN DOGS WITH AND WITHOUT PULMONARY VALVE STENOSIS.

A. To, E.T. Hostnik, J.D. Rhinehart, and B. A. Scansen. Of Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio, USA. Department of Clinical Sciences, College of Veterinary Medicine & Biomedical Sciences, Colorado State University, Fort Collins, Colorado, USA.

Pulmonary valve stenosis (PS) is the most common congenital heart defect of dogs. Currently, transthoracic echocardiography (TTE) is the standard modality used to evaluate PS. Image acquisition by TTE can be challenging in some brachycephalic breeds of dogs. The use of electrocardiography-gated computed tomography angiography (ECG-gated CTA) in veterinary medicine is limited. This retrospective method comparison study investigated right and left ventricular outflow diameters by sedated ECG-gated CTA and unsedated TTE in 14 brachycephalic dogs with PS and 12 brachycephalic dogs without PS. Measurements of ventricular outflow structures were made in early systole and late diastole for both modalities and then compared for significance between systolic and diastolic phases, as well as between the two modalities. Ratios of the pulmonary trunk diameter to the aorta at different locations (aortic valve, aortic annulus, and ascending aorta) and in different planes (transverse, sagittal) were compared between dogs with PS and without PS, as well as within dogs, by both TTE and ECG gated CTA. Transthoracic echocardiography and ECG-gated CTA both detected significantly greater pulmonary trunk to aorta ratios in dogs with PS at all aortic locations ($p < 0.05$). Pulmonary valve to aortic valve ratios were significantly smaller in dogs with PS ($p < 0.05$). Pulmonary trunk to aorta and pulmonary valve to aorta ratios were achieved with good anatomic detail using ECG-gated CTA. A ratio of pulmonary trunk to aortic valve in the transverse plane/aortic annulus in the sagittal plane greater than 1.5 or a pulmonary valve to aortic valve ratio of less than 0.8 supports PS with secondary pulmonary trunk dilatation when measured by ECG-gated CTA. Ratios of the pulmonary trunk and pulmonary valve relative to the aorta may be useful to evaluate for PS using a modality that is underutilized for cardiac assessment.

Keywords: Brachycephalic, Canine, Aorta, Pulmonary Trunk

CR – 22

SAFETY AND SEROLOGIC RESPONSE TO A HAEMONCHUS CONTORTUS VACCINE IN ALPACAS. G. VanHoy, M. Carman, G. Habing, J. Lakritz, C.A. Hinds, A. Niehaus, R. Kaplan, A.E. Marsh

Haemonchosis in camelids remains a challenging disease to treat and prevent due to anthelmintic resistance. Barbervax® is an adjuvanted vaccine containing native H-11, H-gal-GP antigens (enterocyte membrane proteins involved in digesting blood meals) obtained from Haemonchus contortus adults. This vaccine is registered for use in Australian sheep and goats. There are no published studies evaluating Barbervax in camelids. The present study monitored the safety profile of Barbervax® in adolescent alpacas. Although originally designed to include efficacy, the experimental infection with viable H. contortus L3 did not result in significant increases in fecal egg shedding and this was a non-terminal study (no worm counts). Twelve alpacas were randomized to vaccination (n = 7) with Barbervax® or no treatment (n = 5). Three doses of Barbervax® were administered at 3 week intervals and investigators involved in animal monitoring and sample collection were blinded to the groupings. Clinical pathologic parameters were evaluated 7 days before vaccination, 1 and 2 months post-vaccination. Daily clinical observations included injection sites (diameter of lesion, heat, pain) and rectal temperatures were recorded in each alpaca twice daily for 7 days following vaccination. Fecal egg counts, packed cell volume, and total plasma protein was recorded following challenge with 1,500 H. contortus L3 on days 42, 46, and 50. Increases in rectal temperature for 2 days was observed post-vaccination. Vaccinated alpacas were lethargic for 2-3 days following vaccination; however, they maintained an appetite and no visible or palpable injection site reactions were observed. All animals maintained normal clinical pathologic parameters. Fecal egg counts increased in controls, but these variables were not significantly different. Vaccinated animals developed titers to the antigen as measured by ELISA. Barbervax® demonstrated safety in this small group of young, healthy alpacas. Additional studies are required to evaluate the efficacy of the vaccine under field conditions. Funding source: The Alpaca Research Foundation.

Keywords: Haemonchus contortus, Alpaca, Vaccine, Barbervax, Resistance

CR – 23

RETROSPECTIVE ANALYSIS OF INFECTIOUS KERATITIS DIAGNOSED AFTER CATARACT SURGERY IN DIABETIC AND NON-DIABETIC DOGS: 95 CASES (1998-2017). H. Wang, A. Gemensky-Metzler, R. Rowen, E. Miller, H. Chandler and G. Mitchell
Department of Veterinary Clinical Sciences

Purpose. To describe prevalence, risk factors and outcomes of infectious keratitis (IK) in diabetic and non-diabetic dogs following cataract surgery.

Methods. A retrospective review included diabetic and non-diabetic dogs that underwent cataract surgery and developed IK between 1998-2017. Clinical features, cytologic and culture results, therapy prior to IK diagnosis, medical or surgical therapy instituted, clinical outcomes and prevalence were analyzed.

Results. Seventy diabetic (85 eyes) and 25 non-diabetic dogs (27 eyes) were included. Keratoconjunctivitis sicca (KCS) had previously been diagnosed in 42/70 diabetic (49/85 eyes) and 3/25 non-diabetic dogs (3/27 eyes). Common topical treatments prior to IK diagnosis were diclofenac (57 diabetic and 15 non-diabetic eyes), 1% prednisolone acetate (43 diabetic and 13 non-diabetic eyes) and 2% cyclosporine (25 diabetic eyes). Fungi were identified on corneal cytology in 5 diabetic eyes (6%). Bacterial cultures were positive in 48 diabetic and 13 non-diabetic eyes. In diabetic and non-diabetic dogs, respectively, 71 (84%) and 26 (96%) eyes received medical therapy and 14 (16%) and 1 (4%) eyes underwent grafting surgeries. Thirteen (15%) diabetic and 6 (22%) non-diabetic eyes were enucleated due to poor prognosis. Upon IK resolution in diabetic dogs, 66% (47/71) and 79% (11/14) eyes and in non-diabetic dogs 21 (81%) and 1 (100%) retained vision remained visual following medical and surgical therapy, respectively. At last recheck, 61% (43/71) and 50% (7/14) diabetic eyes and 14 (54%) and 1 (100%) retained vision following medical or surgical therapy, respectively. Between 2006 and 2017, the prevalence of IK was 11% (79/987) overall, 16% (76/490) in diabetic dogs and 7% (3/497) in non-diabetic dogs,

Conclusion. Pre-existing KCS and topical anti-inflammatory and immunosuppressive therapy may be risk factors for IK after cataract surgery. Surgical therapy may provide a better short-term outcome compared to medical treatment. However, long-term prognosis for vision retention is guarded.

Keywords: canine, corneal ulcer, infection, phacoemulsification, inflammation, immunosuppression

CR – 24

RADIOGRAPHIC EVALUATION OF CARDIAC SILHOUETTE IN CLINICALLY HEALTHY HUMBOLDT PENGUINS (SPHENISCUS HUMBOLDTI). K. Yunker, E. Hostnik, J. Johnson, and I. Giatis. Dept. of Veterinary Clinical Sciences (Yunker, Hostnik), Animal Health Center, Columbus Zoo and Aquarium (Johnson), MedVet Medical & Cancer Center For Pets (Giatis)

Wild populations of Humboldt penguins (Spheniscus humboldti) on the coasts of Chile and Peru have been declining due to food scarcity caused by the El Niño Southern Oscillation, and human interference. Part of conserving this vulnerable and threatened species is maintaining the health of penguins within zoo collections. A variety of cardiovascular diseases have been reported in individuals from the Spheniscidae family including ventricular septal defects, Dirofilaria immitis infection, pulmonary hypertension, and valvular dysplasia ultimately resulting in congestive heart failure. An accurate clinical picture of cardiovascular disease in Humboldt penguins requires diagnostics tailored to this specific species. The aim of this study was to establish a routine methodology for evaluating the cardiac silhouette of clinically healthy Humboldt penguins using vertebral heart scale (VHS), cardiocoelomic width ratio (CCWR), and a novel cardiac silhouette to keel ratio (CKR). Ventrodorsal and right lateral radiographs were taken of 10 mature Humboldt penguins during routine health evaluations. An echocardiographic exam of each penguin was performed to confirm that there was no evidence of cardiac structural remodeling from disease. Two penguins were excluded based on echocardiographic findings; therefore, data from eight penguins were used to calculate objective cardiac measurements for the clinically healthy population. Right lateral radiographs were used to determine VHS (7.4v-10.4v) and CKR (3.4-4.4). Ventrodorsal radiographs were used for calculating CCWR (0.45-0.59). The excluded penguins had CCWRs which were outside the 95% confidence interval for the range generated by this study. This initial work supports that standardizing radiographic views provides objective measures for cardiac silhouette evaluation in this species. Further research in larger populations and comparison with birds having confirmed cardiac disease is needed to determine the value of these three measurement techniques in Humboldt penguins.

Keywords: Zoological medicine, Diagnostic imaging, Radiology, Avian

EPIDEMIOLOGY AND APPLIED RESEARCH

EAR – 1

THE DISSEMINATION OF ANTIMICROBIAL RESISTANT BACTERIA IN THE ENVIRONMENT OF A NATIONAL EQUESTRIAN COMPETITION. R. Adams, D. Mollenkopf, D. Mathys, and T. Wittum.

Elite equine competitions create a unique opportunity for the natural exchange of bacterial flora, which may harbor clinically important and mobile antimicrobial resistance genetic elements, between horses from geographically diverse locations. This may facilitate the transmission of geographically localized resistance genes into naïve equine populations. Our aim was to generate pilot data evaluating the prevalence of antimicrobial resistant Enterobacteriaceae in the feces and environment of athletes at the 2016 Quarter Horse Congress (QHC.) Feces were obtained from distinct areas within large communal manure piles during and upon conclusion of QHC. Electro-static-cloths were utilized to sample environmental surfaces commonly contracted by horses or people throughout the Ohio Expo Center on the last day of competition. Samples were enriched and inoculated onto selective media to identify Salmonella spp. and extended-spectrum cephalosporin, carbapenem, and fluoroquinolone resistant bacteria. 86%, 79%, and 67% of 43 environmental samples and 95%, 96%, and 90% of 100 fecal samples generated Enterobacteriaceae resistant to 2nd generation cephalosporin, 4th generation cephalosporin, and fluoroquinolone antimicrobials. Horse-associated surfaces harbored more resistant organisms compared to human surfaces. No Salmonella were identified from environmental sampling; 8% of fecal samples yielded Salmonella isolates. Prevalence of resistant organisms and Salmonella was greater in fecal samples obtained during ongoing competition at QHC compared to those collected on the last day of competition, although the disparity was minimal. An unusually high prevalence of Salmonella spp. and resistant Enterobacteriaceae were observed compared to our previous findings within hospital and local community equine populations. Therefore, we plan to further evaluate the colonization and fecal shedding of bacteria expressing antimicrobial resistant genotypes from sport-horses participating in national and international competitions to corroborate our results. Promoting biosecurity protocols and reducing environmental contamination at equine events will promote the welfare of competitors and safeguard public health.

Keywords: antimicrobial resistance, equine, Enterobacteriaceae

EAR – 2

THE EFFICACY OF PULSED ELECTRIC FIELD TO REDUCE ANTIMICROBIAL RESISTANT BACTERIA IN WASTEWATER. A. Albers, D. Mollenkopf, D. Mathys, T. Wittum. Department of Veterinary Preventive Medicine

Antimicrobial resistant bacteria are of concern in both human and veterinary medicine. Pulsed Electric Field uses an electric current to lyse bacterial cells and is currently used to reduce wastewater coliform counts in several wastewater treatment plants. Our objectives were to determine the efficacy of Pulsed Electric Field (PEF) as an intervention to be used in high risk environments to reduce the amount of viable carbapenemase-producing bacteria in raw influent before entering the wastewater treatment plant. Untreated influent samples were collected from a wastewater treatment plant serving Columbus, Ohio twice weekly for 24 weeks. The samples were strained to remove any large particles prior to treatment. A small aliquot of sample was assessed for conductivity, pH, and turbidity. After treatment with PEF, a 100 µl aliquot from both influent and treated samples was spreadplated onto both CHROMagar, and petrifilms to quantify the reduction of E. coli, coliforms, Pseudomonas sp., and Acinetobacter sp. The results indicate approximately a one log reduction of both coliforms and carbapenemase-producing bacteria following treatment. We observed an additional reduction of coliforms and carbapenemase-producing bacteria by using a longer treatment time. These results suggest the potential for PEF treatment to reduce the discharge of carbapenemase-producing bacteria from high risk environments such as hospital ICUs into wastewater flows and ultimately into the environment.

Keywords: Pulsed Electric Field, Antibiotic resistant bacteria, Wastewater

EAR – 3

RECOVERY OF CARBAPENEM-RESISTANT ENTEROBACTERIACEAE FROM WILDLIFE, AGRICULTURE, AND THE ENVIRONMENT IN THE SCIOTO RIVER, OHIO WATERSHED. Ballash, G., Mollenkopf D, Mathys D, Sechrist E, Albers A, Symonds D, Sullivan M, Zimmerman B, Wittum T.

Carbapenem-resistant Enterobacteriaceae (CRE) are a significant public health concern. Currently, these bacteria are isolated from individuals with recent healthcare exposure, but their prevalence in the environment and animals is unknown. Here we collected soil and crop samples, wildlife and dairy cattle fecal samples, and fish and bird vent swabs from within the Scioto River watershed, a watershed in Columbus, OH that receives treated wastewater originating from a major medical center. Samples were screened for reduced susceptibility to meropenem and carbapenemase production. Carbapenemase-producing isolates were classified using MALDI-TOF and subjected to PCR and gene sequencing, if the isolate was suspected to carry a transferable carbapenemase gene. The prevalence of carbapenemase producing isolates was 8.33% (3/36) of wildlife isolates, 26% (8/50) of crop isolates, 15.2% (10/66) of soil isolates, 11.2% (36/322) of dairy cattle isolates, 34.5% (10/29) of bird isolates and 16.2% (73/450) of fish isolates. Carbapenemase-producing isolates from the soil, crops, dairy cattle, wildlife and birds were primarily species with chromosomally-encoded carbapenemase genes conferring intrinsic resistance such as Stenotrophomonas sp. and Pseudomonas otitidis. However, 12 fish isolates were members of Enterobacteriaceae suspected to carry transferable carbapenemase genes on mobile plasmids. These findings suggest that carbapenem-resistant Enterobacteriaceae have spread beyond the healthcare setting and into the environment, likely as a result of waterborne transmission. The public health significance of these findings is not yet fully understood.

Keywords: Carbapenem, Antimicrobial resistance, Enterobacteriaceae, Wildlife, Agriculture, Environment

EAR – 4

PRESENCE AND RISK FACTORS ASSOCIATED WITH STAPHYLOCOCCUS AUREUS CARRIAGE AMONG VETERINARY HEALTH CARE WORKERS. L. Bookenberger, A. Hoet, J. Van Balen Rubio

Staphylococcus aureus is an opportunistic pathogen causing significant morbidity in both human and veterinary health care settings. Veterinarians may be at increased risk of colonization due to occupational exposures. However, risk factors associated with S. aureus carriage among veterinary health care workers are vastly uncharacterized. The purpose of this study was to determine prevalence of S. aureus among veterinary health care workers at the OSU-VMC and the associated risk factors. If the prevalence of S. aureus is indeed higher among veterinary health care workers, then the associated risk factors involved can be identified to target preventative practices. In this cross-sectional analysis, a risk assessment questionnaire was distributed and nasal and hand swabs were taken from 202 participants. Univariate analysis of associated risk factors determined from the questionnaire and positive sample swabs was completed and significant associations were reported. Of the risk factors examined, being hospital personnel, a 4th year veterinary student, and hand hygiene were significantly associated with S. aureus carriage. Odds of staph carriage among hospital personnel were 2.06 times that of those that were not hospital personnel (95% CI: 1.01, 4.17; P: 0.0393). Odds of staph carriage among fourth year veterinary students were 3.17 times that of veterinary students of any other year (95% CI: 1.14, 8.82; P: 0.0221). Odds of staph carriage among those that never or rarely washed hands between patients was 18.9 times the odds of carriage among those that reported frequently washing hands between patients, and 22.2 times that among those that reported always washing hands between patients (95% CI: 2.96, 370.5; P: 0.0010 & 95% CI: 2.46, 436.6; P: 0.0005, respectively). Other significant risk factors were identified and reported. This study helps to identify practices that can be targeted for intervention strategies to decrease S. aureus transmission in veterinary health care settings.

Keywords: staphylococcus aureus, epidemiology, occupational hazard

EAR – 5

THE EFFECT OF LONG-TERM EXPOSURE TO CONCRETE OR RUBBER FLOORING ON LYING BEHAVIOR IN DAIRY CATTLE. E. Bratton, S. Eicher, J. Marchant-Forde, M. Schutz, and K. Proudfoot. OSU Dept. of Veterinary Preventive Medicine, USDA-ARS Livestock Behavior Research Unit, and Purdue University Department of Animal Sciences

Many dairy cows are housed on concrete flooring despite softer alternative flooring surfaces such as rubber. The long-term effects of flooring type on cow comfort indicators such as lying behavior have not been determined. The aim was to determine the effect of concrete or rubber flooring on lying behavior in lactating dairy cows. Forty pregnant heifers (n = 20/treatment) were enrolled before their first lactation and randomly assigned to either a pen with grooved concrete flooring or a pen with rubber flooring; the composition of pens was dynamic as cows entered and left based on calving date. At calving, cows were fitted with 3D accelerometers (AFI Pedometer Plus®) to record daily lying time, number of lying bouts, and lying bout duration for their first two lactations. Accelerometer data from 15 cows on concrete and 14 cows on rubber were included due to missing data. Eight cows per treatment were video recorded on approximately day 90 of their second lactation to measure the time it took them to transition from standing to lying (lying movement duration), and vice versa (rising movement duration), averaged across at least 3 bouts/36 h period. Accelerometer data were analyzed using mixed models and T-tests were used to analyze video data. Cows on rubber flooring had fewer (P = 0.025) and longer lying bouts (P = 0.002) per day compared to cows on concrete, regardless of lactation. There was a trend approaching significance for cows on concrete to have longer lying movements (P = 0.12), but there was no effect of flooring on rising movement duration (P = 0.73). Results suggest that cows alter their lying behavior depending on the flooring surface. The impact of flooring surface on cow comfort has broad welfare implications; further research should address the impact of flooring using a larger sample size.

Keywords: Cow comfort, Lying bouts

EAR – 6

HOST FACTORS AND TESTING MODALITY AGREEMENT ASSOCIATED WITH SNAKE FUNGAL DISEASE IN A FREE-RANGING SNAKE POPULATION IN SOUTHEAST OHIO, USA. R.B. Dalton, D. Love, K.E. Seeley, S. Patel, M.C. Allender, M.M. Garner, and J. Ramer. Dept. of Veterinary Preventative Medicine, The Wilds, University of Illinois Wildife Epidemiology Lab, and Northwest ZooPath.

Snake fungal disease is an emerging mycotic dermatitis caused by Ophidiomyces ophiodiicola, and has been demonstrated to negatively impact snake populations of conservation concern in the United States. Individuals present with lesions such as ulceration, nodules, hyperkeratosis, and erythema. The purpose of this study was to evaluate the prevalence of and factors associated with snake fungal disease in free-ranging snakes on the 9,000+ acre property of the Wilds, a safari park and conservation facility located in southeast Ohio. We swabbed and performed skin biopsies on wild-caught snakes to test for snake fungal disease via qPCR, culture and histopathology. Snout-vent length, sex, body weight, and presence of gross lesions were recorded for each individual. Several individuals across 3 species tested positive via swab and/or tissue qPCR for snake fungal disease. These individuals were distributed across multiple sites on property. Females and males were affected in similar proportions, there was no clear association between snout-vent length or weight and test result, a higher proportion of individuals with gross lesions tested positive than those without gross lesions, and the majority of individuals that tested positive were caught in April or May. A low level of agreement was observed across testing modalities. Our findings support the potential of this pathogen to affect individuals broadly across species and size, highlighting the relevance of this disease for snake conservation efforts, and suggest that while not much agreement was observed across test modalities, the use of multiple modalities is a more ideal method for characterizing prevalence and distribution of SFD.

Keywords: Ophidiomyces, Snake fungal disease, Host factors, Wildlife, Testing modalities, Reptile

EAR – 7

FIELD-TESTING MOSQUITO SURVEILLANCE TRAPS IN LOS ROBLES, JINOTEGA, NICARAGUA. H. Fox and R. Garabed Dept. of Veterinary Preventive Medicine

In Nicaragua, vector-borne diseases have become a growing public health concern. Outbreaks of dengue, chikungunya, and Zika in Nicaragua were reported in 2016 by the Nicaragua Ministry of Health (MINSA), and while the number of cases of these diseases in 2017 have decreased, these vector-borne diseases remain endemic in some areas of Nicaragua and pose a risk for future epidemics. MINSA has attempted to combat disease spread through surveillance, public outreach, and prevention. In order to aid in vector surveillance, a mosquito trap was previously designed and tested in a laboratory setting that targeted gravid female mosquitoes. This research poses the question: Can this designed mosquito trap be utilized as a low-cost surveillance technique in Los Robles, Jinotega, Nicaragua for mosquito vectors of emerging infectious diseases. The mosquito trap design was recreated and tested in three different environments with four different attractants in Los Robles, Jinotega, Nicaragua. Environments for the traps were near humans, near animals, and near the lakefront of Lago de Apanas, a known mosquito-heavy location by locals, while the attractants utilized were guinea pig feed in water, light, lactic acid in water, and Fabuloso cleaner in water, an attractant recommended by locals. Eighty-four traps were placed in total, with seven different locations used for each of the three environments. Traps were checked for mosquitoes 24 hours after placement and 48 hours after placement, and then the traps were removed. While this trap design was reported to have captured mosquitoes in a laboratory setting, no mosquitoes were captured by the traps in this field experiment.

Keywords: Vectors, Nicaragua, Surveillance

EAR – 8

CORE GENOME MULTILOCUS SEQUENCE TYPING (CGMLST): TAKING MLST TO THE NEXT LEVEL USING NEXT-GENERATION SEQUENCING. M. Ghanem and M. El-Gazzar. Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210, USA

Mycoplasma synoviae (MS) is a significant poultry pathogen with increased prevalence and virulence reported in recent years resulting in reduced production efficiency of commercial poultry. MS strain identification is essential for epidemiological outbreak investigation, prevention and control efforts. Currently, multiple multilocus based sequence typing schemes have been developed for MS, yet the resolution of these schemes could be insufficient for outbreak investigation. The cost of whole genome sequencing became close to that of sequencing the seven MLST targets; however, there is no standardized method for typing MS strains based on whole genome sequences. In this paper, we are proposing a core genome multilocus sequence typing (cgMLST) scheme as a potential standardized and reproducible method for typing and differentiation of MS whole genome sequences. A diverse set of 25 MS whole genome sequences were used to identify 302 core genome genes as cgMLST targets (35.5% of MS genome) and a total of 44 whole genome sequences of MS isolates from six countries in four continents were typed using this scheme. cgMLST based phylogenetic trees displayed a high degree of agreement with core genome SNP based analysis and available epidemiological information. cgMLST allowed evaluation of two conventional MLST schemes of MS. Moreover, the high discriminatory power of cgMLST allowed differentiation between samples of the same MLST type. cgMLST represents a standardized, accurate, highly discriminatory, and reproducible method for differentiation between MS isolates. Additionally, it provides stable and expandable nomenclature, allowing for comparing and sharing the typing results between different laboratories worldwide.

Keywords: Mycoplasma synoviae, Molecular typing, Multilocus sequence typing (MLST), Whole genome, Next-generation sequencing

EAR - 9

FIBULAR OSTEOTOMY TO FACILITATE PROXIMAL TIBIAL ROTATION DURING TIBIAL PLATEAU LEVELING OSTEOTOMY. J. Zuckerman, J. Dyce, A. Arruda, C. Kramer, R. Ben-Amotz. Departments of Veterinary Biosciences and Veterinary Clinical Sciences

Cranial cruciate ligament (CCL) rupture is a common cause of hindlimb lameness in the dog. The tibial plateau leveling osteotomy (TPLO) is a frequently performed procedure to treat CCL rupture, the success of which depends on proper positioning, rotation, and fixation of the tibial plateau segment. It has been anecdotally noted that in cases of difficult proximal tibial rotation, a fibular osteotomy may be performed as an adjunct procedure. There is currently no published literature regarding the procedure of fibular osteotomy. This retrospective case-control study aims to identify risk factors for rotational constraints that may require a fibular osteotomy, and document the effects of fibular osteotomy on TPLO outcome.

Medical records and radiographs of 23 dogs undergoing TPLO-FO and 49 control dogs undergoing a routine TPLO were reviewed. Data collected were breed, sex, weight, age, preoperative tibial plateau angle (TPA) and mechanical medial proximal tibial angle (mMPTA), immediate postoperative and recheck TPA and mMPTA, ratio of fibular to tibial width (FW:TW), presence of tibiofibular synostosis, tibial osteotomy location, use of additional implants, and recorded complications.

Dogs with tibiofibular synostosis had 61.61 times greater odds to have rotational constraints requiring a fibular osteotomy than dogs without synostosis. Dogs with FW:TW greater than 0.24 were also 7.76 times more likely to exhibit these rotational constraints. In both cases, a fibular osteotomy successfully allowed for adequate tibial plateau rotation. Additionally, dogs receiving a fibular osteotomy had a greater change in TPA (rockback) upon recheck if a single plate was used to stabilize the site than compared to those dogs whose TPLO was stabilized with an additional plate.

Keywords: TPLO, Fibular Osteotomy, Tibial Plateau Angle, Synostosis, Retrospective study, Case-control study

EAR – 10

ROLE OF OXYTOCIN IN THE BLACK RHINOCEROS FOLLOWING HUMAN AND ANIMAL INTERACTIONS. D. M. Lang, P. M. Dennis. Department of Veterinary Preventive Medicine, The Ohio State College of Veterinary Medicine and Cleveland Metroparks Zoo.

Often termed the “love hormone” in humans, oxytocin is a neuroendocrine hormone that has been associated with pair bonding and maternal behavior in both humans and animals. Recent studies have investigated the importance of oxytocin in human-animal interactions, specifically measuring the oxytocin response in animals following interaction with a human counterpart. Studies thus far have demonstrated the role of oxytocin in domestic animals, yet none has investigated the role in exotic species under human care, such as the black rhinoceros (Diceros bicornis). We wish to explore the role of oxytocin in in the black rhinoceros in a variety of pair settings, with fellow members of the same species and with humans, compared to solitary settings. In order to investigate this, we first needed to validate an ELISA assay for oxytocin in the black rhinoceros. Saliva samples from our two females (one pregnant, and one who recently gave birth and is nursing) were collected at various timepoints throughout the day. Salivary oxytocin levels were measured using an equine oxytocin ELISA kit that was validated in this species. We hypothesize that there will be a significant difference in oxytocin levels from the pregnant female and the lactating female, demonstrating biological validation and a correlation of oxytocin in pair bonding between mother and calf. Validating the oxytocin assay and verifying its relevance in the black rhinoceros will complete the first step of looking at the role of oxytocin in pair bonding with fellow conspecifics and human keepers. Understanding the role of oxytocin in an exotic species in a zoological setting could help provide insight into zoo animal welfare and human-animal bonding.

Keywords: Oxytocin, Black rhinoceros, human-animal bond, zoo and conservation medicine

EAR – 11

POST HOC ANALYSIS OF LABORATORY INFLUENZA A VIRUS DETECTION AND ISOLATION DURING SURVEILLANCE IN SWINE POPULATIONS. J. Lorbach, S. Nelson, J. Nolting, and A. Bowman. Department of Veterinary Preventive Medicine.

Influenza A virus (IAV) has bidirectional zoonotic potential and has occasionally spilled over from swine to humans as seen during the 2009 H1N1 pandemic. Surveillance provides timely identification and characterization of IAVs in swine that are crucial for preventive medicine efforts and response to outbreaks. Our current testing approach includes molecular screening of samples for IAV (RT-PCR) and subsequent isolation of virus (cell culture) from screening-positive samples. Manufacturers of RT-PCR IAV detection kits provide cycle-threshold (Ct) cutoff values for result interpretation (Ct < 38 = positive; 38 < Ct < 40 = suspect; Ct > 40 = negative). In our lab, experience has shown samples with Ct values of approximately 35 or higher tend to fail isolation attempts. This study aimed to examine the relationship of RT-PCR Ct values in IAV detection-positive samples with success or failure of virus isolation using data generated over eight years of surveillance. We hypothesized that this analysis would inform our decision-making process to avoid unnecessary use of resources. From 2011-2018, IAV was detected in 7,140 samples by RT-PCR and isolation attempted. Virus isolation was successful in 44.8% of samples (n=3,199), representing a mean Ct value of 27.1 (95% CI 26.9, 27.2). Of isolation positive samples, 95.0% had an initial Ct < 35.56. Virus isolation failed in 55.2% of samples (n=3,941), representing a mean Ct value of 34.6 (95% CI 34.5, 34.7). A theoretical Ct value cutoff of 35.56 was determined based on 95% recovery of positive isolates below this value (n=3,039). The proportion of isolation failures with Ct values above 35.56 was 43.9% (n=1,729). Adoption of this Ct threshold would theoretically eliminate 1,729 unsuccessful and 160 successful isolation attempts. These results suggest modifying testing parameters could decrease resource use with minimal impact to overall isolate production.

Keywords: influenza, swine, surveillance, detection, isolation, characterization

EAR – 12

EFFECT OF PREPARTUM BEHAVIORAL ACTIVITY ON STILLBIRTH IN TRANSITION DAIRY HEIFERS AND COWS. B.T. Menichetti, Piñeiro, J.M., A.A. Barragan, A. Relling, S. Bas, and G.M. Schuenemann. Depts. Of Veterinary Preventive Medicine and Animal Sciences,

The objective of this study was to assess the effect of behavioral activity on stillbirth in transition dairy heifers and cows. A behavioral activity index (BAI) was computed (Titler et al., 2015 J. Dairy Sci. 98: 5304-5312) for every animal taking into account the number of steps (no./d), standing time (min/d), lying time (min/d), and lying bouts (LB, no./d). A total of 387 Holstein dairy cows (110 primiparous and 277 multiparous) in 3 commercial dairy herds were enrolled at 7 d prior to calving until 14 d post-calving. Weekly, a cohort of 10 to 15 heifers and cows were enrolled at each farm, and electronic data loggers (IceQube, IceRobotics, Edinburgh, UK) were fitted to the hind leg of individual animals to assess their behavioral activity. Pre-partum heifers and cows were moved into prepartum pens 21 d before the expected calving date. All heifers and cows were housed in similar prepartum free-stall barns and moved into a contiguous individual maternity pen for parturition. Stillbirth was defined as a calf born dead or died within 24 h after birth, and with normal gestation length. The BAI was computed for the last 7 d prior to parturition to assess differences among primiparous and multiparous cows. Data were analyzed using MIXED procedure of SAS. Primiparous cows ($P<0.05$) had greater BAI compared to multiparous cows, and as cows progress in lactation (mature), they become less active around parturition. Regardless of parity, cows with a stillborn calf had less pre-partum lying time (10.5 h/d; $P<0.05$) at calving compared to cows with a calf born alive (12.15 h/d). These results suggest that monitoring a combination of pre-partum behavioral metrics, such as lying time and BAI, could be used to predict stillborn calves in transition heifers and cows.

Keywords: Stillbirth, Behavior, Dairy Cattle

EAR – 13

ENVIRONMENTAL PERSISTENCE OF FOOT AND MOUTH DISEASE VIRUS AND THE IMPACT ON TRANSMISSION CYCLES IN ENDEMIC REGIONS. S. R. Mielke, R. Garabed, Dept. of Veterinary Preventive Medicine

Extensive research of Foot and Mouth Disease virus (FMDv) has been conducted concerning basic etiology and transmission potential of livestock and livestock products; with a subset of research determining optimal conditions for environmental survival. However, this subset was conducted in the early to mid-20th century in Northern Europe and the United States which is not easily generalized to today's endemic locations. A systematic literature review found 20 studies that could be used in a Cox Proportional Hazard analysis for evaluation of FMDv survival parameters. Analysis suggests that temperature alone does not describe survival and the interaction of relative humidity (RH) and temperature reveal a broader impact across various temperature, RH, and matrices. For instance, FMDv would be expected to persist longer in the environment during the wet season versus the dry season or comparatively in the UK versus the Southwestern, US. Within the dataset 5 studies reported both RH and temperature resulting in a small number of data points. This reveals a lack of data to establish the "best" parameter estimates for modeling environmental transmission of FMDv transmission cycles in endemic regions. However, this analysis reveals important environmental measurements to include in future studies. Additional questions remain about spatial and temporal patterns of environmental persistence of FMDv in outbreak locations such as Cameroon, West Africa, where both sedentary and mobile cattle populations exist. Data collected at FMD outbreak sites in Ngaoundéré, Cameroon will be used to determine these transmission patterns and the theoretical implications of environmental persistence of FMDv in tropical climates. The consequences of Foot-and-Mouth Disease greatly impact regional economies and food security through animal mortality and morbidity, trade restrictions on developing economies, and burdens to veterinary infrastructure. Therefore, understanding environmental transmission can impact disease prevention and elimination strategies for a disease considered to be economically devastating.

Keywords: FMDv Transmission, Environmental Survival, Survival Analysis, Disease Model, Cameroon, West Africa

EAR – 14

CHARACTERIZATION OF PLASMID MEDIATED CARBAPENEMASE-PRODUCING ENTEROBACTERIACEAE FROM WASTE AND SURFACE WATERS. D.F. Mollenkopf, D.A. Mathys, S.M. Feicht, A.L. Albers, E.K. Sechrist, G.A. Ballash, R.J. Adams, D.B. Stuever, S. Lee, J. Lee, T.E. Wittum

Wastewater from large medical centers routinely transports carbapenemase-producing Enterobacteriaceae (CRE) to wastewater treatment plants where they are maintained and ultimately discharged into the environment in surface water, posing an important public health risk. We characterized over 400 non-chromosomally mediated carbapenemase-bearing isolates recovered from both influent and effluent wastewater at a local wastewater treatment plant (WWTP) which receives effluent from multiple metropolitan hospitals and from surface water collected both upstream and downstream of the WWTP discharge. Identified by MALDI-TOF, bacterial species not associated with chromosomal carbapenemase gene carriage were assessed using conventional PCR and Sanger sequencing for the predominant carbapenem resistance gene, blaKPC. blaKPC-negative isolates were characterized using whole genome sequencing. To date, over half of the plasmid mediated CRE isolates have been identified as Enterobacter sp. (58%), followed by Klebsiella sp. (17%), Escherichia coli (12%), Citrobacter sp. (7%), and Raoultella sp. (5%). blaKPC is the dominant carbapenemase gene, detected in 84% of these isolates with most harboring the blaKPC-2 gene. However, we identified blaKPC in only 24% of E. coli and 64% of Raoultella isolates. While WWTP reclamation does appear to reduce the prevalence of CRE between the influent and effluent flows, the downstream collections are more reflective of the effluent CRE prevalence than the prevalence of CRE detected upstream of the discharge and poses an important risk to the downstream watershed.

Keywords: Carbapenemase-producing Enterobacteriaceae, wastewater, surface water

EAR - 15

DISSEMINATION OF CARBAPENEM-RESISTANT ENTEROBACTERIACEAE FROM HEALTHCARE FACILITIES INTO OHIO SURFACE WATERS. J. Nystrom, G. Ballash, J. Lee, S. Lee, D. Mollenkopf, J. Van Balen Rubio, M. Sullivan, D. Symonds, T. Wittum, B. Zimmerman. Department of Veterinary Preventive Medicine

Carbapenem-resistant *Enterobacteriaceae* (CRE), are a critically important health concern, listed as an urgent public health threat by the CDC and WHO. CRE are associated with hospitalized patients, and are resistant to a wide range of antimicrobials. Jackson Pike Waste Water Treatment Plant filters water from the city of Columbus which includes five major hospitals. If CRE are discharged from the WWTP into the surface water, they may disseminate to intensively-managed animal agriculture facilities. The presence and concentrations of clinically-relevant isolates – CRE, extended-spectrum β-lactamase (AmpC and ESβL phenotypes) producing *Enterobacteriaceae,* and polymyxin-resistant *Enterobacteriaceae* carrying mcr-1 – were examined in the Scioto River. Environmental samples of surface water, sediment, detritus, and periphyton, collected, along with 25 fish vent swabs from fish that represent the species diversity in that portion of the river, were collected from 24 locations along the Scioto River. Sampling sites were selected to target, upstream and downstream, the WWTPs along the river corridor which discharge effluent into the river. A composite of the environmental samples were cultured using selective media for CRE, AmpC, ESβL, and polymyxin-resistant *Enterobacteriaceae*. The same culture procedure was used for the fish vent swabs. Of the 94 environmental samples collected, 34 clinically relevant CRE were recovered at 18 of the sampling sites. Of the 625 fish vent swabs that were collected, 31 clinically relevant CRE were recovered at 9 sites. AmpC-producing *Enterobacteriaceae* were recovered from 82 (87%) of the collected environmental samples. Fish vent swabs yielded 67 (11%) phenotypic AmpC isolates. Phenotypic ESβL producers were recovered from 73 (78%) of the environmental samples and were recovered from 48 (8%) of the fish vent swabs. The process of confirming bacterial species and the *bla*$_{CMY}$ and *bla*$_{CTX-M}$ alleles are ongoing. Suspect polymyxin-resistant *Enterobacteriaceae* are being screened for mcr-1 using conventional PCR. To date, all isolates have been negative.

Keywords: Enterobacteriaceae, carbapenem- resistance, extended-spectrum β-lactamases, polymyxin resistance

EAR – 16

TRENDS IN ANTIMICROBIAL SUSCEPTIBLITY OF SALMONELLA RECOVERED FROM CATTLE DIAGNOSTIC SAMPLES IN OHIO. K. Orso, A. Arruda, R. Andridge, J. Cui, B. Byrum, Y. Zhang, G. Habing, Dept. of Veterinary Preventive Medicine, College of Public Health - Division of Biostatistics, and Ohio Department of Agriculture - Animal Disease Diagnostic Laboratory

Antimicrobial resistance in Salmonella affects human and livestock health. Resistance limits treatment options, and can worsen and lengthen infections. Cattle are an important reservoir of Salmonella for humans through the consumption of beef and dairy products and direct contact. The objective of this study was to retrospectively evaluate the trends in antimicrobial susceptibility of Salmonella isolates from cattle in Ohio between 2006 and 2017. We hypothesized that there were significant changes in proportions of non-susceptible isolates. Veterinarians submitted diagnostic samples from ill cattle suspected of salmonellosis to the Ohio Department of Agriculture. Isolation and susceptibility testing was completed at the Animal Disease Diagnostic Laboratory, and serotyping was completed by the National Veterinary Services Laboratory (NVSL). In total, 948 isolates were included in the analysis and temporal trends in non-susceptibility to ampicillin, ceftiofur, tetracycline, enrofloxacin, florfenicol, sulfadimethoxine, gentamicin, trimethoprim sulfamethoxazole, and neomycin were analyzed. To facilitate statistical comparisons, the years were categorized into four-time periods (2006-08, 2009-11, 2012-14, and 2015-17). Chi-Square tests and Fisher Exact Tests ($p<0.05$) were performed to evaluate the significance of observed overall and within-serotype changes in proportions of non-susceptible isolates between time periods. The most frequently recovered serotypes were Cerro (32.8% of isolates), Dublin (12.3%), Typhimurium (10.7%), and Newport (5.8%). Among all isolates, there were significant increases in susceptibility to ampicillin, ceftiofur, tetracycline, florfenicol, and neomycin; and significant but small decreases in susceptibility to enrofloxacin and trimethoprim sulfamethoxazole. There were few within-serotype changes in susceptibility. Serotypes Cerro and Dublin showed a significant decrease and increase, respectively in susceptibility to sulfadimethoxine and neomycin. During the analyzed time period, there were substantial increases in overall susceptibility of Salmonella isolates. With few within-serotype changes in resistance, overall temporal changes in the susceptibility are mostly due to changes in the populations of Salmonella serotypes with characteristic resistance patterns.

Keywords: antimicrobial, antibiotic, Salmonella, Salmonellosis, cattle, dairy, Ohio, Epidemiology, resistance

EAR – 17

IMPLICATIONS OF HUMAN ANIMAL BOND AND INTERACTIONS ON MICROBIOME DEVELOPMENT OF CHILDREN IN RURAL NICARAGUA.

P. Oruganti, V. Hale, B. Piperata, J. Lee, R. Garabed. Depts of Veterinary Preventive Medicine, Anthropology and Environmental Health Sciences

Diarrheal disease is the second leading cause of infant mortality in developing countries worldwide and many causes are zoonotic in origin, meaning they are transmitted between humans and animals. Previous studies have examined the impact of the human-animal bond on health outcomes of pet owners in western countries, but literature regarding the impact of human-animal bond on human health cross-culturally is limited. In looking at developing countries and other cultures outside of the United States, it is necessary to describe the nature of human-animal interactions, identify whether there is a concept of the “human-animal bond”, and ultimately how these factors contribute to microbiome formation, which may have implications for diarrheal disease. This research uses ethnographic surveys, interviews, observations and microbiome diversity of child fecal samples to understand the interrelationships between domestic animals, humans, and the environment in context of microbiome and disease risk. This study took place in rural Los Robles, Jinotega, Nicaragua in the summer of 2017. The “human-animal bond” was observed, with animals having emotional, economic and nutritional value and importance. Common animal health concerns and treatments were discerned, and children’s interactions with animals and the environment were used to describe a cultural model, microbiome diversity was compared among the children in the sample and to bacterial species expected in animal microbiomes.

Keywords: human-animal bond, microbiome, Nicaragua, ethnography

EAR – 18

CLOSTRIDIUM DIFFICILE ON OHIO SWINE FARMS: A COMPARISON OF SWINE AND HUMAN ENVIRONMENTS AND ASSESSMENT OF ON-FARM RISK FACTORS

R. O'Shaughnessy, G. Habing, A. Hoet, W. C. Miller, J. Stull

Swine are a known reservoir for Clostridium difficile, a pathogen that causes disease in both humans and animals. Although transmission of C. difficile from swine to human farm workers is strongly supported by previous studies, the role of farm environmental surfaces, specifically worker breakrooms, as an important route of transmission has not been explored. This study aimed to (1) Characterize and compare the prevalence of C. difficile, and distribution of PCR-ribotypes in swine and human environments on swine farms and (2) Determine associations between biosecurity protocols and worker hygiene practices and the presence of C. difficile on farms. Between May and August 2015, 13 Ohio swine farms were visited and surveys (farm owner, worker) and environmental samples collected. Samples (n=629) were collected from swine (e.g. floors, gates, piglet feces in farrowing and nursery units) and worker breakroom surfaces (e.g. refrigerators, counters); 19 worker fecal samples were collected. Farm owner (n=12) and worker (n=14) surveys were collected. Responses from surveys were modeled against C. difficile recovery using mixed effects logistic regression. C. difficile was recovered from all farms, with farrowing units exhibiting the highest prevalence (60.1%, 107/178), followed by breakrooms (50.0%, 69/138), and nursery units (9.3%, 12/129). Ribotypes 078, 412, 005, and 596 were recovered from swine and human environments on farms. C. difficile recovery in breakrooms was associated with high prevalence in farrowing units (OR=3.2, p=0.027). This is the first study to characterize C. difficile in swine farm breakrooms. The association between contamination of swine and human surfaces and high prevalence and virulent zoonotic ribotypes found on surfaces in breakrooms implicate these surfaces in C. difficile transmission to swine farm workers.

Keywords: Clostridium difficile, swine, farm worker, occupational safety, epidemiology

EAR – 19

A MULTI-SITE RANDOMIZED FIELD TRIAL TO EVALUATE THE INFLUENCE OF LACTOFERRIN ON HEALTH OF DAIRY CALVES WITH DIARRHEA. J. A. Pempek, L. R. Watkins, C. E. Bruner, and G. G. Habing, Department of Veterinary Preventive Medicine, The Ohio State University

Calf diarrhea remains the most common cause of mortality and antimicrobial use in dairy calves. Reduced overall antimicrobial use necessitates research on viable alternative therapies for calf diarrhea. Lactoferrin, an iron-binding protein found in colostrum, has been shown to improve growth and reduce mortality in pre-weaned heifer calves. The objective of this study was to investigate the effect of lactoferrin on the health of heifer calves with diarrhea. This randomized controlled field trial was conducted on five commercial dairy farms in Ohio. In total, 485 calves (≤ 21 d of age) were enrolled after first diagnosis of diarrhea, and randomly assigned to receive an oral dose of lactoferrin (3 g lactoferrin powder dissolved in 30 mL water) or water (control) once daily for 3 consecutive days. Health assessments were conducted on the day of enrollment and 1, 2, 3, 7, 14, 21, 28, and 35 d post-enrollment. A poisson regression model was used to test differences between treatments in frequency of disease through 35 d post-enrollment. Median calf age at enrollment was 11 d, and ranged from 1 to 21 days of age. On the day of enrollment, 51.3% (123/240) and 52.2% (128/245) of calves in the control and lactoferrin treatment groups, respectively, were diagnosed with severe diarrhea. Diarrhea frequency 35 d post-enrollment did not differ between control and lactoferrin treatment groups (RR: 1.01, 95% CI: 0.93-1.08; P = 0.87). Further, depression was not different for calves supplemented with lactoferrin compared to calves in the control group (RR: 0.85, 95% CI: 0.59-1.22; P = 0.37). This study suggests supplementing lactoferrin to dairy calves with diarrhea does not influence the frequency of diarrhea or depression 5 wk post-diagnosis; however, analyses of secondary outcomes and future research may uncover longer-term benefits of supplemental lactoferrin on morbidity and mortality in dairy heifer calves.

Keywords: calf diarrhea, lactoferrin, antimicrobial alternative

EAR – 20

EFFECT OF KETOSIS ON BEHAVIORAL ACTIVITY IN TRANSITION DAIRY COWS.

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The objective was to assess the effect of ketosis status on behavioral activity in transition dairy cows. A behavioral activity index (BAI) was computed for every animal taking into account the number of steps (no./d), standing time (min/d), lying time (min/d), and lying bout (LB, no./d). Holstein dairy cows (110 primiparous and 277 multiparous) from 3 commercial dairy herds were enrolled at 7 d before calving until 14 d post-calving. Monthly, 20 to 36 cows was enrolled at each farm and electronic data loggers (IceQube, IceRobotics, Edinburgh, UK) were fitted to a hind leg of individual cows to assess their behavioral activity. Postpartum cows were screened for ketosis (KET) at 7 and 14 DIM by measuring β-hydroxybutyrate in serum samples (Nova Vet Ketone Test Strips). A case of KET was recorded when lactating cows had serum concentration of β-hydroxybutyrate ≥1.2 mmol/L. Cases of metritis, retained placenta, milk fever, or mastitis during the study period were recorded and lactating cows were allocated into 1 of 4 groups: 1) non-disease (ND, n = 248; cows without KET and any other health conditions), 2) cows with only KET (n = 64), 2) sick cows experiencing ≥ 1 health conditions, but without KET (SICK, n = 59), or cows with KET plus at least 1 health condition (KET+H, n = 17). The BAI was computed for the first 7 DIM to assess differences among cow health groups. Data were analyzed using MIXED procedure of SAS. Primiparous cows had significantly greater BAI compared with multiparous cows. Cows experiencing KET, SICK and KET+H had reduced BAI (93.1, 56.6 and 95.5, respectively) compared with ND cows ($P < 0.05$). These results suggest that monitoring a combination of behavioral metrics, such as the BAI, could contribute to consistently.

Keywords: behavior, health, dairy cattle

EAR – 21

FREQUENCY AND GENUS DISTRIBUTION OF INTRINSICALLY CARBAPENEM RESISTANT ENTEROBACTERIACEAE IN SURFACE WATER. E. Sechrist, S. Lee, D. Mollenkopf, T. Wittum Depts. of Veterinary Preventive Medicine and Environmental Health Sciences

Multiple genus within the Enterobacteriaceae family are intrinsically resistant to carbapenem antimicrobials through chromosomally encoded carbapenemase genes. These carbapenem-resistant Enterobacteriaceae (CRE) lack the epidemiologic significance of CRE with plasmid-mediated carbapenemase genes, but they can be highly relevant clinically for individual patients. When patients are treated with carbapenems, the resulting selection pressure strongly favors resistant bacteria. We believe that these resistant pathogens eventually spread into the community due to a patient's fecal shedding, eventually entering wastewater, where they are maintained and released into the downstream surface water. CRE in public waterways pose both a direct and, more problematically, an indirect threat to public health. Over the course of 12 months, wastewater was collected at the WWTP servicing a large metropolitan tertiary care hospital in order to assess the degree of hospital-associated CRE environmental contamination. Influent and effluent water samples from the WWTP were collected, as well as water samples upstream, downstream, and further downstream of the WWTP. Collection sites were analyzed to determine the frequency and genus distribution of chromosomally-mediated CRE. We found that the far downstream site produced the highest number of chromosomally-mediated CRE compared to all other sampling sites. We identified a total of 106 CRE isolates with chromosomal carbapenemase genes, consisting of four different genus with Aeromonas the most frequent. This outcome suggests that hospitals may be contributing to the spread of clinically relevant CRE with chromosomal carbapenemase genes into the environment in surface water.

Keywords: Carbapenem, Resistant, Enterobacteriaceae, Wastewater

EAR – 22

CANINE LEPTOSPIROSIS IN THE UNITED STATES (2009 - 2016): USE OF PCR TESTING TO UNRAVEL COMPLEX SPATIAL, TEMPORAL, HUMAN- AND ANIMAL-LEVEL RISK FACTORS. A.M. Smith, A.G. Arruda, T.E. Wittum, J.W. Stull. Dept. of Veterinary Preventive Medicine

Canine leptospirosis is a reemerging zoonotic disease of concern, yet information on its epidemiology is lacking. Recent wide-scale clinical uptake of a new PCR test, which offers greatly improved sensitivity, may provide greater insight into this complex disease. This study aimed to describe the temporal and spatial distribution of PCR-positive canine leptospirosis cases in the U.S., and to identify environmental, dog and human-level factors associated with infection. Data from 40,118 canine leptospirosis PCR tests run in the U.S. between 2009 and 2016 were acquired from IDEXX Laboratories. Data on weather, urban influence, human income and education were obtained from public databases. Maps were created to identify high-risk areas and generalized (univariable, followed by multivariable) mixed logistic regression models accounting for county and state were used to identify significant predictors. Overall test-positive prevalence across the U.S. was 5.4%. Prevalence varied temporally and regionally: prevalence in the western and southwestern regions remained relatively constant, while prevalence in the midwest, northeast, and southeast peaked in the fall/winter. In the final multivariable model, dogs were at higher odds of testing positive with each calendar year increase (OR=1.08, $P < 0.001$). For every 1 unit increase on the Palmer Drought Severity Index (i.e., increasing environmental moisture), dogs were at higher odds of testing positive (OR=1.04, $P < 0.001$). Urban dogs were at higher odds of testing positive compared to non-urban dogs (OR=1.33, $P = 0.008$). Females were at lower odds of testing positive compared to males (OR=0.76, $P < 0.001$). With each year increase in age, dogs were at lower odds of testing positive (OR=0.900, $P < 0.001$). No human factors were significant. These results highlight important factors based on newly available PCR testing, allowing for an improved understanding of this complex disease and targeted education and prevention efforts at clients/dogs with greatest disease risk.

Keywords: Canine, Leptospirosis, Zoonosis, PCR testing

EAR – 23

LYING BEHAVIOR AS AN INDICATOR OF DIARRHEA AND NAVEL INFLAMMATION IN VEAL CALVES. M. Studds, L. Deikun, D. Sorter, and K. Proudfoot. OSU Dept. of Veterinary Preventive Medicine

Veal calves arrive at growing facilities with inadequate immunity, leaving them susceptible to diseases including diarrhea and navel inflammation. Observing calf lying behavior can be used as a tool to understand calf health. This study investigated the effect of navel inflammation and diarrhea on lying behavior in veal calves. A total of 125 calves from 3 cohorts were included in the study. Calves were housed individually on slatted flooring (Tenderfoot®) in 3 rooms of a mechanically ventilated barn. On d 4 after arrival, calves were fitted with 3D accelerometers (HOBO® Pendant Data Loggers) on their hind legs to continuously measure lying time, number of lying bouts, and lying bout duration. Health exams were conducted twice weekly for 2 wk starting on the day after arrival. Exams included a rectal temperature, a navel score (0=normal, 1=mildly inflamed, 2=moderately inflamed, 3=severely inflamed), and a fecal score (0=normal, 1=semi-formed, 2=loose, 3=watery). Calves were considered to have “navel inflammation” (n=22) or “diarrhea” (n=15) if they scored a 2 or greater during at least 3 of the 4 health exams. “Normal” calves (n=18) had no signs of illness and rectal temperatures < 39.4°C during 3 of the 4 health exams. Data were analyzed using a generalized linear model with repeated measures. There was an effect of health status on lying time (P=0.03), whereby normal calves spent more time lying (18.9±0.2 h/d) compared to calves with inflamed navels (18.0±0.2; P=0.01) and diarrhea (18.1±0.2; P=0.05). There was no effect of health status on the number of lying bouts per day (P=0.62), nor on the duration of lying bouts (P=0.36). Results indicate that veal calves with navel inflammation and diarrhea may be less comfortable than calves without these conditions. Veal producers should consider adapting facilities to create more comfortable lying environments to help calves better cope with illness.

Keywords: calf comfort, sickness behavior, calf housing

IMMUNOLOGY
AND
INFECTIOUS DISEASES

IMID – 1

BZLF1-DEC205 FUSION PROTEIN ENHANCES EBV-PROTECTIVE IMMUNITY.

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Epstein-Barr virus (EBV), a B-lymphotrophic gamma herpes virus, infects over 90% of the world's population. In immune competent individuals, EBV-infected B cells become immortalized but are then controlled by a highly efficient EBV-specific cytotoxic T lymphocyte (CTLs) response. Transplant patients receive immunosuppressive medications to prevent graft rejection and are at highest risk of developing EBV-associated lymphoproliferative disease. Post-transplant lymphoproliferative disease (PTLD) is a significant complication of organ transplantation and is strongly associated with poor prognosis.

We are targeting immediate-early EBV protein, BZLF1, as a vaccine immungen. BZLF1 initiates the activation of lytic stage in EBV-infected cells and promotes B-cell transformation. BZLF1-specific CTL expansion correlates with PTLD tumor regression and improved survival. Here we show that the BZLF1-DEC205 fusion protein is capable of driving improved immune responsiveness. DEC205 is endocytic receptors expressed at high levels on Dendritic Cells (DCs). DCs were generated from donor monocytes, mature DCs were then loaded with DEC205-BZLF1. DCs in the control arm were loaded with DEC205-HCG (Human chorionic gonadotropin). DCs were co-cultured with autologous peripheral blood leukocytes (PBLs). Cells were analyzed by flow cytometry using HLA-tetramers. To test the EBV vaccine in-vivo, we are using the human-murine chimeric model of EBV. In this model, severe combined immune deficient (SCID) mice are engrafted with PBL from EBV+ individuals leading to a spontaneous EBV-lymphoproliferative disease of human B cell origin that is similar to PTLD. Mice were immunized with DCs loaded with DEC205-BZLF1 or DEC205-HCG at time of engraftment.

DEC205-BZLF1 loaded DCs stimulated EBV-specific CTL expansion in the co-culture. DEC205-BZLF1 also promoted IFN-gamma secretion in CTLs. Upcoming results from the in vivo experiment will be subjected to Kaplan-Meier analysis for survival. In our DCs-based vaccine platform, we expect DCs to efficiently present peptide to T cells, restoring immune responsiveness and delaying or preventing the development of EBV-LPD.

Keywords: Epstein-Barr virus (EBV), Post-transplant lymphoproliferative disease, Vaccine, BZLF1

IMID – 2

Platform Presentation

IDENTIFICATION AND CHARACTERIZATION OF NOVEL TAX-1 INTERACTING PROTEIN, SNX27, AND ITS ROLE IN HTLV-1 PATHOBIOLOGY

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HTLV-1 and HTLV-2 are highly related viruses, with differential pathogenic outcomes in humans. While HTLV-1 is associated with several diseases, such as adult T cell leukemia, HTLV-2 is not associated with disease. The trans-activator of HTLV-1, Tax-1, has higher transforming potential then its HTLV-2 homolog, Tax-2. It is believed that this difference in transforming capacity plays a pivotal role in HTLV-1 pathogenesis. We propose that Tax-1 interacts with cellular gene products via domains lacking in Tax-2, and that these interactions contribute to pathogenesis. We performed proteomic screens of Tax-1, Tax-2, and Tax-1 mutant binding partners to identify specific interactions and the critical binding domain. Novel interactions were confirmed and mapped by co-immunoprecipitation studies and further characterized by biochemical and biologic assays. We identified a novel cellular interacting partner of Tax-1, Sorting Nexin 27 (SNX27). SNX27 regulates the localization and expression of transmembrane proteins via interactions with its PDZ domain. SNX27 has been demonstrated to regulate glucose transporter 1 (GLUT1), and SNX27 knock down in HeLa cells results in a dramatic redistribution of GLUT1 from the cell surface to the lysosome. GLUT1 serves as one of three receptor molecules for HTLV-1. We proposed that Tax-1 alters GLUT1 localization post-infection via its interaction with SNX27. We confirmed that Tax-1 and SNX27 interact via their PDZ domain binding motif and PDZ domains, respectively. We further show that SNX27 expression levels are inversely related to virus release and that GLUT1 surface localization and glucose transport function is reduced by Tax-1 overexpression. This work demonstrates a novel mechanism by which HTLV-1 regulates a surface receptor molecule post-infection and this interaction could serve as a target to inhibit viral spread.

Keywords: HTLV, Tax, SNX27, GLUT1

IMID – 3

THE SERINC5 PROTEIN ENHANCES EBOLAVIRUS (EBOV) INFECTIVITY BY CHANGING THE CONFORMATION OF GP X.T. Bai, Y.M. Zheng, and S.L. Liu. Department of Veterinary Biosciences.

SERINC5 is a transmembrane protein that has been recently shown to impair HIV infectivity by incorporating into infectious viral particles. Here, we provide evidence that SERINC5 increases the Ebolavirus (EBOV) infectivity and virus spread. By using VP40-based and VP40-Blam virus-like particle (VLP) systems, we showed that SERINC5 is incorporated into virions and promotes EBOV entry into target cells. In addition, we demonstrated that SERINC5 is co-localized with VP40 and EBOV GP on the plasma membrane of viral producer cells. Interestingly, we observed that the presence of SERINC5 in the VLP increases the GP cleavage by thermolysine, as well as its sensitivity to neutralization by KZ52 antibody, suggesting that SERINC5 alters the conformation of GP. Taken together, we show that, distinct from HIV, SERINC5 enhances EBOV infectivity by interacting with and changing the conformation of GP, thereby promoting its entry into host cells for replication.

Keywords: Ebolavirus, SERINC5, GP, conformation

IMID – 4

RANDOM MUTAGENESIS OF EHRLICHIA SP. HF STRAIN FOR IDENTIFICATION OF VIRULENCE GENES. H. Bekebrede, M. Lin, Y. Rikihisa. Department of Veterinary Biosciences.

Ehrlichia spp. (E. canis, E. ruminantium, E. ewingii, and E. chaffeensis) are tick-borne obligatory intracellular bacteria that infect a variety of mammals including dogs, ruminants, deer, and humans, causing severe and sometimes fatal systemic disease. Research to identify virulence factors of Ehrlichia spp. is hampered by the lack of small laboratory animal models. The Rikihisa laboratory isolated and completed whole genome sequencing on a novel Ehrlichia species named “HF strain” from Ixodes ovatus ticks in Japan. The HF strain is most closely related to E. chaffeensis human isolates, and kills laboratory mice within 10 days. My research seeks to analyze gene function of the HF strain using Himar transposon mutagenesis. A random mutant HF strain library is being generated in canine macrophage DH82 cells. Mutant HF strains are being cloned, and genomic loci of transposon insertion are being identified by semi random two-step PCR (ST-PCR). Mutants are used to infect mice; mutated genes in mutants that cannot infect mice are considered virulence factors. I have so far isolated fifty stable mutants expressing mCherry fluorescence as an indication of transposon insertion. We expect these studies to elucidate virulence factors of the HF strain. Because Ehrlichia spp. share homologus genes, the proposed study will help understanding virulence factors of other Ehrlichia spp. as well. One of these mutants disrupts virB2-4, a gene in the type IV secretion system (T4SS) needle protein. Preliminary in vitro and in vivo data suggest that the virB2 mutant is significantly attenuated in mammalian cell infection. This could imply that VirB2 is an ehrlichial virulence factor.

Keywords: Ehrlichia, HF strain, obligatory intracellular bacteria, virulence, mutagenesis

IMID – 5

THE DEVELOPMENT OF RECOMBINANT VESICULAR STOMATITIS VIRUS VECTORS EXPRESSING HUMAN RESPIRATORY SYNCYTIAL VIRUS F PROTEIN FOR USE IN VACCINE STUDIES. K. Brakel, B. Binjawadagi, and S. Niewiesk. Department of Veterinary Biosciences.

Human respiratory syncytial virus (RSV) is a leading cause of respiratory disease in infants, the elderly, and immunocompromised inidviduals. There is no approved vaccine, and antibodies produced after natural infection do not elicit long-term immunity. Both the F (fusion) and G (attachment) protein have been used in vaccine development, with monoclonal antibodies produced against the F protein showing more effective neutralization properties than those against the G protein. The F protein has both a pre-fusion and a post-fusion conformation. In this project, we have created recombinant vesicular stomatitis virus (rVSV) vectors expressing the pre-fusion F (pre-F), the post-fusion F (post-F), both the G and F protein (G-F), or a pre-fusion form of F derived from a clinical isolate of RSV (HEK-pre-F). Flow cytometry results indicate that the G-F and HEK-pre-F recombinants are most highly expressed, along with a positive control rVSV expressing an unmodified F protein, while pre-F and post-F recombinants produce little detectable F protein on the surface of infected cells. Western blots will be performed on all recombinants to confirm the production of the F proteins, regardless of their localization to the cell surface. When inoculated into cotton rats in a pilot study, none of the recombinants provided neutralizing antibody titers against RSV. This pilot study will be repeated and modified by boosting with all rVSVs four weeks after initial immunization. We expect that the HEK-pre-F, G-F, and unmodified F recombinants will produce neutralizing antibodies, while the pre-F and post-F recombinants will not be expressed on the cell surface and will therefore not produce neutralizing antibodies. Ultimately, these recombinants will be used in immunization studies to determine their value as pre-clinical vaccine candidates.

Keywords: Cotton rat, Human respiratory syncytial virus, Immunology, Virology Vaccines

IMID – 6

SOX9 DEFICIENCY IN GUT EPITHELIUM REGULATES ANTIBODY RESPONSES AND ALLERGIC SENSITIZATION. D J Burnett, E Kim, and P N Boyaka. Department of Veterinary Biosciences.

SOX9 is a transcription factor regulating the differentiation and maturation of Paneth cells within the intestinal crypts. The loss of function of the SOX9 gene in the gut epithelium of SOX9ΔIEC mice results in a lack of Paneth cells. Consistent with the fact that Paneth cells contribute to innate immunity primarily through production of antimicrobial peptides and other antimicrobial products, SOX9ΔIEC mice exhibit a profound dysbiosis of the gut microbiota. Furthermore, SOX9ΔIEC mice develop stronger allergic symptoms after oral sensitization. We addressed the relative contribution of the dysbiosis of SOX9ΔIEC mice on the production of mucosal and systemic antibodies. Fecal IgA levels were increased in SOX9ΔIEC mice compared to control wild-type mice. Bacteria-free fecal material extracts (FME) from SOX9ΔIEC mice significantly increased expression of costimulatory molecules (CD40 and CD86) by spleen cells after 24-hour incubation in vitro. Addition of bacteria-free FME from SOX9ΔIEC mice to mesenteric lymph node cells cultured in the presence of anti-CD40 and IL-4 increased the frequency of B cells expressing IgE (IgE + B220+ cells). Fecal material transplantation (FMT) of SOX9ΔIEC enhances allergen sensitization in the gut and allergic responses to subsequent allergen challenges in recipient germ-free mice. Ongoing studies will analyze the profile of metabolites present in the fecal material of SOX9ΔIEC mice to identify molecules that enhance allergic sensitization and allergic responses in germ-free mice. In summation, the findings of this study provide further insight into the relationship between the antimicrobial function of Paneth cells and host susceptibility to allergic responses.

Keywords: Microbiota, Dysbiosis, Allergic Sensitization, Allergic Response, Paneth Cells

IMID – 7

CHARACTERIZATION OF ALPACA IMMUNOGLOBULINS FOLLOWING IMMUNIZATION WITH A COMMERCIAL HAEMONCHUS CONTORTUS VACCINE, BARBERVAX®. M. Carman, G. VanHoy, J. Lakritz, G. Habing, D. Smith, R. Kaplan, and A. E. Marsh. Depts. of Veterinary Preventive Medicine (Carman, Habing, Marsh) and Veterinary Clinical Sciences (VanHoy and Lakritz), Moredun Research Institute Scotland (Smith), and Department of Infectious Disease University of Georgia (Kaplan)

Haemonchus contortus is arguably one of the most significant current challenges facing the viability of the camelid industry. Due to widespread drug resistance, limited genetic pool, and other factors, there are significant impacts of H. contortus infections on long term productivity. Protection against this parasite can involve the host's immune response. In addition to the conventional antibodies similarly produced in sheep and goats, camelids generate a unique type of antibody named heavy-chain antibodies (HCAb) that have a smaller molecular size and altered structure. The small size and conformational structure of camelid HCAbs are thought to better inhibit enzyme complexes and may specifically target the H-gal-GP multiprotease protein of H. contortus to a greater degree than conventional antibodies generated in vaccinated sheep or goats. Barbervax is a vaccine that demonstrates protection and is available for sheep in Australia. Our study assessed the immunologic response of alpacas (Vicugna pacos) following immunization. This study utilized serum of non-vaccinated alpacas and alpacas that received 4 immunization doses of Barbervax (H-gal-GP; native parasite antigen). Controls included similarly vaccinated and non-vaccinated sheep. Immunoglobulin production was assessed using indirect ELISA and Western blot. Serum and molecular sized fractions for heavy-chain antibodies were evaluated for their ability to bind H. contortus cross-sections and the H-gal-GP protein in situ. The study outcome indicates significantly elevated H-gal-GP antibodies in vaccinated alpacas as compared to non-vaccinate alpacas. Overall antibody titers in the alpacas were greater than the sheep and maybe associated with the alpaca HCAbs to the parasite protein. These results indicate the potential for Barbervax utilization as an adjunct to production management practices resulting in reduced reliance on therapeutic antihelmintics.

Keywords: Haemonchus contortus, Camelids, Heavy-chain antibody, H-gal-GP

IMID – 8

A NOVEL LAB-BASED VACCINE CANDIDATE FOR HUMAN NOROVIRUS.

K. Craig[1], L. Rosas[1], Z. Attia[1], M. Lu[1], A. Li[1], M. Xue[1], M. Sun[2], X. He[2], P. N. Boyaka[1], R. Jennings[1], A. Niehaus[3], J. Li[1]

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Human noroviruses (HuNoVs) are responsible for more than 95% of the non-bacterial acute gastroenteritis epidemics in the world. The CDC estimates that every year 21 million individuals suffer from HuNoV-induced gastroenteritis in the U.S. Development of an effective vaccine has been seriously hampered by the lack of an efficient cell culture system for HuNoVs and a suitable small animal model. Currently, there is no FDA-approved vaccine for HuNoVs. The goal of this study is to develop lactic acid bacteria (LAB) as a vector to deliver HuNoV antigen. To do this, a LAB bacteria strain (Lactococcus lactis) carrying VP1 gene of a HuNoV GII.4 virus (LAB-VP1) was constructed. Western blot confirmed that VP1 protein was highly expressed by the LAB vector. A novel microencapsulation technology was developed to enhance the stability of LABs in low and high pH environments. To test whether LAB-based HuNoV vaccine is immunogenic, 4-day-old gnotobiotic piglets were orally inoculated with various doses of LAB-VP1 either with or without microencapsulation. It was found that LABs were persistent in the small intestine of piglets and shed in pig feces for at least 25 days post inoculation. Live LABs or LAB DNA were found in mesenteric lymph nodes and spleen tissue in LAB-VP1 inoculated groups. HuNoV-specific IgG and IgA were detectable in serum and feces at day 13 post-inoculation, respectively, and further increased at late time points. In addition, LAB-based vaccine also triggered HuNoV-specific T cell immune responses in the piglets. After challenge with HuNoV GII.4 strain, a large amount of HuNoV antigens were observed in duodenum, jejunum, and ileum sections of intestine in the LAB control group. In contrast, significantly less or no HuNoV antigens were detected in the LAB-VP1 immunized groups. Collectively, these results demonstrate that LAB-based HuNoV vaccine induces protective immunity in gnotobiotic piglets.

Keywords: lactococcus lactis, lactic acid bacteria, human norovirus, vectored vaccine, gnotobiotic pig model

IMID – 9

HOUSE DUST MITE ALLERGY MODEL IN COTTON RATS (SIGMODON HISPIDUS).

K. Davis, G. Green, M. Martinez, K. Brakel, and S. Niewiesk. Department of Veterinary Biosciences.

Cotton rats are used as a model of human viral respiratory infections. In this study, we aimed to establish a reproducible protocol for respiratory allergies in the cotton rat. This model will allow future studies to investigate how allergies may complicate the severity of viral respiratory infections. Cotton rats were sensitized to house dust mite antigen (HDM) and subsequently challenged with HDM. The adjuvant used to induce an allergic reaction was aluminum phosphate (APhos) or monophosphoryl lipid A (MPLA). Studies in mouse models have shown that these adjuvants will produce either a Th1 response characterized by secretion of IFNγ or a Th2 response characterized by secretion of IL-4. PBS served as the adjuvant control. The allergic response was assessed by bronchoalveolar lavage, histology, ELISA, and lung wet to dry ratios. Contigs derived from an RNA sequencing project were used to design primers for PCR to demonstrate the presence of eosinophil recruitment factors CCL11 and IL5 and eosinophil survival factors FM-CSF and IL33. Intraperitoneal sensitization to HDM with PBS, APhos, or MPLA was sufficient in eliciting an allergic immune response upon intranasal challenge with HDM. This was characterized by an increase in eosinophils, an increase in lung mucous, and detectable levels of CCL11, IL5, GM-CSF, and IL33. Quantitative analysis of these recruitment and survival factors is ongoing. Animals exposed to intranasal HDM without previous sensitization had significantly fewer eosinophils in their lungs. Similar levels of IL-4 and IFNγ were measured in APhos, MPLA, and PBS with HDM sensitized animals. Infection with respiratory syncytial virus (RSV) elicited a primarily lymphocytic response with little mucous in the lungs. Animals infected with RSV and challenged with HDM developed an eosinophilic response with the presence of mucous indicating that this allergy protocol can be used in future studies to investigate the interaction between allergies and respiratory infection.

Keywords: Allergies, Cotton Rat, House Dust Mite, Respiratory

IMID – 10

HIGHER SUSCEPTIBILITY AGAINST RESPIRATORY SYNCYTIAL VIRUS INFECTION IN GERIATRIC COTTON RATS. O. Harder and S. Niewiesk. Department of Veterinary Biosciences.

Human respiratory syncytial virus (RSV) is the leading casue of respiratory disease in infants and young children worldwide. RSV has also been recognized as a serious health risk in elderly individuals and can lead to chronic respiratory disease. The increasing suseptibility and incidence of RSV infection highlights the need for an effective animal model to represent RSV infection in the elderly age group. With an effective model, the immune system can then be manipulated to aid viral clearance within this age group.

Cotton rats (Sigmodon hispidus) are the preferred small animal model for clinically relevant research on human respiratory viruses. Previous literature has shown that cotton rats over the age of 9 months old have a decrease in their ability to clear infection when compared to adult cotton rats. According to the literature, the virus grows to higher titers in geriatric animals. We wanted to confirm these results and determine the mechanism of increased susceptibility.

Our results demonstrated that virus grew to similar titers in the geriatric cotton rats as in adult rats, but had prolonged clearance rates in geriatric rats. After immunization with RSV, the geriatric cotton rats were not fully protected and had minimal neutralizing antibody titers. Cytotoxic lymphocytes were also manipulated and results showed that they are essential for RSV clearance. Pharmacological suppression of inflammation resulted in a reduction in viral growth and complete protection after immunization. It appears that in geriatric animals, the immune system works similarly as in adults animals, but not as effectively and that anti-inflammatory therapy may restore immune function.

Keywords: Respiratory Syncytial Virus, Virology, Immunology, Cotton Rat

IMID – 11

EPIDEMIOLOGY AND GENOMIC CHARACTERIZATION OF MDR SALMONELLA FROM DOMESTIC ANIMALS AND WILDLIFE. B. Jourdan, E. Tigabu, J. O'Quin, G. Habing, and W. Gebreyes. The Ohio State College of Veterinary Medicine, The Ohio State University, Columbus, OH (Jourdan, O'Quin, Habing, Gebreyes, Binkley), Ethiopian Public Health Institute, Addis Ababa, Ethiopia (Tigabu)

As human populations expand, the gap between natural and anthropogenic landscapes disappears bringing wild and human populations into closer contact. Antimicrobial resistant Salmonella strains identified in Ethiopian livestock are known to spread to humans via consumption of contaminated water and food. Resistant Salmonella strains may be similarly transmitted between wild and domestic animals. Existing research on the distribution of Salmonella in Ethiopia has primarily focused on livestock. Consequently, the prevalence of multi-drug resistant (MDR) Salmonella strains among Ethiopian wildlife populations has not been investigated and the role of wildlife in transmission of these strains to humans remains unknown. The objective of this study was to determine the prevalence of MDR Salmonella in Ethiopian wildlife to identify the role they play in the transmission cycle. A cross-sectional study was conducted to assess the prevalence and antimicrobial resistance profiles of Salmonella identified in Ethiopian wild and domestic species. Once the presumptive Salmonella isolates are confirmed, they will be tested for MDR using the Kirby-Bauer disk diffusion method and will undergo whole genome sequencing. Results will be compared to previous data from domestic and wild species as well as from human origin to determine the extent of phenotypic and genotypic similarity. The findings of this study will help inform future research and ultimately improve our understanding of MDR Salmonella transmission among these populations. Such information will be vital for the development of management strategies that can interrupt the transmission of MDR strains when animal and human health could be at risk.

Keywords: Salmonella, multi-drug resistance, zoonotic, Ethiopia

IMID – 12

CHARACTERIZING IMMUNE CELL INFILTRATES IN THE TUMOR MICROENVIRONMENT OF CANINE ORAL MELANOMA. J. Kendziorski, R. Jennings, W. Hendricks, G. Lorch

Understanding the tumor microenvironment is crucial for developing potential therapeutics against cancer. Due to similarities between spontaneous canine oral melanoma tumors and a subset of human melanoma tumors, the dog is a suitable model for translational research for this tumor type. One of the emerging concepts in cancer therapeutics is the development of targeted immunotherapies for both humans and animals. As part of the Canine Comparative Oncology and Genomics Consortium (CCOGC), the aim of this study was to characterize the immune cell infiltrates (ICIs) in canine oral melanoma tumors. For patients with known history, the average survival time was 194.3 ± 45.3 days. Out of 25 cases, 19 developed metastases to either lung or lymph node, 8 received chemotherapy, 10 received radiation, and 15 received at least one dose of the melanoma vaccine. The ICIs were identified via immunohistochemical techniques and included cytotoxic T cells (CD8+), T helper cells (CD4+), activated T cells (CD3+), regulatory T cells (FoxP3+), M2 macrophages (CD163+), and an inhibitory checkpoint antigen (CTLA4+). The most abundant ICIs were CD4+ and CD8+ T cells and lowest level of ICIs was FoxP3+ cells. CD3+ cells were lower than both CD4+ and CD8+ cells, suggesting that T helper and cytotoxic T cells were present in the tumor microenvironment but some were not activated. CD163+ and CTLA4+ cells were present at low levels. This study provides the initial characterization necessary for comprehending the complex microenvironment in canine oral melanoma tumors and is the first step in developing targeted immunotherapeutics for both canines and humans.

Keywords: Melanoma, Canine, Immunology, Immunotherapeutics, Translational

IMID – 13

PANETH CELLS REGULATE ALLERGIC SENSITIZATION IN THE GASTROINTESTINAL TRACT. E. Kim[1], D. J. Burnett[1], A. J. Rudinsky[1], Y. Mori-Akiyama[2], E. Cormet-Boyaka[1], P. N. Boyaka[1]
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The gut immune homeostasis plays a major role in controlling sensitization to food allergens and the development of allergic responses in the gastrointestinal tract, but also to skin or lung. Epithelial cells sensing of commensal microbes is crucial for the maturation of the gut immune system and antibiotic treatment during early life is believed to have contributed to the increased incidence of food allergy. Paneth cells secrete antimicrobial products in the crypts of the small intestine, but their role in food allergic remains elusive. We found that SOX9$^{\Delta IEC}$ mice, which lack Paneth cells, display dysbiosis with increased microbial diversity and the presence of a large number of unclassified bacterial species. Upon oral sensitization with OVA as a model food antigen in the presence of cholera toxin, SOX9$^{\Delta IEC}$ mice developed similar levels of allergen-specific serum IgA and IgG responses than control wild-type mice with the exception of IgG3 responses, which were elevated in SOX9$^{\Delta IEC}$ mice. Interestingly, SOX9$^{\Delta IEC}$ mice exhibited significantly increased allergen-specific serum IgE responses, and exacerbated signs of allergic responses (i.e., swelling, hypothermia, impairment of lung functions) after nasal allergen challenge. Transplantation of fecal materials from SOX9$^{\Delta IEC}$ mice transfered the hyper-allergenic phenotype to control wild-type mice confirming the role the microbiota in the enhanced allergic responses.

Keywords: Paneth cells, Microbiome, Gut homeostasis, Allergy

IMID – 14

EVALUATION OF THE EFFECTS OF CD3E-IMMUNOTOXIN-MEDIATED LYMPHODEPLETION ON VARIOUS BODY ORGANS. A. Kim, S. Kim, and S. Kim. Dept. Of Veterinary Biosciences.

Recent advances in Chimeric Antigen Receptor (CAR) T-cell therapy have generated tremendous hope for the treatment of incurable diseases, including HIV/AIDS. Cytotoxic lymphodepletion preconditioning improves the efficacy of CAR T-cells by creating a favorable “lymphoid space” for the enhanced survival and function of the transferred T-cells via depletion of T-cells and “cytokine sink” cells. Lymphodepleting preconditioning is now included in most CAR T-cell therapies, but the current chemotherapeutic regimen has yielded inconsistent results among patients, and the risk of premature implementation has been poignantly demonstrated by the recent deaths in CD19 CAR T-cell preclinical trials. CD3e-immunotoxin (CD3e-IT), an anti-CD3e monoclonal antibody conjugated with diptheria toxin (DT), has been developed to specifically ablate T-cells in the body. In order to evaluate CD3e-IT as a potential lymphodepletion preconditioning regimen, we analyzed the cytotoxic effects of CD3e-IT on various organs and compared them to the effects of Cyclophosphamide (CTX), a commonly used chemotherapeutic regimen, and the diphtheria toxin (DT)-mediated T-cell depletion system. In particular, we focused on analyzing CXCR5+ follicular T cell (Tfh) populations, a key target in CAR T-cell therapy for HIV-1 and follicular T-cell cancers. Our study demonstrated that CD3e-IT treatment was highly specific and efficient in removing T-cells, showing approximately 14- to 20- and 18- to 29-fold reduction rates for CD4+ T-cells and CD8+ T-cells. Interestingly, CXCR5+ T-cells were notably enriched after the CD3e-IT treatment. Unlike CD3e-IT, CTX treatment showed non-specific cytotoxic effects on lymphoid cells, with a relatively high efficiency in the killing of B-cells. Our data suggest that CD3e-IT is a highly specific T-cell depletion agent and may provide a safer and more effective option for host preconditioning prior to any adoptive T-cell therapies. Higher intensity or combination lymphodepletion therapy may be necessary to effectively remove Tfh in HIV-1 or follicular T-cell cancer patients.

Keywords: CAR T-Cell, HIV/AIDS, Follicular T-cell Cancer, Lymphodepletion, CD3e-immunotoxin, Diptheria Toxin, Cyclophosphamid, Fludarabine, Follicular T cells

IMID – 15

A VACCINE SUPPLEMENTATION APPROACH FOR INDUCTION OF MUCOSAL IGA BY INJECTED VACCINES. Z. Attia, J. C. Rowe, E. Kim, H. E. Steiner1, E. Cormet-Boyaka1, and P. N. Boyaka.

Most current vaccines are injected vaccines that contain alum as adjuvant. These vaccines promote good antibody with predominantly Th2 responses in the bloodstream and they have helped to limit many infectious diseases worldwide. Since, most infectious agents enter the host via mucosal surface of the gastrointestinal, respiratory or genito-urinary tracts, induction of secretory (SIgA) in these sites could provide a first line of defense. Previous work in our laboratory has demonstrated an inverse relationship between the ability of a sublingual vaccine to recruit neutrophils and induction of SIgA. Since alum was shown to recruit Gr1+ cells secreting IL-4, we examined whether inhibition a neutrophil function could help alum-based vaccines to induce serum IgA responses and perhaps, SIgA in mucosal tissues. For this purpose, groups of mice were immunized IP with antigen alone (Ovalbumin + protective antigen of anthrax), antigen plus alum, or antigen plus alum and an inhibitors. Our results show that enhanced the magnitude of antigen-specific serum IgG1, but also IgG2a responses. This profile of serum IgG responses was consistent with increased frequency of IFNg+ CD4+ and IL-17+CD4+ T cells in the spleen of mice that received the inhibitors, promoted antigen-specific serum IgA responses, as well as SIgA in mucosal secretion of the gastrointestinal tract, but not in the genito-urinary tract. Taken together our results show that presence of extended neutrophil function associated with Alum based vaccines down regultaes Th1 immune responses and imapire SIgA by injected vaccines.

Keywords: Alum, Th1, Cytokines, Neutrophils, injectable vaccines

IMID – 17

ROLE OF CHROMATIN INSULATOR CTCF IN HTLV-1 RETROVIRAL PATHOGENESIS. M. Martinez[1], J. Al-Saleem[1], A. Panfil[1], L. Ratner[2], and P. Green[1]
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[2]Department of Medicine, Washington University, St. Louis, MO

Human T-cell leukemia virus type 1 (HTLV-1) is the etiologic agent of adult T-cell leukemia and the neurological disorder HTLV-1 associated myelopathy/tropical spastic paraparesis. Approximately 5-10% of infected individuals will develop disease after a prolonged latency period. The exact mechanisms through which latency and disease progression are regulated are not fully understood. CCCTC-binding factor (CTCF) is an 11-zinc finger, sequence-specific, DNA-binding protein with thousands of binding sites throughout mammalian genomes. CTCF has been shown to be a regulator of chromatin structure and gene expression through homodimer formation. A CTCF-binding site was recently identified within the HTLV-1 provirus and was shown to act as an enhancer blocker, regulate proviral gene expression, and interact with the flanking host genome. Therefore, we propose to study the epigenetic effects of CTCF on HTLV-1-induced *in vitro* immortalization and *in vivo* persistence. Using an HTLV-1 proviral molecular clone, a ΔCTCF mutant was generated using site-directed mutagenesis. The ΔCTCF mutant is transcriptionally similar to WT, as demonstrated by LTR-based reporter gene assays and ELISA for viral gag release. *In vitro* immortalization capacity was assessed via short-term co-cultivation of irradiated, stably transfected, viral producer cells with freshly isolated peripheral blood mononuclear cells (PBMCs). This showed comparable immortalization capacity and viral gag release between the ΔCTCF mutant and WT infected cells. Lastly, *in vivo* viral persistence was assessed using an established rabbit model of infection. Rabbits were inoculated with irradiated viral producer cells followed by serial collection of blood samples. We will assess viral infection, proviral load (qPCR), immune response (ELISA/Immunoblot), and retroviral gene expression (RT-qPCR) to determine the role CTCF plays in the early stages of HTLV-1 infection. Ultimately, understanding epigenetic regulation of HTLV-1 pathogenesis could provide meaningful insights into mechanisms of immune evasion and novel therapeutic targets.

Keywords: CTCF, HTLV-1, ATL

IMID – 18

MEASURING PULMONARY FUNCTION IN COTTON RATS AFTER RSV INFECTION

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Human respiratory syncytial virus (RSV) is a leading cause of bronchiolitis and viral pneumonia in infants and young children worldwide, as well as a major cause of respiratory disease in the elderly. RSV is associated with increased airway resistance, decreased compliance, and increased airway hyperresponsiveness. The gold standard for measuring airway resistance, compliance and hyperresponsiveness is through forced oscillation technique (FOT), which has not been previously performed using the best small animal model for RSV infection, the cotton rat. Our goal was to characterize pulmonary function in the cotton rat, as well as 2, 4, 6 and 8 days post-RSV infection, using FOT. Our hypothesis was that post-RSV infection cotton rats would develop similar alterations in pulmonary function as seen in affected infants. There was a significant increase in female airway resistance at maximum methacholine dosage 4 days post-infection (dpi) when compared to males, indicating a gender difference in airway hyper-responsiveness after RSV infection. Female cotton rats 4 dpi had a significant increase in baseline airway resistance, significant decrease in baseline pulmonary compliance, and demonstrated airway hyper-responsiveness. This time point corresponds to peak viral replication in the cotton rat lungs. Therefore, the female cotton rats at 4dpi resembles some alterations of pulmonary function observed in RSV infected infants. Our data suggest that FOT is a sensitive technique that can potentially be used to measure a vaccine candidate's ability to protect against alterations of lung function.

Keywords: Human respiratory syncytial virus, cotton rat, airway resistance, compliance, airway hyperresponsiveness

IMID – 19

THE HD DOMAIN OF SAMHD1 IS REQUIRED FOR ITS SUPPRESSION OF INTERFERON REGULATORY FACTOR 7 (IRF7)-MEDIATED TYPE I INTERFERON INDUCTIONZ. Z. Qin, S.Chen, C. St. Gelais, S.Kim, S. Bonifati, L. Wu. Depts. Of Veterinary Biosciences

Homozygous mutations of the gene encoding sterile alpha motif and HD domain containing protein 1 (SAMHD1) can cause Aicardi-Goutières syndrome characterized by the induction of type I interferon (IFN-I), indicating that SAMHD1 is a negative regulator of the innate immune responses. Our recent studies identified that SAMHD1 interacts with IRF7 and inhibits IRF7-mediated IFN-I induction, resulting in suppression of innate immune responses to HIV-1 or Sendai virus infection. However, the domain of SAMHD1 responsible for the IRF7 interaction and IFN-I suppression have not been identified. We hypothesize that SAMHD1 and IRF7 interaction is required for the suppression of IRF7-mediated IFN-I induction by SAMHD1. To map the site of SAMHD1-IRF7 interaction, we generated a series of truncated SAMHD1 mutants and tested their interactions with full-length IRF7 through co-immunoprecipitation (co-IP) in HEK293T cells. We found that mutants lacking the HD domain did not interact with IRF7, suggesting that HD domain of SAMHD1 is important for IRF7 interaction. We then determined the contribution of the HD domain to suppression of IRF7-mediated IFN-I induction using an IFN-sensitive response element (ISRE) reporter assay. Our data showed that the HD domain of SAMHD1 is necessary and sufficient for IRF7 interaction and suppression of INF-I induction. Our ongoing work is to examine the exact aa within HD domain responsible for the interaction with IRF7 and the suppression of IRF7-mediated ISRE activation using additional SAMHD1 mutants through co-IP assay and dual luciferase assay. Overall, our findings reveal the domain of SAMHD1 contributing to IFN-I inhibition, which help define the mechanisms of SAMHD1 in suppressing the innate immunity.

Keywords: SAMHD1, IRF7, type I interferon, innate immune responses

IMID – 20

ENGINEERING OF IMMUNE TARGETED MUCOADHESIVE CHITOSAN BASED SALMONELLA NANOVACCINE FOR ORAL DELIVERY IN POULTRY. S. Renu, A.D. Markazi, S.Dhakal, Y.S. Lakshmanappa, R. Shanmugasundaram, R.K. Selvaraj and G.J. Renukaradhya, Food Animal Health Research Program

Salmonellosis in poultry is a serious economic burden. The major concern is the public health hazard caused by consumption of Salmonella contaminated poultry meat and egg. Currently used Salmonella vaccines in poultry are ineffective in combating the Salmonellosis, warranting the need of a potent vaccine, especially an oral vaccine that can elicit robust local intestinal immunity. Biodegradable and biocompatible natural polymers are FDA approved vehicles for vaccine delivery. We prepared a Salmonella candidate subunit chitosan nanoparticles (CS NPs) based vaccine containing the entrapped immunogenic outer membrane proteins (OMPs) and flagellar (F) protein, and surface decorated with F-protein (OMPs-F-CS NPs). Physicochemical and biocompatibility properties of OMPs-F-CS NPs were studied in detail including its stability in stomach pH conditions. Salmonella targets the microfold (M) cell in the Peyers patches (PPs) of chicken ileum. We designed OMPs-F-CS NPs which targets ileal PPs to induce local immune response when delivered orally, and demonstrated that by ex vivo and in vivo studies. The OMPs-F-CS NPs vaccinated layer chickens had significantly higher OMPs-specific mucosal IgA and lymphocytes proliferation responses. OMPs-F-CS NPs upregulated the expression of toll-like receptor (TLR)-2, TLR-4, IFN-gamma, TGF-beta and IL-4 mRNA expression in the cecal tonsils of layer chickens. In conclusion, chitosan based oral Salmonella vaccine was found targeted to intestinal PPs immune cells of birds, and induced antigen specific B and T cell responses. Thus, our candidate oral Salmonella vaccine has the potential to mitigate Salmonellosis in poultry.

Keywords: Chicken, Salmonella, Chitosan, Oral delivery, Mucosal

IMID – 21

FUNCTIONAL MATURATION OF THE COTTON RAT IMMUNE SYSTEM THROUGH SEQUENTIAL VIRAL INFECTIONS. A. M. Romano, S. Niewiesk. Department of Veterinary Biosciences.

Laboratory animals are raised in specific pathogen free environments. Recent research has demonstrated that the immune system of laboratory mice is phenotypically as immature as that of a human infant. Exposing lab mice to mouse pathogens leads to a phenotypical maturation of their immune system. Cotton rats (Sigmodon hispidus) are an ideal model to study the pathogenesis of human respiratory viruses, and are used for the development of vaccines and anti-virals. We hypothesized that the immune system of cotton rats are similarly immature as that of mice, and that infecting cotton rats with different viruses would lead to maturation as measured by differences in resistance to virus infection, generation of antibody, and T-cell responses.

Cotton rats were infected with three human viruses (i.e. influenza, parainfluenza, and measles viruses) or inoculated with keyhole limpet hemocyanin dinitrophenyl in different combinations in weekly intervals and then challenged with respiratory syncytial virus (RSV). Viral titers in the lung post RSV challenge indicated significant reduction in viral titers of infected versus untreated cotton rats. The same trend was visible in nasal titers. T-cell proliferation assays four weeks post infection indicated significant reductions in T-cell responses. Neutralization assays showed a trend in infected cotton rats having a higher antibody response against RSV compared to untreated cotton rats.

It appears that prior infection changes the resistance of cotton rats against infection with RSV. Subsequent studies will evaluate whether cotton rats with an artificially matured immune system will be better models for vaccination studies, and what the mechanisms behind the maturation process are.

Keywords: Cotton rat, Respiratory syncytial virus, Immune system

IMID – 22

A NOVEL SUPPLEMENTATION APPROACH TO ENHANCE HOST RESPONSE TO SUBLINGUAL VACCINATION. J. Rowe, Z. Attia, E. Kim, E. Cormet-Boyaka, P. Boyaka. Department of Veterinary Biosciences

Sublingual immunization is emerging as an alternative to nasal immunization and induction of mucosal IgA responses. Using *Bacillus anthracis* edema toxin (EdTx) as an adjuvant, we previously showed that innate responses triggered after sublingual immunization could limit generation of IgA responses. We tested whether co-administration of a neutrophil elastase inhibitor (NEI) could rescue the ability of EdTx to induce broad antibody responses, including mucosal IgA. NEI supplementation of sublingual vaccines containing EdTx promoted antigen-specific serum IgA responses but also enhanced serum IgG1, and IgG2b responses. This enhancing effect of NEI did not extend to all antibody isotypes and IgG sublclasses, since it reduced serum IgE responses and did not affect IgG2a/c and IgG3 responses. The NEI also promoted anti-*Bacillus anthracis* protective antigen (PA) neutralizing antibodies and enhanced high affinity IgG1 and IgA antibodies. In addition to serum IgA, NEI supplementation stimulated antigen-specific mucosal IgA responses in the GI tract, and enhanced antigen-specific IgG responses in vaginal washes. Analysis of CD4+ T helper cell responses revealed that co-administration of NEI broadened the profile of cytokine responses, by stimulating Th1, Th2, Th17, and Tfh cytokines. We also noted that NEI had a higher stimulatory effect on IL-5, IL-10, IL-17 responses.

Keywords: Sublingual Vaccination, Vaccine Supplementation, Immunoglobulin Class Switch

IMID – 23

CONCURRENT BUT CONSECUTIVE VACCINATION OF MODIFIED LIVE PRRSV-1 & PRRSV-2 PROVIDES BETTER PROTECTION IN NURSERY PIGS. Y.Shaan Lakshmanappa[1]*, P. Shang[2], S. Renu[1], S. Dhakal[1], B. Hogshead[1], P. Bernardo[1], X. Yan[2], Y. Fang[2] and R. Gourapura[1] , [1]Food Animal Health Research Program (FAHRP), OARDC, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, OH 44691, USA. [2]Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, KS, USA

Porcine reproductive and respiratory syndrome virus (PRRSV) causes significant economic loss to the swine industry worldwide. Conventional PRRSV free nursery pigs were vaccinated with modified live virus (MLV) of both type 1 and type 2 PRRSV genotypes. However, the vaccination efficacy against PRRSV-1 in the simultaneously manner is less desirable. Therefore, in current study, a concurrent and consecutive vaccination strategy was adopted in order to improve the protective efficacy against both PRRSV species. The PRRSV-1 and PRRSV-2 were administered intramuscularly, either 3 days apart (PRRSV-1 followed by PRRSV-2, consecutive) or together on the same day (concurrent). At day 42, half of the pigs in each group were separately challenged with homologous PRRSV-1 or PRRSV-2. The study was terminated at 10 days post challenge. Quantitative RT-PCR result showed that PRRSV-1 was detectable from days 3-42 in consecutive, but days 28-42 in concurrent vaccinated pigs. The PRRSV-2 was detectable from days 3-42 in both groups at comparable levels. By day 42, genomic copies were <2 log and replicating virus was undetectable in both the pig groups. In post-challenge stage, viral load in serum was lower in pigs vaccinated by PRRSV-1 MLV in consecutive manner than that of concurrently vaccinated pigs, while replicating PRRSV-2 was undetectable in both immunization groups. In the recall lymphocyte response study, TBLN mononuclear cells restimulated with the respective challenge virus had enhanced IFN-g secreting T-helper/memory and cytotoxic T lymphocytes responses in both groups. In PBMCs, only IFN-g positive T-helper/memory cells in PRRSV-2 challenged animals were observed. Consecutive vaccine received pigs showed significantly higher VN titer (>6 log2) against PRRSV1/PRRSV2 challenge, whereas concurrent vaccine received pigs had >3 log2 VN titer against PRRSV2 challenge. In conclusion, consecutive vaccination with PRRSV-1 first followed by PRRSV-2 provides better viral clearance and induced better adaptive immune responses in pigs.

Keywords: Porcine reproductive and respiratory syndrome, MLVs, Protective Immunity, PRRSV-1/2 restimulation, TBLN

IMID – 24

RATIONAL DESIGN OF HUMAN RESPIRATORY SYNCYTIAL VIRUS LIVE ATTENUATED VACCINES BY INHIBITING VIRAL MRNA CAP METHYLTRANSFERASES. M. Xue[1], R. Wang[1], Y.Zhang[1], M. Lu[1], O. Harder1, X. Liang[1], M. Peeples[2], S. Niewiesk[1], J. Li[1]

Human respiratory syncytial virus (RSV) is the leading causative agent of pediatric respiratory tract disease worldwide. Currently, there are no vaccines or antiviral drugs to combat this virus. A live attenuated vaccine is one of the most promising vaccines for RSV. However, it has been a challenge to identify an attenuated RSV strain that has an optimal balance between attenuation and immunogenicity. The 5' end of the mRNA of pneumoviruses contains a unique cap structure that is typically methylated by guanine N-7 (G-N-7) and ribose 2'-O methyltransferases (MTases). The objective of this study is to rationally design RSV live attenuated vaccines by inhibiting viral mRNA cap MTase . The S-adenosylmethionine (SAM) binding sites (G1853 and G1857) in the MTase region of the large (L) polymerase protein of RSV was mutated to alanine, and recombinant RSV(rRSV) carrying these mutations were recovered from an infectious cDNA clone. All three recombinant viruses (rRSV-G1853A, G1857A, and G1853A-G1857A) were defective in mRNA cap methylation and were genetically stable and highly attenuated in cell culture. These recombinant viruses had significant defects in spread and replication in primary, well differentiated, human airway epithelial (HAE) cultures, a near in vivo model for RSV infection. Finally, the replication, pathogenesis, immunogenicity, and capacity to induce protection of these rRSVs were examined in cotton rats, the best available small animal model for RSV infection. The results showed that all three recombinant viruses were highly attenuated in replication in the upper and lower respiratory tracts of cotton rats. Importantly, these recombinant viruses elicited high levels of neutralizing antibody and provided complete protection against RSV infection. In addition, no enhanced pulmonary disease was observed. Taken together, these results demonstrate that targeting the viral mRNA cap MTase is a novel, new approach to rationally attenuate RSV for vaccine purposes.

Keywords: Respiratory syncytial virus(RSV), Vaccine, Methylation

IMID – 25

Platform Presentation

ZIKA VIRUS DOWN-REGULATES AXL AND TIM-1 FOR OPTIMAL SPREAD.

J. Yu, Y.M. Zheng, and S.L. Liu. Depts. Of Veterinary Biosciences

The interplay between the virus and host largely determines the outcome of viral infections. The phosphatidylserine (PS) receptors, AXL and TIM-1, have been recently shown to be candidate entry factors for ZIKV infection in vitro. However, if and how ZIKV infection regulates these entry factors remains unknown. Here we examined AXL and TIM-1 expression following ZIKV infection of human alveolar basal epithelial cell line A549, glioblastoma cell line U87, as well as primary neuron progenitor cell and primary trophoblasts. Our results showed that both the Asian strain ZIKV strain FSS13025 and the African ZIKV strain MR766 down-regulate AXL, and to a lesser extent, TIM-1 expression in viral infected cells. Furthermore, we observed that multiple ZIKV proteins, including E, NS2A, NS3 and NS4B, are responsible for this down-regulation. Additional experiments showed that down-modulation of AXL and TIM-1 can be overcome by treating cells with lysosomal inhibitor NH4Cl and autophagy inhibitor 3-MA. Moreover, we showed that the downregulation of AXL and TIM-1 expression reduces ZIKV superinfection, and diminishes host cell death and innate immune signaling. Our findings reveal that ZIKV down-regulates their candidate entry factors, thus allowing for optimal viral spread and pathogenesis.

Keywords: ZIKV, AXL, Tim1, Down-regulation

IMID - 26

PRMT5 IS UPREGULATED IN ACTIVATED T CELLS & IS A NOVEL THERAPEUTIC FOR AGVHD. N.C. Zitzer[1], K. Snyder[2], R. Garzon[2], R.A. Baiocchi[2], P. Ranganathan[2]
Departments of [1]Veterinary Biosciences and [2]Internal Medicine

Acute Graft-versus-host disease (aGVHD), a T cell mediated immunological disorder is the leading cause of non-relapse mortality in patients receiving allogeneic bone marrow (BM) transplants. Protein arginine methytransferase 5 (PRMT5) catalyzes symmetric dimethylation of arginine (R) residues on proteins. Recently it was shown that T cells are sensitive to R methylation & PRMT5 promotes activation of memory T helper cells.
Objectives: To investigate 1) mechanisms by which PRMT5 regulates T cell function & 2) PRMT5 inhibition as a therapeutic strategy for aGVHD.
Methods: Splenic T cells were isolated from mice that received either T cell depleted BM (TCD BM) or TCD BM with C57/BL6 (B6) allogeneic splenocytes. *In vitro* activation of B6 T cells was achieved with CD3/CD28 Dynabeads or co-culture with allogeneic BM derived dendritic cells. PRMT5 expression (RT-PCR, western blot) & function (H3R8me2s western blot) were evaluated. PRT220, a novel PRMT5 inhibitor, was used to evaluate PRMT5 inhibition on T cell function. We assessed proliferation (Cell Trace Violet, Ki67), apoptosis (Annexin V), cytokine secretion (ELISA, flow cytometry), cell cycle (PI incorporation & Fucci mice), & cell signaling (western blot).
Results: PRMT5 expression & activity is increased following T cell activation. Treatment of T cells with PRT220 reduces symmetric dimethylation indicating impaired PRMT5 activity. Inhibition of PRMT5 reduces T cell proliferation by arresting T cells in G0/G1 phase. Blocking PRMT5 activity leads to reduced effector function & perturbation of ERK signaling. Finally, PRT220 shows potent biological activity *in vivo* by reducing T cell absolute numbers, proliferation, effector function.
Conclusions: PRMT5 expression & activity are upregulated in activated T cells & blocking PRMT5 enzymatic activity significantly impairs multiple facets of T cell response. These findings both *in vitro* & *in vivo* make PRMT5 an exciting novel therapeutic target for aGVHD.

Keywords: aGVHD, T cells, PRMT5, immunology

IMID - 27

ULTRA-HIGH-ACCURACY, LONG-RANGE TARGET SEQUENCING FOR HIV-1 WHOLE GENOME ANALYSIS. H.Yu*, A.Baek*, S.H. Kim and S.Kim. Departments of Veterinary Biosciences (*equal contribution)

HIV/AIDS remains a global epidemic. The genetic diversity and continual hyper-evolution of HIV-1 have presented major challenges in controlling the epidemic. Meanwhile, HIV genotyping has been hampered by the short-read length (100-800bp) and frequent errors (1/100~1/1000) of current sequencing platforms. Nanopore sequencing (Oxford Nanopore technologies), a 3rd generation sequencing platform, has several revolutionary features, including an extremely long-read length (up to 350kb), real-time data output, and pocket-size mobility. However, its application is significantly limited by its high error rates (1/20~1/10). We have developed a novel Tandem Twin barcode (TTB) method that can eliminate read errors in Nanopore single-molecule target sequencing. Our method can correct read errors via (i) unique tagging of individual target DNA with a TTB, (ii) PCR amplification and high-throughput sequencing of the PCR products, (iii) accurate barcode reading (by cross-comparison of the twin barcodes in the same read), and (iv) correction of read errors in the parental template through cross-comparison of sequences with an identical TTB. We have successfully generated a high-quality TTB library specific to HIV-1 and sequenced 24 TTB-labeled, clonal HIV-1 plasmid DNA (pNL4.3) molecules and 3 proviruses with an accuracy as high as 99.99%. Multiple Sequence Alignment (MSA) that cross-compare raw data (with an identical TTB) improved the average read accuracy from 80-95% to an average 98.74%. An additional homopolymer error correction further improved the average read accuracy to 99.73%. The development of this new sequencing method will enable high-fidelity genotyping of full-length HIV DNA in a high-throughput fashion. Our goal is to achieve an accuracy of > 99.99% with a read depth of < 20. This technical advancement will have a broad impact on diverse areas of biomedical sequence analysis. We are particularly interested in investigating the genetic diversity of HIV-1 provirus to better understand HIV-1 latency and persistent infection in patients.

Keywords : HIV/AIDS, Tandem twin-barcode(TTB) library, long-read sequencing, barcoding-mediated error correction, Nanopore sequencing

MOLECULAR
AND
CELLULAR BIOLOGY

MCB - 1

IL-6/GP130-RELATED SIGNALING IN A NOVEL MODEL OF SUPPORTING LIMB LAMINITIS. M.A. Bernard, M.R. Watts, A.W. van Eps*, J.K. Belknap. Depts. Of Veterinary Clinical Sciences, University of Pennsylvania School of Veterinary Medicine*

In equine laminitis, structural failure of the lamellae occurs due to lamellar epidermal stretching and dysadhesion from the underlying dermal lamellae. Supporting limb laminitis (SLL) is a type of laminitis that occurs in a limb undergoing excessive weight bearing due to an inability to weight bear on the contralateral limb. Previous studies on the other types of laminitis (sepsis-related and endocrinopathic laminitis) indicate that mTORC1-related signaling may play a role in lamellar failure. Due to these results and increased lamellar IL-6 mRNA concentrations present in an SLL model, we hypothesized that IL-6/gp130-mediated activation of mTORC1/p70S6K/RPS6 and JAK2/STAT3 signaling occur in the lamellar epidermis in SLL. In the current study, laminitis was induced in Standardbred horses by use of a V-shaped shoe (unloaded limb) to cause unilateral/preferential weight-bearing to the opposite forelimb (supporting limb). Mounted ground sensors were used to confirm preferential weight-bearing of the supporting limb. After 72 hours of preferential weight bearing, lamellae were harvested and snap frozen for protein analysis via Western blot and immunofluorescence. The supporting limb lamellae had increased concentrations (vs. control animals) of phospho (P)-RPS6 (Ser 240/244 [p=0.0140] and Ser 235/236 [p=0.0023] moieties) and P-STAT3 (Ser 727 [p=0.0012] and Tyr 705 [p=0.0082] moieties). The majority of signaling was localized to lamellar epidermal cells on immunofluorescence. There was not a strong correlation between lamellar IL-6 and lamellar P-RPS6 or P-STAT3 concentrations (i.e., r=0.357, p<0.444 for P-RPS6 235/6 moiety). These findings, while not supporting a major role for IL-6 in the downstream signaling assessed in this project, provide a basis for further evaluation of mTORC1/p70S6K/RPS6 and JAK2/STAT3 signaling in an effort to discover potential therapeutic targets to prevent lamellar failure in horses with SLL.

Keywords: IL-6/gp130 signaling, supporting limb laminitis, JAK2/STAT3, equine Immunofluorescence, immunoblotting

MCB - 2

EFFICIENT CONCENTRATION OF PLASMA AND PLATELET-WHITE BLOOD CELL-RICH PLASMA PROTEINS USING A POLYACRYLAMIDE DEVICE. S. M. Muir[1], N. Reisbig[1], M. Baria[2], C. C. Kaeding[3], A. L. Bertone[1]
[1]Veterinary Clinical Science, College of Veterinary Medicine; and [2]Physical Medicine & Rehabilitation, College of Medicine. Jameson Crane Sports Medicine Institute, The Ohio State University.

Background: Currently platelet-poor plasma (PPP) is a discarded waste product of platelet-rich plasma (PRP) and may contain valuable anabolic proteins. Our goal was to evaluate the potential benefit of concentrating PPP as a therapy for osteoarthritis.

Hypothesis: Our hypothesis was that a novel polyacrylamide concentration device would efficiently concentrate insulin-like growth factor-1 (IGF-1) from PPP and would concentrate transforming growth factor-beta (TGF-beta) and interleukin-1 receptor antagonist (IL-1ra) from PRP.

Study Design: A laboratory study was conducted with human clinical plasma and equine blood samples.

Methods: Samples and products were characterized for platelet, white blood cell, and growth factor / cytokine content and quantified by enzyme-linked immunosorbent assays specific for IGF-1, TGF-beta, IL-1beta and IL-1ra as representatives of cartilage anabolic and inflammatory mediators.

Results: IGF-1 was significantly concentrated by the device from both human and equine PPP and was even greater than the levels found in PRP. TGF-beta, IL-1beta and IL-1ra were concentrated in PRP, not in concentrated PPP. The polyacrylamide concentrator device highly concentrated white blood cells and plasma proteins over PRP and whole blood, most dramatically TGF-beta (29-fold over blood) and IL-1ra (70-fold over plasma) also resulting in a 2000-fold increase in IL-1beta/IL-1ra ratio over plasma and 1668-fold increase in the ratio over PRP. Patients with osteoarthritis had a lower anabolic protein profile and a higher inflammatory-related protein profile compared to equine athletes without OA.

Conclusion: Concentrated PPP is a unique resource for IGF-1 not found in standard PRP. Further concentration of PRP can produce greater anabolic factors such as TGF-beta and greater anti-inflammatory proteins such as IL-1ra.

Clinical Relevance: The polyacrylamide device efficiently concentrated PPP to create a unique source of IGF-1 that may supplement orthobiologic procedures.

Keywords: Platelet-poor plasma, platelet-rich plasma, IGF-1, autologous protein solution, concentrated platelet-poor plasma

MCB - 3

CHARACTERIZING THE ROLE OF WWOX DYSREGULATION IN CANINE OSTEOSARCOMA. J. Breitbach, F. Xu, C. London, J. Fenger. Depts Of Veterinary Biosciences and Veterinary Clinical Sciences; K. Huebner. OSU College of Medicine

WW domain-containing oxidoreductase (WWOX) is a tumor suppressor gene that is frequently deleted or reduced in human osteosarcoma (OS) tumors and loss of WWOX in OS cells promotes a highly tumorigenic phenotype. Western blotting demonstrated that WWOX protein is absent or reduced in primary canine OS tumors and canine OS cell lines. To assess the functional consequences of WWOX deletion on normal osteoblast behavior, the canine OSA8 and Abrams OS cell lines which express low levels of WWOX were transduced with WWOX lentiviral constructs resulting in high levels of WWOX expression as demonstrated by real time PCR and Western blotting. Restoration of WWOX in canine OS cells resulted in decreased cellular proliferation and invasion. These data provide evidence supporting a role for WWOX in canine OS cell proliferation and invasion, and suggest that dysregulation of WWOX may be fundamental to the disease process in both human and canine OS.

Keywords: Osteosarcoma, WWOX

MCB - 4

ACUTELY LETHAL H1N1 INFLUENZA A VIRUS INFECTION IMPACTS ALVEOLAR TYPE II CELL MITOCHONDRIAL STRUCTURE AND FUNCTION IN MICE. L. Doolittle[1], E. Calomeni[2], D. Guttridge[3], and I. Davis[1]
[1]Department of Veterinary Biosciences, [2]Department of Pathology, and [3]Department of Cancer Biology and Genetics

Rationale: Alveolar type II (ATII) cells are the primary site of influenza A virus (IAV) replication in the distal lung. ATII cells perform a number of processes critical for lung homeostasis, including synthesis and secretion of pulmonary surfactant components and vectorial ion transport to regulate alveolar lining fluid depth. These processes require ATP, which is primarily generated by mitochondrial oxidative phosphorylation (OxPhos). Recent studies indicated that IAV infection significantly alters ATII cell phospholipid content. Since phospholipids are essential components of mitochondrial membranes, we hypothesized that infection-induced alterations in ATII cell phospholipids would have detrimental effects on mitochondrial structure and function.

Methods: C57BL/6 mice were inoculated intranasally with virus diluent or 10,000 pfu IAV A/WSN/33 (H1N1), which induces severe lung injury in mice by 6 days post-inoculation (dpi). Lungs were fixed in glutaraldehyde at 6 dpi for transmission electron microscopy and ImageJ analysis. Alternatively, ATII cells were isolated at 6 dpi. ATII cell cardiolipin content was determined by flow cytometry with nonyl acridine orange dye. Rates of glycolysis and mitochondrial OxPhos were measured on a Seahorse XFe Analyzer. Gene and protein expression of electron transport chain (ETC) complexes were analyzed by qRT-PCR and Western blotting.

Results: Relative to mock-infected controls at 6 dpi, ATII cells from infected mice contained fewer and smaller mitochondria. Levels of cardiolipin, a mitochondria-specific phospholipid, were reduced. Basal rates of glycolysis and mitochondrial respiration decreased, as did the rate of mitochondrial ATP production by ATP synthase. ATII cells showed altered gene and protein expression of several ETC enzymes post-infection, including multiple subunits of Complex IV (cytochrome c oxidase).

Conclusions: Infection-induced alterations in ATII cell phospholipid content were associated with abnormal mitochondrial morphology and impaired OxPhos. Decreased ATP availability may impair ATII cell function and contribute to development of severe lung injury.

Keywords: Alveolar type II cells, Mitochondria, Influenza A virus, Lung function
Glycolysis, Oxidative phosphorylation

MCB - 5

MAMMARY TUMOR AND MASTECTOMY SYNERGISTICALLY PROMOTE CHRONIC NEUROINFLAMMATION IN A BREAST CANCER SURVIVOR MODEL. K. Emmer, W.H. Walker II, A. Smith, N. Zhang, T. TinKai, J. Fitzgerald and A.C. DeVries; Depts. of Veterinary Preventive Medicine and Neuroscience

Breast cancer accounts for 15% of all new cancer cases in the United States, and among those diagnosed almost 90% will survive 5 years. These breast cancer survivors are at an increased risk of cognitive sequelae such as depression and anxiety and little is known regarding the mechanism. Activated (primed) microglia trigger enhanced secretion of inflammatory cytokines when challenged with a secondary immune stimulus and offer a potential explanation for this cognitive dysfunction. This study examined whether mammary gland tumors prime microglia and augment the inflammatory profile of mice. 67NR tumor cells were injected orthotopically into the mammary gland of BALB/c mice and a mastectomy was performed after 16 days of growth to remove the mammary tumor. Non-tumor-bearing animals comprised a control group who received sham surgeries and a surgical control group who received mastectomies. At the time of mastectomy, there were no significant differences in cytokine concentrations in the hippocampus or serum apart from increased serum CXCL1 chemokine in tumor-bearing compared to non-tumor-bearing animals. However, 14 days following surgery, hippocampal mRNA expression of proinflammatory cytokines IL-1β and TNFα along with microglial priming marker CD68 were increased in animals who had mastectomy tumor removal relative to control animals. The surgical control group displayed intermediate mRNA levels, suggesting a synergistic effect of both tumor and surgery on neuroinflammation. Together these data demonstrate that tumors may prime microglia resulting in an exaggerated proinflammatory response to mastectomy surgery and consequently promoting chronic neuroinflammation. Further, these data provide insight into a potential pathogenesis for cognitive deficits experienced by breast cancer survivors.

Keywords: breast cancer, mastectomy, neuroinflammation, priming, microglia

MCB - 6

CARBAPENEMASE CONTAINING INTEGRONS.

S.V. Grooters, D. Mollenkopf, T. Wittum. Dept. of Veterinary Preventive Medicine

Antimicrobial resistance is a critical public health threat recognized by the U.S. Centers for Disease Control and Prevention, the World Health Organization, the United Nations and all member states. Regulations require that certain antibiotics be preserved only for use in human medicine – in the U.S., this includes carbapenems. Without selective pressure from this class of drugs, bacteria possessing resistance to carbapenems are not expected in a livestock environment. Considering that environmental, livestock, and human health are all interconnected under a One Health paradigm, we recovered carbapenemase-producing bacteria from three different farm environments, as well as from a local waterway. Phenotypic evidence from 14 isolates tested suggested resistance to meropenem, a carbapenem. Initial genetic screening by PCR was conducted which confirmed the presence of the carbapenemase gene *bla*$_{IMP}$ in 10 of the isolates prior to whole genome sequencing. In order to correctly identify the genes responsible for phenotypic antimicrobial resistance, and to accurately annotate chromosomes, mobile genetic elements, plasmids, transposons, and integrons, we utilized next generation sequencing, and a variety of bioinformatic tools. Short-read sequences were assembled and annotated, with eight isolates showing significant similarities in the type2 integron and gene cassette structure. Four of these isolates differed in a single nucleotide base pair that translated into a different amino acid, thus a slightly different gene *bla*$_{IMP-64}$, despite their similar surrounding genomic map. The two additional isolates harbored *bla*$_{IMP-27}$ contained within a different integron structure. Dairy, swine, and waterway samples were significantly associated by genomic characteristics surrounding the *bla*$_{IMP}$ genes. Our study highlights a One Health approach and use of genomic epidemiologic tools to analyze the public health threat from dissemination of antimicrobial resistance.

Keywords: Antibiotic Resistance, Plasmid, Integron, beta-lactamase IMP

MCB - 7

INVESTIGATING THE ROLE OF EXOSOMAL MIR-9 IN CANINE AND HUMAN OSTEOSARCOMA. M. M. McKinney, O. Stephenson, J. M. Fenger. Department of Veterinary Clinical Sciences

Osteosarcoma (OS) is the most common bone tumor in children and dogs and remains a fatal disease for 30% of children and over 90% of affected dogs. MicroRNAs (miRNAs) are non-protein coding RNAs that regulate gene expression and their dysregulation is well documented in cancer. Circulating miRNAs embedded in small (30-100 nm) membrane-derived vesicles called exosomes are detectable in body fluids such as serum and plasma and recent studies demonstrate that tumor-specific exosomal miRNAs are capable of mediating tumorigenesis and metastasis. Our laboratory found that overexpression of miR-9 in OS cells promotes cell motility and invasiveness, consistent with data implicating miR-9 as a key player in promoting metastasis in human cancer cells. We hypothesize that high levels of miR-9 will be detected in exosomes derived from canine and human OS cell lines. We further hypothesize that serum exosome miR-9 will be increased in dogs with OS compared to healthy controls and that tumor-associated miR-9 levels will decrease following therapeutic intervention. Exosomes were isolated from media from canine and human OS cell lines and canine serum and analyzed using NanoSight™ imaging and Western blotting to detect exosomal marker expression. Real time PCR demonstrated that exosomal miR-9 expression is higher in canine and human OS cells compared to normal osteoblasts, suggesting that OS cell-derived exosomal miR-9 may play a role in mediating tumor-stroma cross-talk. MiR-9 is detectable in canine serum-derived exosomes; however, studies are underway to evaluate serum exosome miR-9 levels in healthy dogs and dogs with OS to determine the feasibility of circulating miR-9 as a non-invasive biomarker in canine OS.

Keywords: Osteosarcoma, MicroRNAs, Exosomes, Metastasis, Non-Invasive Biomarker

MCB - 8

CHONDROGENIC POTENTIAL OD SYNOVIUM-BASED CELL-SCAFFOLD CONSTRUCTS *IN-VITRO* AND *IN-VIVO* IN RAT OSTEOARTHRITIS MODEL.

N Reisbig, E Pinnell, L Scheuerman, H Hussein and A L Bertone. Comparative Orthopedics Research Laboratory, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio 43210

Osteoarthritis (OA) is a progressive disease associated with cartilage injury and its inherently limited repair capability. Synovium-based cell constructs can be used to promote cartilage repair and reduce OA. Decellularized synovium-based extracellular matrix scaffolds (sECM) were seeded with synovium-derived mesenchymal stem (signaling) cells (sMSC), engineered to express green fluorescent protein (GFP), or bone morphogenetic protein-2 (BMP-2), to assess survival, distribution, and chondrogenic potential of constructs *in vitro* and *in vivo*. Synovium-based cell constructs in co-culture with chondrocytes increased chondrocyte proliferation, viability, and collagen type II production ($p<0.01$), greatest in BMP-2 constructs ($p<0.01$). Chondrocyte presence increased the production of hyaluronic acid, proteoglycan, and BMP-2 in a positive feedback loop ($p<0.01$). Rat knees had full-thickness cartilage defects drilled bilaterally and randomly assigned synovium implants of suture alone or sECM (controls); GFP- or BMP-2 (cellular constructs) placed adjacent to the defect. At 5 weeks, gross and cartilage OA scores; peripheral, central and cartilage defect size; and subchondral bone damage scores were significantly greater for the controls and significantly reduced ($p<0.01$) in cellular constructs, greatest with the BMP-2 constructs ($p<0.001$). Immunohistochemistry demonstrated migration of endogenous cells into the sECM, with greater cellularity ($p<0.01$) in the constructs with intense positive GFP staining confirming engraftment of implanted sMSC and continued gene expression. In summary, exposing cartilage to synovium-based scaffold-cell constructs was chondrogenic *in vitro* and *in vivo*, and resulted in reduced OA *in vivo*. This effect was mediated, in part, by soluble ECM and cell factors and upregulation of anabolic growth proteins, such as BMP-2.

Keywords: mesenchymal stem cells, osteoarthritis, in vivo, cartilage, extracellular matrix scaffolds

MCB - 9

MKLP2 INHIBITION AS A NOVEL ANTIMITOTIC FOR GLIOBLASTOMA.

M.S. Schrock, P. Dickinson, M. Venere, and M. Summers

Background: Inhibition of motor kinesins, proteins which travel along microtubules and perform specific mitotic, meiotic or cellular transport functions, is a growing area of cancer drug development. There are over 20 proteins in the kinesin superfamily and inhibition of kinesins with mitosis-specific functions have the potential to circumvent neurotoxicity associated with traditional antimitotics. In combination with published literature, our preliminary data suggests a previously undescribed role for mitotic kinesin-like protein 2 (MKlp2) in early mitosis. The goal of this study is to further define this novel function of MKlp2 and to determine the efficacy of MKlp2 inhibition in canine and human glioblastoma.

Methods: Applying cell synchronization techniques, we used IncuCyte ZOOM live cell imaging to determine the effects of parotrain, a commercially available MKlp2 inhibitor, on short-term cellular proliferation and duration of metaphase in canine and human glioblastoma cell lines. Clonogenic assays were used to determine long term survival.

Results: Our data indicate that MKlp2 is overexpressed in human GBM and that MKlp2 inhibition significantly lengthens metaphase in human GBM cell lines. In addition, MKlp2 inhibition with paprotrain significantly decreased short-term and long-term survival in canine and human GBM cell lines.

Conclusions: Altogether our data suggests expression of MKlp2 is important for recovery from the spindle assembly checkpoint and progression to anaphase. Decreased short-term and long-term cellular proliferation suggest MKlp2 inhibition could be an effective next-generation antimitotic for glioblastoma patients. However, more work is needed to determine the clinical relevance of the canine glioblastoma model for human glioblastoma, particularly in the context of MKlp2 expression.

Keywords: spindle assembly checkpoint, KIF20A mitotic kinesin, antimitotic, glioblastoma

MCB - 10

EHRLICHIA CHAFFEENSIS TBC-LIKE MOTIF-CONTAINING EFFECTOR RETAINS RAB5 TO PARASITOPHOROUS VACUOLES. Q. Yan, M. Lin, Y. Rikihisa. Department of Veterinary Biosciences.

Ehrlichia chaffeensis is an obligatory intracellular bacterium that can infect human monocytes/macrophages and causes Human monocytic ehrlichiosis. E. chaffeensis lives in an early endosome-like membrane-bound compartment (inclusion) retaining early endosomal marker RAB5 GTPase, which can protect E. chaffeensis from being degraded in phagolysosomes. How E. chaffeensis keeps RAB5 GTPase to its inclusion is poorly understood. The Rikihisa laboratory recently identified an ehrlichial type IV secretion system (T4SS) effector and named it as Etf-2. Etf-2 contains a Tre2-Bub2-Cdc16 (TBC)-like motif, which is a universal conserved motif in RAB GTPase-activating proteins (RABGAPs). My hypothesis is Etf-2 is responsible for retaining RAB5 to ehrlichial inclusions. To test this hypothesis, I cloned Etf-2, and Etf-2 mutants-tagged with green fluorescent protein (GFP), and determined the localization of these proteins in E. chaffeensis-infected and uninfected cells. I also used HA-tagged RAB5, and constitutive active (CA) and dominant negative (DN) RAB5 mutants to investigate whether Etf-2 associates with active RAB5 (membrane-bound RAB5). My result showed that Etf-2-GFP localizes on ehrlichial inclusion membranes. Etf-2-GFP colocalized with RAB5-CA, but not RAB5-DN. Mutation of TBC-like motif in Etf-2 impaired localization of Etf-2 to RAB5-decorated early endosome membrane. To study whether Etf-2 modulates endosome maturation process, latex beads were coated with EtpE-C (C terminus of Entry-triggering protein of Ehrlichia, an invasin of E. chaffeensis) and added to host cells to mimic infection of E. chaffeensis. Etf-2 localized to the membranes of beads-containing endosomes, and significantly delayed RAB5 dissociation from or RAB7 (late endosome marker) localization to, and blocked RAB5-specific GAP (RABGAP5) localization to the bead-containing endosomes. Over expression of Etf-2 facilitated ehrlichial infection. Thus, association of Etf-2 to active RAB5 appears to delay RAB5 inactivation by impeding RABGAP5 localization to endosomes, and provide a safe haven for E. chaffeensis proliferation inside host cells.

Keywords: Ehrlichia chaffeensis, endosome, TBC motif, RAB5, T4SS effector, RABGAP5

STRUCTURE/FUNCTION

SF - 1

FLUOROSCOPIC KINEMATIC COMPARISON BETWEEN CRANIAL CRUCIATE LIGAMENT-RUPTURE SUSCEPTIBLE AND STABLE DOGS. T. Fiorini, S. Jones. Department of Veterinary Clinical Sciences.

Cranial cruciate ligament (CCL) rupture is the most common cause of hindlimb lameness in dogs. The Labrador is considered an “at risk” breed for CCL rupture, while the Greyhound is considered a “genetically-safe” breed, almost never developing naturally-occurring CCL rupture. We hypothesize that tracking stifle motion during ambulation will demonstrate specific kinematic differences between the two breeds that will help elucidate why Greyhounds are considered a genetically safe breed for CCL disease. Six normal Greyhounds will ambulate on a treadmill while fluoroscopic images of the stifle are acquired at both the walk and the trot. Three-dimensional (3D) bone models of each dog’s femur and tibia, generated from a CT scan of the hindlimbs, are assigned an anatomic coordinate system. Femorotibial kinematics are determined by matching the 3D bone models to the corresponding fluoroscopic images using a 3D to 2D shapematching technique. These data are then compared to previously described Labrador kinematics. Preliminary results demonstrate that stifle range of motion in flexion and extension is similar between both breeds. Tight coupling between flexion and internal tibial rotation ($r = 0.92$) and between flexion and caudal tibial translation was detected in the Greyhound ($r = 0.94$). This coupling is tighter than what was seen in the Labrador. Interestingly, despite the difference in prevalence of CCL disease between these two breeds, preliminary data suggests that kinematic patters are markedly similar. Unfortunately, we had to reject our null hypothesis. Significant kinematic differences between these two breeds did not elucidate some of the pathomechanisms behind CCL disease and rupture.

Keywords: Cranial cruciate ligament, Joint kinematics, Fluoroscopy

CHEMOPREVENTIVE TARGETING OF THE ANDROGEN RECEPTOR IN HEPATOCELLULAR CARCINOMA.

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[1]College of Veterinary Medicine [2]University Laboratory Animal Resources [3]Comprehensive Cancer Center [4]College of Pharmacy, The Ohio State University, Columbus, OH, 43210

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the second most common cause of cancer death worldwide (1). Men are three times more likely than women to acquire and die from the disease. In rodent models of HCC, surgical castration delays onset and reduces tumor burden implicating a pro-carcinogenic role for testicular derived androgens. This effect is mediated through the hepatic androgen receptor (AR) as AR knockout mice exhibit a similar phenotype (2). The benefits of AR knockout are limited to early carcinogenesis, however, as HCC in hepatic AR knockout mice is ultimately more invasive and metastatic compared to AR replete animals (3).
Beyond carcinogenic outcomes, very little is known about the timing, distribution, and mechanistic contributions of the hepatic AR to hepatocarcinogenesis. To gain a better understanding of its influences, we orchiectomized (ORX) or pretreated male rats with enzalutamide (ENZ) – a potent non-steroidal AR antagonist – and induced HCC by combined intraperitoneal diethylnitrosamine (DEN) and partial hepatectomy (PH). We saw a trending, but statistically insignificant, decrease in the total number of pre-neoplastic foci in both ORX and ENZ treatment groups. The hepatic AR, however, is robustly upregulated four weeks following carcinogenic challenge. Preliminary immunohistochemical analysis of the AR in liver reveals minimal to mild hepatocyte nuclear staining with immunoperoxidase staining present within periportal cells. DEN enhances cytoplasmic staining within dysplastic hepatocytes supporting a role for the AR in early, pre-cancerous lesions. Microarray analysis finds upregulation of cholesterol biosynthesis pathways with pharmacologic inhibition of the AR, and transition metal homeostasis pathways with castration identifying two separate mechanisms of action between the two methods of antagonism of androgen signaling. ENZ antagonism of the AR results in robust downregulation of DEN bioactivator CYP2e1 suggesting androgen regulated metabolism of this commonly used model carcinogen.

Keywords: Hepatocellular carcinoma, carcinogenesis, chemoprevention, androgens, androgen receptor, Enzalutamide, castration, Rattus norvegicus.

SF - 3

PHARMACOKINETICS AND PHARMACODYNAMICS OF ORAL TAPENTADOL HYDROCHLORIDE IN DOGS. J Howard, DVM; TK Aarnes, DVM, MS; J Dyce MA, VetMB, MRCVS; P Lerche, BVSc, PhD; L Wulf, PhD; JF Coetzee, BVSc, PhD; and J Lakritz, DVM, PhD. From the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio 43210 (Howard, Aarnes, Dyce, Lerche, Lakritz); and the Department of Veterinary Diagnostic and Production Animal Medicine (VDPAM) - ISU Pharmacology Analytical Support Team (ISU-PhAST); 2448 Lloyd Vet Med Center, College of Veterinary Medicine; 1800 Christensen Drive, Ames, IA, 50011-1134 (Wulf, Coetzee). Dr. Coetzee's current address is: Department of Anatomy & Physiology, College of Veterinary Medicine, Kansas State University; 228 Coles Hall, Manhattan, KS 66506-5802

Objective: To evaluate the pharmacokinetic and pharmacodynamic characteristics of three oral doses of tapentadol hydrochloride in dogs.

Animals: Six healthy adult mixed breed dogs.

Procedures: In a prospective, randomized, cross-over study design, dogs were assigned each of three doses (10, 20, and 30 mg/kg) of oral tapentadol with a one week washout period between each administration. Plasma concentrations and physiologic parameters were measured over 24 hours. Samples were analyzed using high performance liquid chromatography tandem mass spectrometry.

Results: Tapentadol was rapidly absorbed after oral administration in dogs. Maximum plasma concentrations observed after 10, 20, and 30 mg tapentadol/kg body weight were 10.2, 19.7, and 31 ng/mL, respectively. Plasma half-life of the terminal phase after tapentadol administration after 10, 20, and 30 mg/kg was 3.5 (2.7 - 4.5), 3.7 (3.1 - 4.0, and 3.7 (2.8 - 6.5) hr, respectively. Tapentadol and its 3 quantified metabolites (tapentadol sulfate, tapentadol-O-glucuronide, and desmethyltapentadol) were observed in all dogs and constituted 0.16%, 2.8%, 97%, and 0.04% of the total AUC, respectively. Plasma area under the concentration versus time curves for tapentadol, tapentadol sulfate, and tapentadol-O-glucuronide increased in a dose dependent manner. Desmethyltapentadol did not increase in a linear manner up to 30 mg/kg. Sedation scores, thermal thresholds, heart and respiratory rates were not significantly affected by dosing or time following administration.

Conclusions and Clinical Relevance:
Oral tapentadol was well-tolerated after oral dosing, with rapid absorption. Significant metabolism occurs in the dog. Adverse events were not apparent in any dogs or doses in this study.

Keywords: Tapentadol, canine analgesia, pharmacokinetic, pharmacodynamics

SF - 4

REDUCED EXPRESSION OF THE CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR LEADS TO INCREASED ALVEOLAR DESTRUCTION FOLLOWING CIGARETTE SMOKE EXPOSURE IN MICE.

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Chronic obstructive pulmonary disease (COPD), the third leading cause of death globally, is a chronic inflammatory disease with no cure, which ultimately results in death from respiratory failure. The two main components of COPD are chronic bronchitis and emphysema. Chronic bronchitis refers to airflow restriction due to smooth muscle constriction, mucus hypersecretion, and airway fibrosis. Emphysema refers to poor gas exchange due to alveolar destruction. Approximately 90% of COPD cases can be attributed to tobacco smoking, however only 10% of smokers develop COPD. This suggests genetic factors may play a key role. Patients with Cystic Fibrosis (CF), an autosomal recessive disorder caused by mutation in the cftr gene (encoding the CF Transmembrane Conductance Regulator), display airway inflammation and mucus plugging similar to that of COPD. Previous studies by us and others have shown decreased CFTR expression in the bronchial epithelium of COPD patients. We also reported that cigarette smoke exposure decreases CFTR expression and function in primary human bronchial cells and the human bronchial epithelial cell line 16HBE. We therefore hypothesize that individuals with reduced CFTR expression, such as CF carriers, may be more susceptible to developing COPD. While CFTR is often studied in the context of airway inflammation, CF patients also develop emphysema (Mets O, et al. PLOS One. 2015;10(6)). To assess the effect of CFTR expression levels on susceptibility to developing emphysema, we exposed wild-type, cftr+/-, and cftr-/- mice to cigarette smoke using whole-body exposure and examined alveolar destruction. Cftr+/- and cftr-/- mice displayed an increased mean linear intercept compared to wild-type mice after 10 months of cigarette smoke exposure, suggesting more sever emphysema. Our findings demonstrate that reduced CFTR expression increases alveolar tissue destruction following cigarette smoke exposure. Therefore, people carrying a mutation in the cftr gene might be more susceptible to developing emphysema.

Keywords: COPD, Emphysema, CFTR, Smoke