



THE OHIO STATE UNIVERSITY

COLLEGE OF VETERINARY MEDICINE

**ADVANCES IN
VETERINARY MEDICINE
RESEARCH**

2 APRIL 2015

**BOOK OF
ABSTRACTS**

PROGRAM

April 2, 2015

POSTER JUDGING

Graduate Student Posters

8:00 am – 10:30 am

(closed session – only open to
those being judged)

AWARDS PRESENTATION

Veterinary Medical Center Auditorium

12:15 pm

GRADUATE STUDENT/POST DOC/RESEARCH SCIENTIST PLATFORM PRESENTATIONS

Dr. Kasia Dembek

Dr. Laura Pomeroy

Dr. Mingqun Lin

KEYNOTE SPEAKER

Veterinary Medical Center Auditorium
immediately following the awards
presentation and platform presentations

DR. PATRICIA CONRAD

Professor and Associate Dean for Global Affairs

UC Davis School of Veterinary Medicine

Co-Director of the One Health Center of Expertise:

Water, Animals, Food and Society in the UC Global Health Institute

***“Otters, Toxoplasma Oocysts, Oceans and
One Health: What’s the Connection?”***

POSTER SESSION

1st and 2nd Floors – Vet Med Academic Building

11:00 am – 5:00 pm

PROGRAM CHAIR

Dr. Jeff Lakritz

ORGANIZED BY

Michele Morscher

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- * Zoetis * CVM Graduate Student Association *
- * Public Health Preparedness for Infectious Diseases *

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

POSTER JUDGING SESSIONS

Wednesday, April 1, 2015
2:00 – 5:00 pm
Veterinary Student Poster Judging

Thursday, April 2, 2015
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 78 posters.

Kate Hayes-Ozello	Garrett Newbound
Stefan Niewiesk	Bill Kisseberth
Mike Oglesbee	Judith Radin
Paivi Rajala-Schultz	Tom Wittum
Carol Robertson-Plouch	Eric Miller
Sharon Stevenson	Luciana da Costa
Joe McCracken	Cheryl London
Matthew Krecic	Kat Ham
Jason Stull	Rebecca Garabed
Patricia Conrad	Barb Wolfe



ADVANCES IN VETERINARY MEDICINE

RESEARCH DAY

Awards Presentation, Graduate Student,
Post Doc, Research Scientist Platforms,
and Keynote Address

Thursday, April 2nd, 2015 Noon - 2 p.m.

Veterinary Medical Center Auditorium



Dr. Patricia A. Conrad

Professor and Associate Dean
for Global Programs

UC Davis School of Veterinary Medicine
Co-Director of the One Health Center
of Expertise: Water, Animals, Food and
Society in the UC Global Health Institute

“Otters, Toxoplasma Oocysts, Oceans and One Health: What’s the connection?”

Poster judging:

April 1st, 2 - 5 p.m. for Professional Students

April 2nd, 8 - 10:30 a.m. for Graduate Students

Sponsored by



Public Health Preparedness for Infectious Diseases

College of Veterinary Medicine Graduate Student Association

GRADUATE STUDENT PLATFORM PRESENTATION
DR. KASIA DEMBEK
MENTOR: DR. RAMIRO TORIBIO
DEPARTMENT OF VETERINARY CLINICAL SCIENCES

MULTIPLE ADRENOCORTICAL STEROID AND STEROID PRECURSORS RESPONSE TO ACTH STIMULATION TEST IN HOSPITALIZED FOALS. K. Dembek¹, K. Timko¹, L. Johnson¹, N. Slovis², S. Reed³, K. Hart⁴ and R. Toribio¹, ¹Department of Veterinary Clinical Sciences, The Ohio State University, ²Hagyard Equine Medical Institute, ³Rood and Riddle Equine Hospital, ⁴College of Veterinary Medicine, University of Georgia

Sepsis remains the main cause of death in neonatal foals. The hypothalamic-pituitary-adrenal axis regulates the response to sepsis-associated stress. We have shown that relative adrenal insufficiency (RAI), characterized by an impaired cortisol response to adrenocorticotropin (ACTH), is common and associated with mortality in foals. Most studies in foals have been focused on cortisol, while other adrenocortical steroid precursors have not been investigated.

We hypothesized that RAI in critically ill foals will involve multiple adrenocortical layers, resulting in decreased glucocorticoid and mineralocorticoid, and increased steroid precursor secretion, which will be associated with severity of disease and mortality. We also proposed that the response of these steroids to ACTH will be altered.

Foals <1 day of age were categorized into 3 groups: 11 septic, 9 sick non-septic and 11 healthy. After baseline blood sample collection on admission (Time 0), each foal received 10 µg of ACTH. Additional blood samples were collected at 30 (Time 30) and 90 min (Time 90) post ACTH. Steroid concentrations were determined by immunoassays. The delta steroid was defined as the percent change in steroid concentration between Time 0 and 30 min (Delta₀₋₃₀).

Septic foals had higher cortisol, pregnenolone, progesterone, 17α-OH-progesterone and androstenedione concentrations at 3 time points and higher aldosterone at Time 0 and Time 30 compared to healthy foals (P<0.05). Non-surviving foals had lower cortisol at Time 0 and Time 30 compared to surviving septic foals. The Delta₀₋₃₀ was lower in septic compared to healthy foals for cortisol, progesterone, aldosterone, and 17α-OH-progesterone (P<0.05), suggesting RAI. Progesterone and 17α-OH-progesterone decreased, however, DHEA increased in response to ACTH at Time 30 in septic foals. ACTH stimulation did not influence pregnenolone and androstenedione concentrations.

In our study, RAI was characterized by altered steroidogenesis in response to ACTH in critically ill foals. Hypocortisolemia was associated with mortality in septic foals.

Keywords: sepsis, equine neonates, endocrinology, adrenal insufficiency

**POST DOC PLATFORM PRESENTATION
DR. LAURA POMEROY
MENTOR: DR. REBECCA GARABED
DEPARTMENT OF VETERINARY PREVENTIVE MEDICINE**

SEROTYPE-SPECIFIC TRANSMISSION AND WANING IMMUNITY OF ENDEMIC FOOT-AND-MOUTH DISEASE VIRUS. L. Pomeroy¹, O. Bjornstad², H. Kim³, R.Garabed¹

¹ Department of Veterinary Preventive Medicine, Ohio State University

² Department of Biology, Penn State University

³ Department of Geography, Ohio State University

Foot-and-mouth disease virus (FMDV) causes morbidity and mortality in a range of mammals and threatens local economies by acting as a barrier to international trade. The outbreak in the United Kingdom in 2001 cost billions to control and highlighted the risk that the pathogen poses to agriculture. In response, government agencies have called for planned disease control policies. However, a lack of understanding of the multistrain etiology and host immune response undermines disease control. Here, we use data from FMDV infections in endemic settings to probe strain-specific transmission and immunodynamics. We use serology data and catalytic models to quantify the force of infection and the rate of waning immunity, and to detect periods of sustained transmission. Five serotypes of FMDV are endemic among cattle in the Far North Region of Cameroon. For serotypes SAT2, SAT3, and type A, a catalytic model assuming life-long immunity fit better. For serotypes SAT1 and type O, the reverse catalytic model fit better, suggesting that immunity may wane over time. Our analysis further indicates that type O has the largest force of infection and the longest duration of immunity. Estimates for the force of infection were time-varying and indicated that serotypes SAT1 and O displayed endemic dynamics, serotype A displayed epidemic dynamics, and SAT2 and SAT3 did not sustain local chains of transmission. These results highlight important differences in transmission and immunity specific to each serotype, which influences disease control decisions. In this setting, vaccination could be appropriate for SAT1, O, and A; however, SAT2 and SAT3 might best be controlled by preventing viral incursion into the population studied. Overall, this work shows that viral serotypes can differ significantly in their epidemiological and immunological characteristics. Patterns and processes that drive transmission in endemic settings must consider complex viral dynamics for accurate representation, interpretation, and control.

Keywords: foot and mouth disease (FMD), waning immunity, multistrain transmission, endemic dynamics

RESEARCH SCIENTIST PLATFORM PRESENTATION
DR. MINGQUN LIN
MENTOR: DR. YASUKO RIKIHISA
DEPARTMENT OF VETERINARY BIOSCIENCES

INFECTION AND RELEASE OF OBLIGATORY INTRACELLULAR PATHOGEN ANAPLASMA REQUIRE ACTIN CYTOSKELETON REGULATION BY TYPE IV SECRETION EFFECTOR ANKA. M. Lin and Y. Rikihisa. Department of Veterinary Biosciences

The obligatory intracellular bacterium *Anaplasma phagocytophilum*, the causative agent of human granulocytic anaplasmosis, primarily invades and replicates within membrane-bound vacuoles in human neutrophils. A type IV secretion effector of *A. phagocytophilum*, AnKA, was previously identified for its essential roles in bacterial infection by activating Abl-1 tyrosine kinases. Bioinformatic analysis showed AnKA consists of 11 Ankyrin-repeat domains clustered at the N-terminus, and 1 actin-binding domain, 6 phospho-tyrosine motifs, and 1 proline-rich domain at the C-terminus. In this study, we demonstrated that native AnKA could interact with actin and actin-regulatory proteins α -actinin 4 (Actn4) and gelsolin using co-immunoprecipitation of *A. phagocytophilum*-infected HL-60 cells using anti-AnKA IgG and protein identification by mass spectrometry. Disruption of actin filaments (F-actin) assembly induced striking release of *A. phagocytophilum*, which were less infectious than spontaneously released bacteria. GST pull-down assay showed that C-terminus of AnKA interacted with actin and gelsolin, whereas the N-terminus of AnKA interacted with Actn4 and Abi-1, an adaptor protein for Abl-1 tyrosine kinases. AnKA colocalized with gelsolin, Actn4, Abl-1 kinase, and cortical F-actin at cell periphery, which required Abl-1 kinase activity. *In vitro* pyrene-actin polymerization assay showed that AnKA proteins enhanced actin polymerization kinetics significantly, whereas ectopically expressed AnKA enhanced *A. phagocytophilum* infection and altered F-actin dynamics in transfected cells. On the contrary, functional inhibition of AnKA by intracellularly delivered anti-AnKA IgG or shRNA-knockdown of Actn4 and gelsolin, reduced F-actin content and enhanced the release of premature *A. phagocytophilum* into culture medium from infected host cells. These results suggest that AnKA could serve as a protein scaffold to assemble actin-regulatory proteins and host signaling molecules to regulate actin dynamics, therefore coordinate bacterial growth, development, and release by preventing premature bacterial release from host cells.

Keywords: Human granulocytic anaplasmosis, *Anaplasma phagocytophilum*, Type IV secretion, AnKA, Actin dynamics

CLINICAL RESEARCH

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OXYTOCIN CONCENTRATIONS IN SEPTIC AND DUMMY FOALS. C. Brown¹, K. Dembek², R.E. Toribio². ¹College of Food, Agricultural, and Environmental Sciences, ²Dept. of Veterinary Clinical Sciences

Sepsis, the leading cause of foal mortality, is a condition where bacteria multiply in the blood and set up widespread infection throughout the body. Septic foals have varying levels of survival depending on severity and duration of infection. In response to stress, the hypothalamus and pituitary gland release multiple hormones, including oxytocin which is typically associated with delivery and milk secretion. Previous studies indicate elevated oxytocin in response to sepsis associated stress and decreased concentration with neurological dysfunction in critically ill human neonates, but limited information exists in newborn foals. Neonatal Maladjustment Syndrome (NMS) occurs in foals during or shortly after parturition and is often associated with septicemia. The syndrome is characterized by abnormal neurologic behavior, loss of suckle reflex, depression, and seizures. The exact cause of equine NMS has not been determined; however, abnormal hormone concentrations have been proposed.

Objectives of this project were to measure oxytocin concentrations in neonatal foals and examine its association with disease severity, NMS, and likelihood of survival. Newborn foals were categorized into 3 groups based on severity of illness: septic, sick non-septic (SNS), and healthy. Foals diagnosed with NMS were included. Plasma oxytocin concentrations were measured by enzyme immunoassay.

Oxytocin concentrations were significantly lower in septic compared to healthy foals but higher in NMS compared to healthy foals ($P < 0.05$). In our study, decreased oxytocin concentrations were associated with septicemia and mortality, while increased oxytocin was linked to NMS. This is the first study to measure oxytocin in sick newborn foals and to demonstrate an association between this hormone with disease and outcome. This information provides additional insight on the pathogenesis of sepsis and neurological function in newborn foals.

Keywords: oxytocin, foal, septicemia

THE BRISTOL CATS STUDY: RECRUITMENT AND CHARACTERISTICS OF THE COHORT. J. Murray, R. Casey, E. Gale, T. Buffington and T. Gruffydd-Jones. Departments of Veterinary Clinical Sciences, Bristol University and The Ohio State University.

The aim of the Bristol Cats Study is to collect prospective observational data to permit identification of risk factors for a range of outcomes in cats, including health, diseases (including FIC, T2D, dental, organ-specific disorders, obesity, and etc.), and problematic behaviors

Recruitment: 8-16 week old pet kittens were recruited to the study between 01/06/13 and 31/12/13. The study was advertised using a variety of methods (e.g., vet practices, pet forums, rescue centers) to attempt to reduce enrollment selection bias.

Data collection: Owners completed extensive questionnaires at recruitment (Q1), and at 6 (Q2), 12 (Q3), and 18 months (Q4), at 2.5 (Q5) and 4 years (Q6), and annually thereafter. Questionnaire completion rates are: 1899 (86.8%) for Q2, and 1725 (78.8%) for Q3. Additional data, and data to validate owner-reported information, are being collected from participating veterinary practices. These data include, body condition scores, oral health scores, veterinary records, buccal swabs (for DNA), and fecal samples (at 6 months and 2.5 years).

The cohort (Q1 data for 2189 cats, some missing data) is comprised of 1138 (52.1%) male and 1051 (47.9%) female cats. Of these, 61.6% (1338) lived in multi-cat households at Q1. 45.1% (988) of kittens were obtained from friends, relatives, neighbors, or from local advertisements, 18.5% (404) from rescue centers, 18.6% (406) from pedigree breeders, 7.7% (168) had been bred by their owner (63 accidentally), 7.3% (159) were stray, feral, or abandoned, and 2.9% (64) were obtained from a pet shop or "other" source.

Most owners are between 25 and 54 years of age, 69% own their home, and have annual household incomes ranging from <\$15,000 (9%) to > \$74,000 (26%).

Data from Q1-Q4 currently are being analyzed to determine risk factors for lower urinary tract signs and obesity; other diseases will follow as the cohort is followed further.

Keywords: Cats, Epidemiology, Health, Housing, Environment.

DEVELOPMENT AND VALIDATION OF A LOW-COST SURGICAL SIMULATION MODEL TO TEACH CANINE OVARIOHYSTERECTOMY. B Carter, C Silveira, M McLoughlin, L Hill, T Motta. College of Veterinary Medicine, The Ohio State University, Columbus, Ohio 43210.

Basic surgical skill has been shown to be considered the most important area of knowledge in new graduates by over half of veterinarians. At The Ohio State University College of Veterinary Medicine, the curriculum of the first two years is based predominantly in lecture and supplemental material with minimal hands-on experience. As a result, third year students often lack surgical skill and experience high levels of anxiety when entering operative procedure labs. In this study, we aim to test the hypothesis that utilization of a low-cost surgical simulation model can help to improve the performance and confidence of students, while decreasing students' perception of stress and anxiety.

To investigate this, a low-cost surgical simulator for canine ovariohysterectomy was created. This model allows students to practice surgical skills including approach and incision, identification of relevant anatomic structures, three clamp technique, disruption of the suspensory ligament, pedicle and uterine body ligation, and closure. Twenty-four students volunteered to participate, all of whom attended a lecture and had unlimited access to supplemental materials and videos online. Half of the students were chosen at random to also receive a low-cost simulator, surgical instruments, and sufficient suture material to practice the procedure up to 5 times. The study culminated for the student volunteers by performing the surgical procedure on a cadaver.

Data acquisition is ongoing. Surgical performance of each student is being graded by faculty using recorded videos and a rubric created specifically for this study. A quiz and questionnaire completed by the participants are being used to evaluate the students' knowledge, performance, comfort level, and perceived model efficacy. We expect to find that students who utilized the surgical simulator will display higher levels of surgical skill and confidence, as well as lower levels of perceived stress and anxiety.

Keywords:

AN ASSESSMENT OF THE SAFETY OF RECUVYRA FOLLOWING TOPICAL ADMINISTRATION IN MICE. A. Darbyshire, V. Bergdall, and D. Coble. University Laboratory Animal Resources.

Mice are commonly used in surgical procedures requiring the use of analgesic medications. Most analgesics require repeat administration leading to suspected animal stress secondary to handling and additional staff time requirements. Recuvyra, a topically-applied fentanyl solution, is currently approved for use in dogs. It is effective for providing up to four days of analgesia with one topical dose. In this study, multiple doses of Recuvyra (5mg/kg, 12.5mg/kg, or 20mg/kg) were topically administered to mice to evaluate the safety and efficacy. We hypothesized that Recuvyra would be safe for use in mice, and provide multiple days of analgesia with one application to the dorsal tail base. Mice were assessed for weight loss, behavior, and nociception for four days following application. Behavioral tests (nest complexity, time-to-integrate-to-nest test, and open field testing) were performed to assess individual mouse behavior. Nociception following application was assessed with the tail flick test. All mice survived dosing with Recuvyra; however one mouse that was dosed at 20mg/kg was euthanized due to lethargy and dehydration. All dosed mice initially lost between 1g and 1.5g of body weight, but regained it prior to the study end. Nest complexity scores decreased with dosing for all groups, returning to normal by day 4. Time-to-integrate-to-nest testing showed a decrease in nesting behavior initially. Tail-flick latencies were increased significantly for all groups at 24hr, and gradually decreased to normal latency time by day 4 for the 12.5mg/kg and 20mg/kg groups. During open field testing, dosed mice exhibited less exploratory behavior, with lower center duration times and less rearing than control mice initially. Defecation during open field testing was decreased on day 1 for all groups, returning to normal day 2. Dosing at 12.5mg/kg was determined to be a safe analgesic for use in mice that remains effective for approximately 3 days.

Keywords: Recuvyra, analgesia

**MICROCIRCULATORY CHANGES USING SIDESTREAM DARK FIELD
MICROSCOPY OF DOGS WITH NATURALLY OCCURRING GASTRIC DILATATION
VOLVULUS. K.Datte, A. Dodd, E. Cooper, Dept of Veterinary Clinical Sciences**

Microcirculatory perfusion (blood vessels $<200\mu\text{m}$) is maintained through a combination of systemic and local factors. However, in states of cardiovascular compromise, such as gastric dilatation volvulus (GDV), microcirculatory blood flow may be altered. In patients with GDV, gastric necrosis has been found to be prognostic and may be challenging to diagnose intraoperatively. The objective of this study was to directly assess gastric microcirculation (GM) with sidestream dark field microscopy (SDM) in dogs with naturally occurring GDV and compare to data obtained from normal dogs. Secondary objectives included evaluation of the sublingual microcirculation (SM) and other measured variables (including heart rate (HR), blood pressure, end-tidal CO₂ (ETCO₂), respiratory rate (RR), pulse oximetry (S_O₂), central venous saturation (ScvO₂) and lactate) to determine correlation with the gastric microcirculation. Data was obtained at admission, after anesthesia induction, prior to gastric derotation, after gastric derotation and in recovery. Vascular analysis was performed to assess total vessel density (TVD), proportion of perfused vessels (PPV), perfused vessel density (PVD) and microcirculatory flow index (MFI). Our hypothesis was that there would be poor correlation between macrohemodynamics and GM while good correlation would exist between GM and SM. There were no significant changes in SM across time points, whereas there was significant improvement in GM after derotation. Further, GM values were significantly decreased compared to normals both pre- and post-derotation, whereas GS showed increased TVD but decreased PPV and MFI. At varying time points it was determined that there was correlation between GM or SM and S_O₂, ScvO₂, HR, lactate and ETCO₂. A significant correlation between GM and SM was found only for MFI at the pre-derotational time point. These results indicate a significant reduction in GM during GDV which improves after derotation. This study suggests utility for intraoperative microcirculatory assessment with SDM for patients with gastrointestinal disease.

Keywords: microcirculation, sidestream dark field microscopy, gastric dilatation volvulus

BIOMECHANICAL EVALUATION AND EFFECTS OF STERILIZATION ON MECHANICAL PROPERTIES OF POLYMETHYLMETHACRYLATE INTERVERTEBRAL DISK SPACERS. BT Dent, BF Hettlich. Department of Veterinary Clinical Sciences

Cervical Spondylomyelopathy (CSM), or wobbler syndrome, is a relatively common cervical vertebral disease of large and giant breed dogs. The pathogenesis is generally thought to involve intervertebral disk protrusion, osseous malformation, soft tissue hypertrophy, dynamic vertebral instability, and/or vertebral canal stenosis, leading to chronic, progressive spinal cord compression. The disease commonly presents with cervical hyperesthesia, radiculopathy, proprioceptive ataxia and paresis, progressing to tetraparesis and incontinence.

A rapidly growing area of research in the surgical management of CSM is cervical distraction and stabilization, which involves ventral discectomy, distraction of the affected disk space, and insertion of a spacer to maintain disk space height. Most implants used for such a procedure are either allogeneic bone grafts or synthetic cage spacers made of materials such as titanium or polyetheretherketone (PEEK) currently not available on the veterinary market.

Such limited options can dramatically affect the availability and cost of appropriate surgical therapy. Polymethylmethacrylate (PMMA) is widely available for use in orthopedic and spinal procedures, but has not been evaluated in the production of ring disk spacers, which would maintain disk space height yet allow bony fusion between vertebral endplates through the ring

This project aimed to develop a technique for the production of intervertebral disk spacers from PMMA. Additionally, it sought to compare the biomechanical properties of the PMMA spacers to those of cortical ring allografts and to verify the preservation of those properties following various means of sterilization.

We hypothesized that a mold system could be developed to produce PMMA intervertebral disk spacers which would perform similarly to currently used cortical ring allografts and retain their compression strength following sterilization.

Keywords: Dog, lumbosacral, dorsal laminectomy, minimally invasive surgery, pipeline retractor

PHARMACOKINETICS OF AMPICILLIN SULBACTAM IN SERUM AND SYNOVIAL FLUID FOLLOWING REGIONAL INTRAVENOUS PERFUSION OF THE DISTAL LIMB IN CATTLE S. Depenbrock, K. Simpson, M. Papich

Digital sepsis is an important cause of lameness, welfare concerns and economic loss in cattle. Regional intravenous perfusion (RIVP) of antimicrobials can be an effective treatment. The β -lactam ampicillin with β -lactamase inhibitor sulbactam is potentially useful for RIVP because it can administered intravenously, MIC data for ampicillin against common pathogens of digital sepsis exist, it is bactericidal, and it is legal to administer extra-label in cattle. The study goal was to determine the time above MIC in synovial fluid and digital circulation which will predict antibacterial success for β -lactam antimicrobials.

Catheters were placed in the dorsal common digital vein (DCDV), metatarsophalangeal joint, and jugular vein of 6 healthy adult dairy cows. An RIVP was performed using 1.5g combined ampicillin-sulbactam (2:1 ratio respectively). Synovial fluid and blood was collected at specified time points over 24 hours. Synovial fluid and serum were analyzed by high-pressure liquid chromatography.

Both drugs reached high concentrations in the synovial fluid and regional circulation. Maximum mean concentration of ampicillin in synovial fluid, digital circulation and systemic circulation was 1692.178 +/- 1047.64 $\mu\text{g/mL}$, 5422.72 +/- 1953.22 $\mu\text{g/mL}$ and 1.85 +/- 1.85 $\mu\text{g/mL}$ respectively. Mean time of ampicillin concentration in synovial fluid above the CSLI breakpoint MIC for ampicillin of 8 $\mu\text{g/mL}$ was 16 +/- 5.6 hours.

Ampicillin-sulbactam is potentially useful as an RIVP for treatment of infectious disorders of the distal limb. The dosage used in this study likely exceeds that necessary for therapy. Further research is needed to assess treatment efficacy in cattle clinically affected by digital sepsis.

Keywords: Bovine, antibiotic, foot

MICROCIRCULATORY ASSESSMENT OF GASTRIC SEROSA DURING ELECTIVE GASTROPEXY USING SIDESTREAM DARK FIELD MICROSCOPY

K. Datte, A. Dodd, E. Cooper, Dept of Veterinary Clinical Sciences

The microcirculation (blood vessels $<200\mu\text{m}$) is extremely important as capillary beds are the main site of oxygen and nutrient exchange between blood and tissues. Previous studies have indicated that microcirculation remains poor for animals experiencing shock even though heart rate, blood pressure, and cardiac output values return to normal reference ranges. This suggests the microcirculation behaves differently than systemic circulation, likely owing to local control mechanisms. We sought to use patients undergoing anesthesia for elective gastropexy to establish a technique for direct intra-operative assessment of gastric serosal (GS) microcirculation using sidestream dark field microscopy (SDM). In addition correlation with sublingual microcirculation (SM) and other measured parameters (including heart rate, blood pressure, end-tidal CO₂ (ETCO₂), respiratory rate (RR), pulse oximetry (S_O₂) and lactate) was assessed. Vascular analysis on all videos to assess total vessel density (TVD), proportion of perfused vessels (PPV), perfused vessel density (PVD) and microcirculatory flow index (MFI) was performed using Automated Vascular Analysis software. We hypothesized that microcirculatory parameters could be obtained and that there would be good correlation with sublingual circulation but poor correlation with other measured parameters. Obtaining GS microcirculatory videos was challenging but an effective aseptic intra-operative technique was established. There was good intraobserver variability and acceptable interobserver variability. Microvascular parameters for GS were established (TVD 21.1 ± 1.29 , PPV 100 (86.8-100), PVD 20.5 ± 1.39 , MFI 3.00(2.75-3.00)) and found to be significantly different than SM (TVD 27.5 ± 0.73 , PPV 94.9 ± 1.16 , PVD 26.2 ± 0.88 , MFI 3.00 (2.54-3.00)) with poor correlation. A correlation was found between ETCO₂, S_O₂ and RR when compared to microvascular parameters, but correlation was poor with all other measured variables. Based on these results, SDM can be used to assess intraoperative gastric microcirculation. As such, this technique may be beneficial in assessing blood flow and viability in disease states like gastric dilatation volvulus.

Key words: microcirculation, sidestream dark field microscopy, gastric serosa, gastropexy

EVALUATION OF AGREEMENT OF FOUR COMMON DIAGNOSTIC TESTS FOR INSULIN RESISTANCE IN ADULT LIGHT-BREED HORSES

Dunbar L, Mielnicki K, Dembek K, Toribio R, Burns T. College of Veterinary Medicine, The Ohio State University, Columbus, OH.

Several tests have been used clinically for quantifying equine systemic insulin resistance. This study evaluated the agreement between four methods of insulin sensitivity testing, including basal serum insulin and glucose concentration, oral sugar test (OST), combined glucose-insulin test (CGIT), and the insulin-modified frequently sampled intravenous glucose tolerance test (FSIGTT; a gold standard test).

Each of the above tests was performed once in random order on 12 healthy light-breed horses. Several morphometric measurements were obtained, including Body condition score (BCS), cresty neck score (CNS), mean neck circumference (MNC), and tailhead fat depth (TFD). Proxy measurements of insulin resistance (reciprocal of the square root of insulin [RISQI], modified insulin to glucose ratio [MIRG], quantitative insulin sensitivity check index [QUICKI], insulin to glucose ratio [I:G], and homeostasis model assessment [HOMA]) were calculated. Significant correlations were observed between the FSIGTT and QUICKI, RISQI, I:G, HOMA, and basal insulin concentration. Additionally, morphometric data were significantly correlated with proxy measurements.

The gold standard test classified 7 horses as insulin resistant ($IR = SI < 1.0 \times 10^{-4} \text{ L} \cdot \text{mU}^{-1} \cdot \text{min}^{-1}$) and 5 as insulin sensitive (IS). In contrast, basal insulin and OST classified all horses as IS. Calculated Kappa statistics for the CGIT and FSIGTT were 0.274 ($IR = PP\text{-}D_{\text{glu}} > 45 \text{ minutes}$) and 0.25 ($IR = \text{Insulin}_{45\text{min}} > 100 \mu\text{IU/ml}$). Sensitivity of the $CGIT_{PP\text{-}D_{\text{glu}} > 45\text{min}}$ was 85.7% and specificity was 40%. Sensitivity of the $CGIT_{\text{Ins} > 100\mu\text{IU/ml} - 45\text{min}}$ was 28.5% and specificity was 100%.

Current cutoff values for the diagnosis of IR using basal insulin concentration and the OST are highly specific but lack sensitivity. The CGIT displayed better sensitivity and specificity than basal insulin concentration and OST; however, more liberal diagnostic cutoff values may be necessary to improve agreement with the gold standard. CNS had the strongest correlation with proxy measurements and may be the most useful morphometric measurement in horses.

Keywords: Insulin Resistance, Equine Metabolic Syndrome

CATHEPSIN K INHIBITION SIGNIFICANTLY SUPPRESSES BONE RESORPTION IN YOUNG EXERCISING HORSES WITH EVIDENCE OF MAINTAINED BONE FORMATION AND ADAPTIVE REMODELING. H. Hussein¹, J. Dulin¹, L. Smanik¹, W.T. Drost¹, D. Russell², M. Wellman², A. Bertone^{1,2}. 1.Dept. of Veterinary Clinical Sciences. 2. Dept. of Veterinary Biosciences. College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA.

Our investigations evaluated VEL-0230, a highly specific Cathepsin K (CatK) inhibitor, which suppressed osteoclast-mediated bone resorption and inflammation. We have optimized an effective oral dose and dose-interval of VEL-0230 in horses. Unlike bisphosphonates, VEL-0230 induced a rapid, short-acting inhibitory effect of bone resorption. We conducted this study with the objectives of: 1. Determining whether repeated dosing of VEL-0230 produced a desired inhibition of the bone resorption biomarker (CTX-1). 2. Determining the effect of repeated dosing on bone homeostasis, structure and dynamics of bone resorption and formation in horses. We used 12 young exercising horses in a prospective, randomized, blinded, controlled clinical trial in which they received 4 weekly doses of VEL-0230 or vehicle. We performed baseline and post-study radiographs for the third metacarpal bone (MCIII), periodical blood sampling and analysis of plasma bone biomarkers (CTX-1 and osteoclastin), post-study bone fluorescent labeling and biopsy. Bone biopsy specimens were further processed for ex-vivo micro-computed tomography, bone histomorphometry, and immunostaining of Catk. Repetitive dosing of this CatK inhibitor transiently inhibited plasma CTX-1 (reflecting inhibition of bone collagen resorption), but did not prevent normal adaptive bone remodeling to exercise which was evident by a radiographic narrowing of the MCIII dorsal cortex and occurred in both treated and control groups. Bone morphology, density and formation in the group treated with VEL-0230 were not different from controls by all intensive measures. VEL-0230-mediated bone inhibition was unique as it produced marked inhibition of bone resorption without reducing osteoclast numbers or affecting bone homeostasis in healthy horses. Valid concerns exist about using current drugs on young athletes (animal and human) that interfere with the necessary normal adaptive bone remodeling to exercise. Therefore, Catk inhibition is a potential therapeutic in different osteo-inflammatory disorders of young athletes, soldiers, and astronauts in which osteoclast activity is increased.

Keywords: Cathepsin k- bone resorption- horse- remodeling- VEL-0230.

SEMEN VITRIFICATION IN FELIDS – A SIMPLIFIED CRYOPRESERVATION METHOD FOR FIELD USE.

JaCiara D. Johnson, BS,^{1*} Helen L. Bateman, MS,² Jackie Newsom,² Lindsey M. Vansandt, DVM, PhD,² and William F. Swanson, DVM, PhD,²

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Assisted reproductive technologies such as semen cryopreservation and AI are becoming increasingly important for genetic management and conservation of endangered felid species. However, current semen freezing methods for felids are not 'field-friendly', requiring advanced reproductive expertise and access to specialized equipment. One alternative to conventional semen freezing may be vitrification, using ultra rapid cooling in liquid nitrogen to form a glass-like state without ice crystal formation. With this approach, wildlife veterinarians and biologists could easily cryopreserve felid semen in the wild for subsequent AI and genetic augmentation of captive populations. In this study, our objectives were to 1) compare post-thaw sperm parameters for domestic cat and nondomestic cat semen preserved with vitrification vs. conventional freezing; and 2) investigate production of viable kittens using vitrified domestic cat sperm for IVF/ET and AI. Semen was collected from domestic cats (n=2) and non-domestic felids (fishing cat, *Prionailurus viverrinus*; n=1; ocelot, *Leopardus pardalis*, n=1). Control samples were extended in a chemically-defined soy lecithin-based medium with 4% glycerol, slow cooled and frozen in straws over liquid nitrogen vapor.³ For vitrification, raw semen was diluted in soy lecithin medium (w/o glycerol) containing sucrose (0.1 M, Trt 1; 0.2 M, Trt 2; 0.3 M, Trt 3), held for 5 min and vitrified by pipetting (30 µl) directly into liquid nitrogen. After thawing, control and vitrified samples were evaluated for acrosome status, progressive motility and fertility in vitro. For IVF, oocytes collected laparoscopically from gonadotropin-treated domestic cats were inseminated with control or vitrified (Trt 2 only) semen and assessed for embryo cleavage. To evaluate in vivo viability, domestic cat embryos (n=8 total) produced with vitrified sperm were transferred into three synchronized recipients, and three additional females were inseminated with vitrified sperm.² In domestic cats, post-thaw sperm motility and acrosome status did not differ ($P > 0.05$) for control, Trt 1 or Trt 2, but values were reduced ($P < 0.01$) for Trt 3. IVF percentages were similar ($P > 0.05$) for control (16/53, 30.2%) vs. vitrified sperm (13/53, 24.5%). In the fishing cat and ocelot, post-thaw motility was slightly decreased for vitrified (20-25%) compared to control (~40%) sperm but acrosome status (43-48% intact, fishing cat; 26-27% intact, ocelot) was similar. IVF of domestic cat oocytes with vitrified sperm resulted in fertilization in both species (5/9, 55%, fishing cat; 2/9, 22%, ocelot). Embryo transfer failed to produce any pregnancies: however, AI resulted in three ongoing pregnancies with multiple (>5) viable fetuses and the pending birth of the first animals ever produced from vitrified sperm AI. These findings suggest that vitrification may be a suitable option for cryopreservation of felid semen in a field situation, especially if combined with newer semen collection methods that do not require electroejaculation.¹

Key Words: Felids, semen, vitrification, cryopreservation, in vitro fertilization, embryo transfer, artificial insemination

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DYNAMICS OF ADRENAL STEROIDS, STEROID PRECURSORS AND NEUROSTEROIDS IN HOSPITALIZED FOALS.

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Sepsis, the main cause of foal mortality in the first week of life, is a systemic inflammatory response to microorganisms and their toxins. An appropriate physiological response to sepsis is activation of the hypothalamic-pituitary-adrenal axis (HPAA). Many critically ill foals exhibit adrenal insufficiency (AI), which has been associated with disease severity and mortality. What remains unclear is the dynamics of adrenocortical hormones during hospitalization and whether these are indicators of disease severity. We propose that AI foals will have atypical concentrations of adrenocortical steroids (steroid precursors and neurosteroids). We hypothesized that septic foals will have greater concentrations of hormones than healthy and sick non-septic (SNS) foals and that steroid precursors/neurosteroids will remain abnormally elevated during hospitalization in non-surviving septic foals compared to surviving septic foals.

Blood samples were collected from 10 healthy, 22 SNS, and 29 septic foals <3 days old on admission (T0), 24h (T24h) and 72h (T72h) post-admission. Radioimmunoassays quantified cortisol, progesterone, aldosterone, dehydroepiandrosterone (DHEA), 17 α -OH-progesterone and androstenedione.

At T0, cortisol, progesterone, aldosterone, DHEA, and 17 α -OH-progesterone were significantly higher in septic foals than healthy foals. Septic foals at T0 had higher DHEA and progesterone than SNS foals. Septic foals had significantly higher cortisol and DHEA and lower 17 α -OH-progesterone than healthy foals at T72h. In non-survivors, cortisol increased 44% from T0 to T24h, and 51% from T0 to T72h, while cortisol in survivors decreased 63% from T0 to T24h and 203% (P = 0.003) from T0 to T72h (P = 0.017).

We conclude that most critically ill foals had an appropriate response to stress characterized by increased concentrations of most adrenal steroids/precursors/neurosteroids. Different steroid dynamics during hospitalization correlated with severity of illness and outcome. Elevated cortisol and DHEA at 72h in septic foals indicates altered adrenocortical secretion. This research will enhance our understanding of the role of steroid precursors and neurosteroids in the pathogenesis of foal sepsis.

Keywords:

EFFECT OF REPETITION ON QUANTITATIVE PARAMETERS OF VERTICAL FORCE PEAK IN LAME HORSES WITH NATURALLY OCCURRING LIMB MUSCULOSKELETAL DISEASES. Mari Kaido; Allison H. Kilborne; Joy Sizemore; Nathalie Reisbig; Turi Aarnes; Alicia L. Bertone. Depts. of Veterinary Clinical Sciences

The effect of repetitions on objective lameness evaluation and the potential for inaccuracies with repetitions has previously been reported in an experimentally induced lameness model. The purpose of this study was to evaluate the effect of repetition within a trial and trial sets in horses with naturally occurring disease to establish standardized procedures for objective lameness assessment. Twenty client-owned horses with confirmed osteoarthritis (n=11), tendonitis/desmitis (n=6), or foot pain (n=3) experienced subjective lameness and kinetic gait analysis (5 repetitions at 0,3,6,12, and 24 hours). Vertical Force Peak (VFP) within trials significantly increased after the 4th repetition in the lame limb and at the 4th repetition in the contralateral limb. Lame limb VFP increased in the first 3 trials (Time 0,3, and 6), decreasing in the last 2 trials. Contralateral limb VFP was stable until the 2nd trial (Time 3), similarly decreasing until the 5th trial (Time 24). In both analyses, Coefficient of Variation (CV)-VFP was low and did not differ from the experimental model. Asymmetry Index (AI)-VFP did not change across the study. Results of the study suggest the use of naturally occurring lameness in research due to its low variability. Additionally, the lameness profile is the most accurate when trial repetitions are limited to 3, with the investigation frequency restricted to greater than 3 hours in the natural disease model. AI-VFP which previously depicted change in weight bearing in the induced lame limb is unable to account for changes resulting from bilateral disease common in naturally occurring disease.

Keywords: Horse, Force plate, Lameness, Exercise, Repetition

VITAMIN D METABOLITES AND THEIR ASSOCIATION WITH CALCIUM, PHOSPHORUS, AND PTH CONCENTRATIONS, SEVERITY OF DISEASE, AND OUTCOME IN HOSPITALIZED FOALS. A. Kamr^{1,4}, K.A. Dembek¹, S.M. Reed², N.M. Slovis³, A. Zaghawa⁴, T.J. Rosol¹, R.E. Toribio¹. ¹ Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH; ²Rood and Riddle Equine Hospital, Lexington, KY; ³Hagyard Equine Medical Institute, Lexington, KY; ⁴Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt

Hypovitaminosis D is a frequent finding in critically ill human patients that has been associated with hypocalcemia, disease severity and poor outcome. However, information on vitamin D metabolites and their association with hypocalcemia, severity of disease and mortality in critically ill foals remains undocumented. The goal of this study was to investigate the prevalence of hypovitaminosis D and its association with calcium, phosphorus, and parathyroid hormone (PTH) concentrations, severity of disease and mortality in critically ill foals.

One hundred newborn foals \leq 72 hours old divided into hospitalized (n=83; 59 septic, 24 sick non-septic [SNS]) and healthy (n=17) groups were included in the study. Blood samples were collected on admission to measure serum 25-hydroxyvitamin D₃ [25(OH)D₃], 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], calcium, phosphorus and PTH concentrations. Data were analyzed by non-parametric statistical methods and univariate logistic regression to calculate odds of mortality.

The prevalence of hypovitaminosis D [defined as 25(OH)D₃ < 9.51 ng/mL] was 63% for hospitalized, 64% for septic, and 63% for SNS foals. Serum 25(OH)D₃ and 1,25(OH)₂D₃ concentrations were significantly lower in septic and SNS compared to healthy foals (P<0.05). Septic foals had significantly lower calcium and higher phosphorus and PTH concentrations than healthy and SNS foals (P<0.05). In hospitalized and septic foals, low 1,25(OH)₂D₃ concentrations were associated with increased PTH but not with calcium or phosphorus concentrations. Septic foals with 25(OH)D₃ < 9.51 ng/mL and 1,25(OH)₂D₃ < 7.09 pmol/L were more likely to die (OR=3.62; OR=5.41, respectively).

We conclude that low concentrations vitamin D metabolites are associated with disease severity and mortality in hospitalized foals. Hypocalcemia and hyperphosphatemia together with low 1,25(OH)₂D₃ and increased PTH concentrations in septic foals indicates that PTH resistance could contribute to the development of these abnormalities.

Keywords: vitamin D metabolites; calcium; phosphorus; PTH; sepsis; mortality; hospitalized foals.

SYNOVIAL FLUID PARAMETERS FROM OSTEOARTHRITIC EQUINE JOINTS TREATED WITH INTRA-ARTICULAR EQUINE DENTAL STEM CELLS OR

CONTROL SOLUTION. R Kopp, A Kilborne, M Kaido, AL Bertone. From the Comparative Orthopedics Research Laboratory, Dept of Veterinary Clinical Sciences, The Ohio State University.

Intra-articular use of dental pulp stem cells may have the potential to decrease lameness and joint inflammation in naturally occurring osteoarthritis in horses. The goal of this project was to investigate the effects of dental pulp derived stem cell therapy on the immune and inflammatory response, specifically cytokine profiles, when administered intra-articular to horses with osteoarthritis. Twenty horses were randomly assigned to receive 1ml of control solution (n=10) or 10 million cells of dental pulp (n=10), exercised on a treadmill, and synovial fluid was evaluated before and at day 14 after injection for cytology and IL-1 β , IL-1 receptor antagonist, IL-6, and IL-10 [Genorise ELISA kit]. Data was analyzed by repeated measure ANOVA for time and treatment and Wilcoxon rank post-test. Statistical significance was set at P< 0.05. Synovial fluid WBC count, protein and cell differentials at Day 0 or 14 did not differ between treated and control and synovial fluid was noted as within acceptable limits with counts > 1000 cells/ul were only seen in samples with blood contamination. The IL-10 percent change from baseline differed and increased in the control joints and decreased in the treated joints. (P<0.03) The control synovial fluid increased in IL-6 (p< 0.03) and IL-6 percent change from baseline increased in the control synovial fluid and decreased in the treated synovial fluid. (P<0.03) No significant difference was noted between groups for IL-1 β and IL-1 receptor antagonist and values were low. Adverse effects were not observed. Intra-articular injection of dental pulp therapy was not pro-inflammatory in the synovial fluid after 14 days and demonstrated persistent anti-inflammatory and immunologic effects as measured by cytokine analysis. Intra-articular administration of dental pulp derived stem cell therapy can be considered a safe treatment option for equine osteoarthritis, with the potential for disease modifying effects.

Keywords: dental pulp stem cells, ELISA, immune response

ANATOMICAL VARIATION AND COMPRESSION OF THE CELIAC TRUNK IN DOGS AND CATS AS ASSESSED BY COMPUTED TOMOGRAPHY ANGIOGRAPHY: RETROSPECTIVE STUDY OF 18 CASES (2009-2014).

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The celiac artery (CA) and cranial mesenteric artery (CMA) are the primary arterial blood supply of the abdominal organs. Anatomical abnormalities are rare, but knowledge of them is critical for surgical or imaging-guided intervention. Celiomesenteric trunk is rarely reported in humans, with fewer reports in animals. Syndromes associated with CA constriction, such as median arcuate ligament syndrome and CA compression syndrome, have been described in humans and are treated by surgical decompression. In veterinary medicine, these syndromes have never been described.

Computed tomography angiography (CTA) scans of dogs and cats that included the cranial abdomen between January 2009 and December 2014 were reviewed. The CA and the CMA were analyzed individually and anatomical variations recorded.

A total of 179 scans were retrieved from 168 dogs and 11 cats. Eighteen abnormalities in the CA axis were observed (10.1%) including nine abnormal arterial origins (5.0%) and nine cases of CA compression (5.0%). Seven celiomesenteric trunks, one case of splenic artery origination from the CMA, and one case of hepatic arterial branches originating from the left gastric artery were observed. Celiac artery compression was diagnosed in 5.4% of the dogs and no cat.

Anatomical variants of CA origin and division occur in dogs and cats, can be evaluated by CTA, and may impact surgical or image-guided intervention. We report CA compression for the first time in dogs, apparently related to constriction by the diaphragmatic crura and associated structures; the clinical relevance of this imaging finding remains uncertain and further study is needed.

Keywords: Celiac artery, celiomesenteric trunk, median arcuate ligament, CTA

ELASTOGRAPHY CHARACTERIZATION AND REPEATABILITY IN 14 NORMAL CANINE SPLEENS. K.A. Mulligan, W.T. Drost, D.M. Auld, J.D. Bonagura. Department of Veterinary Clinical Sciences.

Ultrasound elastography is a technology that measures the elasticity of tissues and can differentiate benign versus malignant conditions in people. Few studies in veterinary patients exist, and only two have investigated characteristics of normal canine spleens. The purpose of this study was to describe elastography characteristics of the normal canine spleen and assess intra-and interobserver repeatability.

Two observers independently performed elastography in 14 healthy dogs. Images were made at minimum, neutral, and maximum compression, and this was repeated two weeks later. Regions of interest were drawn on the spleen at each compression point and pixel color percentages determined. A strain score was calculated for each spleen, using a weighted average of the percentages. Friedman's test compared the three compression points, and Spearman's rank-order correlation coefficients assessed intra-and interobserver repeatability. Waveform height was compared using a Mann-Whitney U test.

Strain scores at neutral compression were significantly lower than maximum compression for both observers ($P < 0.05$). Intraobserver correlation was moderate and statistically significant for observer one ($R_s^2 = -0.548$, $P = 0.043$), but variation existed between individual subjects. For observer two, there was weak to moderate correlation which was not significant ($R_s^2 = 0.433$, $P = 0.122$), indicating poor intraobserver repeatability. Interobserver correlation for the first time point was weak to moderate and not significant ($R_s^2 = -0.464$, $P = 0.095$), and lower correlation was found at the second time point ($R_s^2 = 0.301$, $P = 0.122$). These values indicate poor interobserver repeatability. Observer two exhibited significantly more compression than observer one ($P = 0.002$).

We recommend measuring strain at neutral compression because repeatability was better. However, inter- and intraobserver repeatability is weak to moderate, and operator compression levels vary, which underscores the need for thorough training and practice if elastography is to become useful in canine splenic imaging. Patient factors such as variable compliance and lack of sedation contributed to non-ideal repeatability.

Keywords: elastography, strain, repeatability, spleen, canine

ECHOCARDIOGRAPHIC ASSESSMENT OF THE RIGHT VENTRICLE IN CATS WITH HYPERTROPHIC CARDIOMYOPATHY. S.I. Savino and K.E. Schober. Dept. of Veterinary Clinical Sciences.

Background and Significance: Hypertrophic cardiomyopathy (HCM) is the most common form of acquired heart disease in cats ultimately leading to high morbidity and mortality. While HCM is phenotypically characterized by left ventricular (LV) hypertrophy, studies in humans with HCM revealed evidence of right ventricular (RV) remodeling. Whether structural abnormalities of the right ventricle are also present in feline HCM is currently unknown. Knowledge on RV involvement would enhance our understanding in the pathophysiology of feline HCM, and likely be useful in explaining the development of right-sided CHF in some cats with HCM.

Approach: The current study is a retrospective observational study that includes 150 healthy cats and 200 HCM cats imaged between 2003–2014. Echocardiograms in three imaging planes are analyzed, and variables measured in triplicate. Measurement variability and the effect of age, sex, and body weight will be determined. A quality control study in 10 cats of either group will prove reproducibility of quantitative data. Parametric and non-parametric statistical tests will be used to compare normal cats to cats with HCM. The proportion of cats with RV hypertrophy will be determined and the link to severity of LV hypertrophy and right-sided CHF investigated.

Preliminary Findings: 40 control cats and 57 cats with HCM have been analyzed. Linear regression analysis identified an effect of body weight on linear variables but not ratio indices. Results are summarized:

Image view	Variable	Control	HCM	<i>P</i>	HCM above mean±2SD of control (%)
1	RVPW (mm)	2.99±0.57	3.92±1.04	<0.001	28
	RVPW:RVD ratio	0.50±0.16	0.70±0.31	<0.001	25
2	RVPW (mm)	2.79±0.48	3.33±0.83	<0.001	25
	RVPW:RVD ratio	0.57±0.13	0.79±0.37	<0.001	37
3	RVPW (mm)	3.08±0.59	3.77±0.86	0.002	32
	RVPW:RVD ratio	0.38±0.13	0.52±0.21	<0.001	8

Conclusions: RV free wall hypertrophy is seen in approximately 25% of cats, indicating that RV involvement is relatively common in cats with HCM.

Keywords: Feline; Hypertrophic Cardiomyopathy; Right Ventricular Hypertrophy

CLINICOPATHOLOGIC RESPONSES TO EXPERIMENTAL ABDOMINAL SURGERY IN HORSES. A. Shore, J. Godman, T. Burns, and M. Mudge. The Ohio State University, Dept. of Veterinary Clinical Sciences.

Colic can result from a variety of disease processes leading to variable degrees of inflammation in the gastrointestinal tract. Previous reports have described the utility of individual variables for prognostication of disease outcome in horses with colic. The goal of this project was to investigate the effects of abdominal surgery and intestinal manipulation on the inflammatory response in venous blood and peritoneal fluid. A prospective clinical evaluation of 10 university-owned adult light breed horses undergoing ventral midline celiotomy was performed. Venous blood and peritoneal fluid samples were collected prior to surgery and 12, 24, 48, 72 hours, 5 days, and 7 days post-operatively; several clinicopathologic tests were performed on these samples (packed cell volume, total protein, lactate and fibrinogen concentrations on venous blood; lactate and total protein concentration on peritoneal fluid). Data were analyzed for normality by the Shapiro-Wilk test. Differences between time points were assessed parametrically using ANOVA or non-parametrically using a Kruskal-Wallis test as appropriate based on normality of the data. Serial venous blood samples showed a decrease in packed cell volume 12 hours after surgery and a subsequent increase at day 5 when compared to pre-operative values ($P < 0.05$). Total protein concentration was noted to be elevated for all evaluations starting 48 hours post-operatively, remaining elevated at day 7 ($P < 0.05$). Lactate was increased 12 hours, 72 hours, and 5 days after surgery ($P < 0.05$), and fibrinogen concentration was elevated for all evaluations after surgery ($P < 0.05$). Peritoneal fluid collection was attempted at each time point; however, sufficient sample was only obtained in approximately 65% of attempted collections. The concentrations of lactate and total protein in peritoneal fluid increased post-operatively, with values peaking between 24 and 48 hours before returning toward baseline; statistical significance could not be evaluated due to the low number of data points. This information may provide insight into the evaluation of post-operative morbidity and ultimately impact the ability to provide an accurate prognosis for surgical abdominal disease in horses.

Keywords: abdominal surgery, celiotomy, clinicopathologic response, horses, post-operative morbidity, colic, inflammation

PROSPECTIVE RANDOMIZED BLINDED CONTROLLED CLINICAL TRIAL FOR THE INJECTION OF LIVING DENTAL PULP CELL PARTICLES FOR THE TREATMENT OF EQUINE LAMENESS CONDITIONS

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Injection of dental pulp cells (DPC) may decrease lameness and pain in natural-occurring disease. Sterile dental pulp harvested from fresh perished foals was processed to produce minimally manipulated, unexpanded allogeneic living cells suspended in extracellular matrix (PulpCyte®, StemLutions). We hypothesized that injection of DPC would improve pain and lameness in horses. We used a prospective, randomized, blinded, controlled clinical trial using natural-occurring cases with the outcomes of lameness, inflammation, pain, and client satisfaction. Forty client-owned horses with confirmed OA (n=20), desmitis (n=14) or tendonitis (n=6) were assigned to receive 2 ml intra-articular (n=20 OA) or intra-lesional (n=20) injection of control vehicle (n=20) or 5×10^6 dental pulp cells (n=20). Horses were acclimated to the treadmill and force plate, had baseline measurements performed, and were injected on Day 0. For 2 weeks, horses were exercised on the treadmill. The horses were evaluated by clinical parameters, AAEP lameness score, gait analysis, edema (score and circumference), pain on flexion or pressure, and clients' scores for pain and discomfort before, and through 45 days after cell injection. Treated horses showed persistent decrease in lameness and induced lameness after flexion (OA) or pressure (desmitis, tendonitis) compared to baseline or control ($P < 0.05$). Cells induced edema on day 1 at the injection site. ($P < 0.05$) Client assessment of lameness and comfort were improved between, before, and 31 or 45 days after cell injection ($P < 0.05$). Clinical improvement with tendonitis and desmitis was greater than OA. PulpCyte® can be considered an effective and safe treatment option for equine lameness due to OA, desmitis or tendonitis.

Keywords: horse, stem cell, osteoarthritis, tendonitis, desmitis,

RESPONSE OF ADRENOCORTICAL STEROIDS AND STEROID PRECURSORS TO ACTH STIMULATION IN CRITICALLY ILL FOALS. K. Timko, K. Dembek, L. Johnson, K. Hart, P. Morreseay, R. Toribio. Department of Veterinary Clinical Sciences.

Septicemia is the leading cause of mortality in newborn foals. During sepsis, the hypothalamus-pituitary-adrenal axis is activated. Adrenocortical hormones regulate energy, electrolytes, blood pressure homeostasis, organ maturation, and immune function. While most foals have an elevation in adrenocorticotropin (ACTH) and cortisol during septicemia, preliminary studies by our group suggest that some sick foals have low cortisol despite elevated ACTH. This is consistent with relative adrenal insufficiency (RAI), which is linked to impaired organ function and mortality in foals. We hypothesized that RAI in foals will involve multiple adrenocortical layers that will be characterized by reduced glucocorticoids and mineralocorticoids and increased steroid precursors/neurosteroids, which will be associated with disease severity and mortality. We proposed that foals with multilayer adrenocortical failure will have an abnormal steroid response to exogenous ACTH stimulation.

Foals <3 days of age were divided into septic (n=11); sick non-septic (SNS) (n=9); and healthy (n=12). After baseline blood sample collection (Time 0), foals received 10 µg of ACTH IV. Additional blood samples were collected at 30 and 90 min post ACTH (Time 30 and 90 Min). Steroid/steroid precursor/neurosteroid concentrations were determined by immunoassays.

Septic foals had higher cortisol, progesterone, 17α-OH-progesterone, pregnenolone, aldosterone and androstenedione concentrations at Time 0, 30 and 90 min than healthy foals (P<0.05). Non-surviving septic foals had lower cortisol concentrations at Time 0 and 30 min compared to surviving septic foals (P<0.05). The percent change from Time 0 to Time 30 min for cortisol, progesterone, aldosterone, and 17α-OH-progesterone (P<0.05) was lower in septic foals compared to healthy foals, suggesting RAI. Progesterone and 17α-OH-progesterone decreased in response to ACTH at Time 30 min in septic foals, indicating abnormal steroidogenesis. We provide evidence that in critically ill foals steroidogenesis is altered, resulting in RAI, which may contribute to severity of illness and mortality.

Keywords: Septicemia, HPA, ACTH, Relative Adrenal Insufficiency (RAI), foal

INTRA-ARTICULAR INJECTION OF AN AUTOLOGOUS PROTEIN SOLUTION FOR TREATMENT OF CANINE OSTEOARTHRITIS: A PROSPECTIVE, RANDOMIZED, DOUBLE-BLINDED, PLACEBO-CONTROLLED CLINICAL TRIAL. A. Wanstrath, B. Hettlich, L. Su, A. Smith, L. Zekas, M. Allen, A. Bertone. Dept. of Veterinary Clinical Sciences

Osteoarthritis (OA) is a common cause of debilitating lameness in dogs. Although osteoarthritis is classified as a non-inflammatory disease, the inflammation process can affect the progression of the disease. As cartilage begins to breakdown, the ratio of naturally occurring inflammatory to anti-inflammatory cytokines becomes imbalanced causing increased breakdown of cartilage. A novel method for up regulation of anti-inflammatory cytokines from whole blood has been reported which produces an autologous protein solution (APS). Injection of APS has been shown to be an effective treatment of OA in horses and has greater anti-inflammatory proteins than platelet rich plasma. The objective of the study was to prospectively evaluate the efficacy of an intra-articular injection of APS for treatment of OA in dogs using the Hudson visual analog scale (HVAS), Canine Brief Pain Inventory (CBPI), and peak vertical force. Twenty client-owned dogs with a unilateral lameness attributable to OA of the elbow or stifle were enrolled and randomly assigned to a joint injection with APS or 0.9% saline solution. Owners and observers performed assessments blinded prior to injection, and at week 2 and 12. Radiographs of the affected joint were obtained prior to injection and at week 12. For dogs that received the APS injection, lameness scores (improved 25.6%; $P < 0.03$), pain scores (improved 15%; $P < 0.05$) and peak vertical force (increased 14.9%; $P < 0.2$) showed significant improvement at week 12 compared with pretreatment values. For control dogs, lameness scores, pain scores and peak vertical force at week 12 were not significantly different from pretreatment values. There was no evidence of radiographic progression of osteoarthritis from week 0 to week 12. A single intra-articular injection of autologous protein solution was an effective means of improving lameness, pain and weight bearing by 12 weeks in dogs with OA involving a single joint.

Keywords: osteoarthritis, joint injection, autologous protein solution

BIOLUMINESCENCE IMAGING OF CANINE OSTEOSARCOMA METASTASIS IN AN ORTHOTOPIC MURINE MODEL. L. Rose, F. Xu and MJ Allen.

Department of Comparative and Veterinary Medicine

Osteosarcoma is the most common primary bone neoplasm in dogs and humans, and behaves aggressively in both species. In canine patients, metastases are frequently present at the time of diagnosis. Bioluminescence imaging has been established as a useful tool to visualize tumors *in vivo*. This technique may further quantify and help monitor the size and abundance of primary and metastatic tumors, as well as tumor responses to chemotherapy. An athymic, nude mouse model of osteosarcoma has been developed to study the responses of lung metastases to chemotherapy. Enhancement of tumor recognition via specific antibody labeling and luminescence may improve metastatic nodule detection and provide a useful clinical tool for studying osteosarcoma behavior. We evaluated luciferase-labeled Abrams osteosarcoma tumor cells in an orthotopic murine model, as well as other neoplastic cell lines, via an *in vivo* imaging system (IVIS). The presence of signal, of primary tumor and metastatic burden, was compared to histology as commonly used in research laboratories, as well as radiography. We ultimately hope to accurately monitor metastatic burden for response to chemotherapeutic protocols.

Keywords: bioluminescence, canine, osteosarcoma, orthotopic

**EPIDEMILOGY
AND
APPLIED RESEARCH**

EXTENDED-SPECTRUM CEPHALOSPORIN, CARBAPENEM, AND FLUOROQUINOLONE-RESISTANT GRAM-NEGATIVE COLIFORM BACTERIA PRESENT ON EQUINE ENVIRONMENTAL SURFACES

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Antimicrobial resistant bacteria are a rapidly growing concern in human and veterinary medicine. The increasing prevalence of extended spectrum beta-lactamase (ESBL), carbapenemase (CRE), and fluoroquinolone-resistant Enterobacteriaceae continually decreases the efficiency of essential antibiotics. Moreover, antibiotic resistant enteric bacteria are zoonotic and can be transmitted between horses and people. Our objective was to evaluate the prevalence of antibiotic resistant bacteria on human contact surfaces in equine environments. Environmental surfaces in 20 Ohio equine barns were sampled using two electro-static-cloths (Swiffer®), yielding a total of 242 samples. Samples were phenotypically screened for AmpC, ESBL, CRE, and fluoroquinolone resistance using selective media. To select for cephalosporinase phenotypes, samples were incubated at 37°C in nutrient broth with 2 ug/mL cefotaxime. This broth was aseptically inoculated to MacConkey Agar with 8 ug/mL cefoxitin, 4 ug/mL cefepime, and 1 ug/mL meropenem to detect AmpC, ESBL, and CRE phenotypes, respectively. Additionally, samples were incubated in nutrient broth containing 16 ug/mL naladixic acid and then inoculated to MacConkey agar with 16 ug/mL naladixic acid and 2 ug/mL ciprofloxacin to detect fluoroquinolone resistance phenotypes. Genotypes were confirmed using standard PCR techniques. Of the Gram-negative coliform bacteria sampled from 242 surfaces, 51 (21.07%) were cefoxitin resistant, 24 (9.92%) were naladixic acid resistant, 13 (5.37%) were cefepime resistant, and 8 (3.31%) were ciprofloxacin resistant. Drains and wash stalls harbored the highest prevalence at 9.92% (n=24), followed by handles of mucking equipment at 5.37% (n=13). These results suggest that equine environmental surfaces are commonly contaminated with resistant bacteria that can potentially be transmitted between human and horse populations. Furthermore, identifying these bacteria on common human contact surfaces suggests that the equine environment can serve as a reservoir for antibiotic resistance genes. Identifying interventions to lower the prevalence of antibiotic resistant bacteria in equine environments will protect both animal and public health.

Keywords: public health, antibiotic resistance, environmental prevalence

UNDERSTANDING ANTIMICROBIAL RESISTANCE AT THE HUMAN-WILDLIFE INTERFACE. G Ballash, T Wittum, D Mollenkopf, P Dennis. Department of Veterinary Preventive Medicine

Antimicrobial resistance (AMR) is a global public and animal health issue. Although resistant isolates are commonly found in domestic animals, a growing concern has emerged as resistant organisms have entered into wildlife populations. This study investigates AMR isolates in white-tailed deer from population dense regions where intensive farming is not prevalent. WTD fecal samples and surface water samples were collected from the Cleveland Metroparks between May 2013 to August 2015. *E. coli* was isolated and tested for AMR to three types of antimicrobials: Second generation cephalosporins (Cefoxitin), third generation cephalosporins (Cefepime) and Fluoroquinolones (Ciprofloxacin). Those isolates resistant to at least one antimicrobial were further characterized by PCR and gel electrophoresis and/or sequencing. The results and their implications in regards to public, veterinary and environmental health will be discussed.

Keywords:

EAR - 3

EFFECTS OF CALVING MANAGEMENT PRACTICES IN DAIRY HERDS.

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Department of Veterinary Preventive Medicine

Calving-related losses (survival, health, and productivity of dams and calves) and welfare practices are known challenges for the dairy industry worldwide, and management practices have been associated with this problem. The objective of the present study was to assess calving and newborn management practices. A total of six large dairy operations (n=15,000 cows; range 900-5,000) were enrolled in the present study. All herds (100%) had calving and colostrum management protocols, and were reviewed at least annually (50%) or when necessary (50%). Appearance of the amniotic sac (83.3%) and feet of the calf outside the vulva (33.3%), including raised tail, filled udder, and dilated vulva, were used as imminent signs of births prior to parturition by calving personnel. The frequency of observation (calving personnel walking the prepartum pen and actually looking for cows with the imminent signs of birth) was every half hour (50%), every 1 hour (33.3%), and 1-2 hours (16.7%). Cows experiencing difficult births were determined by the appearance of 1 foot outside the vulva (100%) and calving progress (<1 hour = 66.7% and >3-4 hours = 33.3%). When assistance was provided during difficult birth, 100% of personnel used protective disposable long-sleeve gloves, 66.7% of hers washed the perineum area, and 66.7% disinfect obstetric chains prior to use. Regarding newborn care practices, 100% disinfected the navel after parturition and fed colostrum within 1 hour after birth. Only 33.3% of herds offered less than 1 gallon of colostrum at first feeding, 50% monitor colostrum quality, and only 33.3% of farms assessed failure of passive transfers (FPT) in calves. Designing and implementing a proactive calving management program (from feed management, cow comfort, record-keeping, to personnel training) will significantly reduce calving-related losses (e.g., stillbirth) and improve the overall welfare of the herd.

Key Words; Dairy, Calving, Stillbirth

THE PREVALENCE OF INFLUENZA A VIRUS IN ENTERING EXHIBITION SWINE AND THE ASSOCIATION OF ON-FARM MANAGEMENT PRACTICES WITH RISK OF INFECTION. N. Bliss, S. Nelson, J. Nolting and A. Bowman. Department of Veterinary Preventive Medicine

Swine play a key role in the evolution and ecology of influenza A virus (IAV) infecting humans as pigs are a host species in which reassortment of the IAV segmented genome commonly occurs. Exhibition swine, due to the distinctive management practices under which they are reared and due to the way they are displayed for show, provide a critical human-swine interface allowing for the bidirectional zoonotic transmission of IAV. Previous IAV surveillance in these unique settings occurred at the end of the fairs and little was known about the incoming prevalence of IAV. In 2014 snout wipes were used to sample pigs during the first day of nine agricultural exhibitions in Indiana and Ohio. Samples were screened using rRT-PCR for the matrix protein gene of IAV. Positive samples were inoculated onto Madin-Darby canine kidney cells for virus isolation. An IAV prevalence of 1.47% (52/3,547) was determined among arriving exhibition swine. Sampling also identified corralling activities to be a possible transmission point for IAV. In addition, a survey concerning the on-farm management history of the exhibition swine was administered. A total of 480 surveys were collected and correlated to 614 snout wipe samples. Participants were found to attend multiple exhibitions (median 2, range 0-50) and the number of exhibitions attended by individual swine ranged 0-30. Hosting as an Open house/sale had 3.93 (CI: 1.10-13.06) times higher odds of having an IAV positive pig. Overall, this research yields a better understanding of the epidemiology of IAV in exhibition swine and allows for improved prevention. This study illustrates that a small number of pigs arrive at the fair shedding IAV, identifies a possible transmission point for IAV, and expands upon the limited knowledge that is known about exhibition swine management.

Key Words: Influenza A virus, Swine, Virus Shedding, Ecology, Animals

NEST LOCATION PREFERENCE IN MICE HOUSED IN INDIVIDUALLY VENTILATED AND STATIC CAGING SYSTEMS. K. Cornelius, S. Lewis, T. Martin, and C. Hendrick; University Laboratory Animal Resources

Mice build nests for more reasons than simply to rear young. Nests hide mice from predators and aid in thermoregulation, especially in the research environment. Recent studies have examined the benefits of scoring mice nests to aid in determining welfare and health statuses. However, environment also plays a role in nest-building and can influence nest score as well as nest location in the cage. In the research environment, cage ventilation especially likely affects nest complexity and location. Consequently, we examined how nest location and scores differed between mice housed in individually ventilated cages (IVCs) versus static cages. We hypothesized that mice in IVCs would build more complex nests at the front of the cage, furthest from air intake. Mice of mixed backgrounds representative of a large scale academic institution (n= 14) were initially housed in unventilated cages and provided with a 5 cm² nestlet. 3 days later, nests were scored, and we recorded nest location (front, middle, or back third of the cage), cage temperature, and cage humidity. The same mice were then moved to ventilated cages with nestlets and the same data was collected in 7 days. Lastly, survey data on nest scores and location was randomly collected on 1769 ventilated cages on day 7 after cage changing. We found that, contrary to our hypothesis, most nests were located in the back third of IVCs. Moreover, nests in IVCs scored significantly higher than nests in static cages. We conclude that design of IVCs should allow mice to build nests in the back and environmental factors, such as air flow, alter nest score and location.

Keywords: mice, enrichment, ventilation, caging

PRESCENCE, DISTRIBUTION AND RISK FACTORS ASSOCIATED WITH ENVIRONMENTAL STAPHYLOCOCCUS SPP. IN A NEWLY ESTABLISHED SMALL ANIMAL VETERINARY SPECIALTY HOSPITAL. N. Diana, J. Van Balen Rubio, A. Hoet, J. Stull. Dept of Veterinary Preventive Medicine

Staphylococci are common inhabitants of skin and mucous membranes with *S. pseudintermedius* and *S. aureus* serving as important pathogens for companion animals and people. The veterinary environment is likely important in nosocomial and zoonotic transmission, yet little is known of the role of within-clinic factors in staphylococci occurrence and persistence. This research examined the prevalence of environmental coagulase-positive staphylococci (CPS) in a new veterinary practice and evaluated the role of environmental, clinic, and patient factors in CPS prevalence. Samples were collected prior to the clinic opening and then every 3 months from 2013 to 2014. At each collection, 67-73 samples were taken from human only and mixed animal/human contact surfaces. Daily cleaning logs, monthly patient visits, and staff employment logs were also collected. Prior to opening, 28.4% of the surfaces were contaminated, followed by 22.5%, 27.4% and 52.9% at subsequent samplings. Methicillin susceptible *S. pseudintermedius* (MSSP) steadily increased over the 4 samplings from 0 to 26.5% of surfaces contaminated. Methicillin susceptible *S. aureus* (MSSA) contamination was the highest at the first and last samplings (25%). The prevalence of methicillin-resistant CPS (MRSA, MRSP) was low ($\leq 1.5\%$) at all samplings. Through the sampling period, the number of staff employed remained stable while the monthly caseload increased and cleaning decreased. The steady increase of MSSP contamination is likely the result of more animals being seen at the hospital coupled with decreased cleaning practices. Additionally, many surfaces commonly contaminated were not specified to be cleaned on the lists provided to the staff. These findings illustrate that as a veterinary practice grows, CPS environmental contamination also increases. Proper cleaning practices with attention to key identified surfaces should be put in place and maintained in order to provide an optimal environment for patients and staff.

Keywords: *S. aureus*, zoonotic, environmental contamination, small animal, MRSA

EAR - 7

INFLUENZA A VIRUS TRANSMISSION IN SWINE AT AGRICULTURAL FAIRS.

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Agricultural fairs and livestock exhibitions create unique interfaces where a diverse population of swine congregate and can transmit diseases within and between species. Influenza A virus (IAV) is a respiratory disease that can cause fever and anorexia, which decrease rate of gain and negatively affect profits. IAV reassortment is common in swine populations, making swine an important source of novel IAVs. Transmission of IAV between swine and people at agricultural fairs has been documented at unprecedented levels in recent years, highlighting the need to decrease this risk to animal and public health. Understanding the spatial dynamics of IAV spread in mixed population of swine will allow swine practitioners to better minimize IAV transmission within swine herds and protect susceptible people. Data collected from this project provides clues as to the most significant routes of spread, such as movement of animals or humans, fomites, airborne transmission, or direct contact. This project is exploring the local spatial dynamics of IAV within fairs using longitudinal data collected during the 2014 fair season. These data were collected by serially testing swine for IAV over time and concurrently mapping their spatial location during the fairs. The hypothesis tested is transmission of IAV in a swine population at an agricultural fair occurs in a spatially clustering fashion. During four Ohio and four Indiana fairs, snout wipe samples from every pig from each fair were collected and mapped daily, placed in transport media and frozen at -80°C until tested. rRT-PCR was used to test for the presence of IAV after which, spatial and aspatial data will be combined for autocorrelation with Moran's I statistic. Statistically-significant clustering of IAV-positive swine related to fair environment conditions and management practices is expected. These data can be translated to commercial swine production where swine outbreaks are common.

Keywords: Influenza A virus, swine, public health, disease outbreaks, livestock

EPIDEMIOLOGY OF *TRYPANOSOMA EVANSI* IN THE UPPER AWASH RIFT VALLEY, ETHIOPIA

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Camels play an important role in the lives of pastoralists and people living in lowland areas in Ethiopia. The Upper Awash Rift Valley of Ethiopia is home to the Afar ethnic group, a large pastoralist community. Camels provide meat and milk, and they serve as primary transportation for the pastoralist community. Animal diseases impose a major burden to the livelihood of the pastoralists. *Trypanosoma evansi* can cause both acute and chronic disease in camels; therefore, it represents a significant threat to the personal livelihood of people in this region. Improving natural habitats and feed for camel herds is difficult due to the nomadic lifestyle of the pastoralists and the climate of the Upper Awash Rift Valley; therefore, it is recommended that short-term disease control programs be employed to increase productivity of camel herds (1). To aid in the study of the epidemiology of this disease in the Afar region, it is imperative to determine the prevalence of this parasite. This study uses molecular diagnosis via PCR and gel electrophoresis to determine the prevalence of *T. evansi* in a pastoralist-owned camel herd. Body condition scores and anemia levels determined by packed cell volume are also evaluated as possible methods for preliminary on-site diagnosis of this disease. Additionally, the effects of formaldehyde on long-term storage of the samples will be evaluated via comparison of treated and non-treated samples of camel blood in order to determine the reliability of Whatman FTA cards for the importation of potentially infectious material. The results of this study will provide important information regarding the prevalence of this disease in Eastern Ethiopia and will lay the groundwork for future epidemiological studies in this area.

- 1) Melaku T. and Fesha G. (2001) A study on the productivity and diseases of camels in Eastern Ethiopia. *Tropical Animal Health and Production*. 33(4): 265-274.

Keywords: Ethiopia, *Trypanosoma evansi*, camels

A 'ONE HEALTH' APPROACH TO *CLOSTRIDIUM DIFFICILE* INFECTION IN HUMANS AND ANIMALS. K. Godden, G. Habing, and J. Stull. Department of Veterinary Preventative Medicine.

Direct or indirect exposure to animal populations harboring *Clostridium difficile* may be a potential source for human infection, which to-date remains unclear. The objective of this study was to assess the risk associated with human-animal interaction, along with factors that may contribute to an individual's susceptibility to *C. difficile* infection (CDI). *C. difficile* has been found in animals with and without clinical diarrheal symptoms, suggesting a potential for environmental contamination and zoonotic spread. We hypothesized that there would be a correlation between the prevalence of *C. difficile* in the animal and human environments on swine and dairy farms. In addition, we hypothesized that indistinguishable *C. difficile* genotypes would be found in both human and animal environments, suggesting the potential for zoonotic transmission. We collected samples from swine and dairy farms, and focused on the environments of the farrowing piglets and pre-weaned calves, and on property break rooms or houses. *C. difficile* Moxalactam Norfloxacin (CDMN) selective media was used to enrich and isolate *C. difficile* from the samples. Confirmation was achieved using characteristic odor, colony morphology and gram-stain appearance. The swine (n=8) and dairy farms (n=4) had an overall prevalence of 74.5% (117/157) and 77.2% (61/79), respectively. The farrowing barn prevalence was comparable to the prevalence in the pre-weaned calves at 81.2% (69/85) compared to 87.5% (35/40). The *C. difficile* prevalence in the human environments, break rooms or houses, were 66.7% (48/72) for the swine operations and 66.7% (26/39) for the dairies. The recovered isolates between people and pigs will be compared using multiple genotyping methods to assess the possibility of zoonotic transmission. A better understanding of *C. difficile* transmission will allow producers to implement measures that may lead to a reduction in animal and human colonization and improvements in animal and human health.

Keywords: *Clostridium difficile*, One Health, farrowing piglets, pre-weaned calves, zoonotic disease

ASSOCIATION OF MILK CESSATION METHOD AND DAILY MILK YIELD AROUND DRY-OFF WITH MAJOR INTRAMAMMARY INFECTIONS AT CALVING. P.N. Gott¹, P.J. Rajala-Schultz¹, G.M. Schuenemann¹, and J.S. Hogan². Department of Veterinary Preventive Medicine¹; Department of Animal Sciences².

Introduction

Dry period prepares mammary glands for optimal milk production and udder health in the following lactation. Increased milk yield near dry-off has been associated with increased risk of intramammary infections (IMI) at calving. Abrupt cessation of milking is widely practiced even though gradual cessation has been shown to improve udder health. The **objective** of this study was to evaluate the impact of milk cessation method and daily milk yield near dry-off on IMI caused by major pathogens at calving.

Materials and Methods

Cows from four Ohio dairy herds were enrolled 7-14d before dry-off and randomly assigned to either abrupt or gradual cessation of milking. GRADUAL cessation cows were milked once daily for the final week of lactation while ABRUPT cessation cows remained on the farm's normal milking schedule. Aseptic quarter foremilk samples were collected at enrollment (PRE), dry-off (DRY), and within 7d of calving (CALV) to determine IMI status. Association between major IMI at CALV and milk cessation method and daily milk yield before dry-off was evaluated using PROC GLIMMIX in SAS, adjusting for clustering of quarters within cows and cows within herds.

Results and Conclusion

Data from 894 quarters were used for these analyses. Daily milk yield at PRE did not differ between treatment groups ($P=0.3543$), but daily milk yield at DRY was significantly lower in the GRADUAL group ($P<0.0001$). Treatment was not a significant ($P=0.5203$) predictor of major IMI at CALV, however, for every 10-lb increase in daily milk yield at DRY, the odds of major IMI at CALV increased 51% ($P=0.0045$).

Treatment significantly reduced milk yield before dry-off and increasing milk yield at DRY was significantly associated with increased risk of major IMI at calving. In conclusion, gradual cessation of milking is a viable method to reduce milk production prior to dry-off and IMI at calving.

Keywords: dairy, dry period, milk cessation method, intramammary infection

EVALUATION OF CANINE-SPECIFIC MINOCYCLINE AND DOXYCYCLINE SUSCEPTIBILITY BREAKPOINTS FOR METHICILLIN-RESISTANT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* ISOLATED FROM DOGS

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Background: Clinical and Laboratory Standards Institute (CLSI) human tetracycline breakpoints to predict minocycline and doxycycline susceptibility of *Staphylococcus pseudintermedius* (SP) isolates from dogs are not appropriate because they do not meet pharmacokinetic/pharmacodynamic data using a standard dose. New breakpoints have been approved for doxycycline and proposed for minocycline. Revised breakpoints are four dilutions lower than tetracycline breakpoints, providing a more conservative standard for classification of isolates.

Hypothesis/Objectives: The objectives of this study were to measure MICs of minocycline and doxycycline of 100 canine methicillin-resistant SP clinical isolates, compare their susceptibilities to minocycline and doxycycline based on current and revised standards, and document their tetracycline resistance genes.

Methods: E-test strips were used to determine MICs. PCR was used to identify tetracycline resistance (*tet*) genes.

Results: Using the human tetracycline breakpoint of $MIC_{\leq 4}$ $\mu\text{g/mL}$, 76 isolates were susceptible to minocycline and 36 isolates were susceptible to doxycycline. In contrast, using the proposed minocycline breakpoint ($MIC_{\leq 0.25}$ $\mu\text{g/mL}$) and approved doxycycline breakpoint ($MIC_{\leq 0.125}$ $\mu\text{g/mL}$), 31 isolates were susceptible to both minocycline and doxycycline. Thirty-one isolates carried no *tet* genes, two had *tet(K)*, and 67 had *tet(M)*.

Conclusions and clinical importance: Use of the human tetracycline breakpoints misclassified 45 and five of the isolates as susceptible to minocycline and doxycycline, respectively. PCR analysis revealed that 43/45 isolates classified as susceptible to minocycline and 5/5 isolates classified susceptible to doxycycline possessed the tetracycline resistance gene, *tet(M)*, known to confer resistance to both drugs. These results underscore the importance of utilizing the proposed minocycline and approved doxycycline canine breakpoints in place of human tetracycline breakpoints.

Keywords: minocycline, doxycycline, methicillin-resistant *S. pseudintermedius*

EXTENDED-SPECTRUM CEPHALOSPORIN RESISTANT NONTYPHOIDAL SALMONELLA RECOVERED FROM CLINICAL HUMAN INFECTIONS IN OHIO, USA. C. King¹, D. Mollenkopf¹, D. Mathys¹, S. Kim¹, R. Adams¹, E. Brandt², T. Wittum¹.
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In the US, nontyphoidal *Salmonella* are a common foodborne zoonotic gastroenteritis pathogen. Invasive *Salmonella* infections caused by extended-spectrum cephalosporin resistant (ESCR) phenotypes are more likely to result in treatment failure and adverse health outcomes, especially in severe pediatric *Salmonella* infections where the extended-spectrum β -lactams are the therapy of choice.

To estimate the prevalence of ESCR *Salmonella* in clinical human isolates received between January, 2012 and June, 2014 at the Ohio Dept. of Health, we screened 3,175 cryopreserved isolates on Mueller-Hinton agar containing 2 μ g/ml cefotaxime. Of these, 70 *Salmonella* isolates (2.2%) expressed reduced susceptibility to 3rd generation cephalosporins. This subset was further screened on Mueller-Hinton agars containing 16 μ g/ml cefoxitin, 8 μ g/ml cefepime, and 2 μ g/ml meropenem to identify the *bla*_{CMY}, *bla*_{CTX-M}, and carbapenem resistance phenotypes, respectively. A majority of these isolates (n=59, 84%, 1.9% overall) expressed the *bla*_{CMY} phenotype with an additional 6 isolates (8.6%, 0.2% overall) having the *bla*_{CTX-M} phenotype, and 7 isolates exhibiting only cefotaxime reduced susceptibility. No *Salmonella* isolates were resistant to carbapenems.

The 59 phenotypic *bla*_{CMY} *Salmonella* isolates represented 15 serotypes, most commonly *S. Typhimurium* (n=14, 24%), *S. Newport* (n=9, 15%), and *S. Dublin* (n=7, 12%). The *bla*_{CTX-M} phenotype included *S. Saintpaul* (n=3, 50%), *S. Agona* (n=1, 17%), and *S. Enteritidis* (n=1, 17%). Of the isolates expressing only reduced susceptibility to cefotaxime, the majority were *S. Enteritidis* (n=5, 72%).

Most *Salmonella* infections are the result of zoonotic foodborne transmission from a livestock reservoir where extended-spectrum cephalosporins are commonly used. Our observed prevalence of ESCR *Salmonella* causing clinical human illness (2.2%) was lower than NAHMS Surveillance ceftiofur resistant *Salmonella* statistics reported for livestock - - Swine, 2006 (14.6%), Dairy, 2007 (4.7%), and Cattle Feedlot, 2011 (7.7%).

Keywords: *Salmonella*, foodborne pathogens, extended-spectrum β -lactams, carbapenem, antimicrobial resistance

AMPHIBIAN MICROBIOMES AS INDICATORS OF INDIVIDUAL AND ENVIRONMENTAL HEALTH. S Leyman¹, B Wolfe¹, P Mouser²

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Amphibians depend on their cutaneous microbial community as a first line of immune defense against disease. However, very few studies have been performed to characterize the bacterial genera found on the skin of different amphibian species and under different water quality conditions. The goal of this study was to classify the bacterial genera present on the skin of two *Lithobates* species living in lakes of highly variant water characteristics on a reclaimed surface mine. A second objective was to develop a baseline frog microbiome library on the site prior to shale gas exploration in order to monitor microbiome changes in association with environmental disturbance. Northern green frogs (*Lithobates clamitans melanota*) and American bullfrogs (*Lithobates catesbeiana*) were caught from 10 different lakes on *the Wilds* in Cumberland, OH. Skin swabs were collected following a sterile saline solution rinse for bacterial characterization and to test for *Batrachochytrium dendrobatidis* (*Bd*), the etiologic agent of amphibian chytridiomycosis. Pharyngeal swabs were taken to test for ranavirus, another emerging disease of amphibians, and blood samples were collected to assess the heterophil-lymphocyte ratio as an indicator of stress. Water quality parameters were documented and water samples collected for chemical analysis at the time of frog capture for each site. The DNA was extracted from the bacterial swabs and sequenced using 454 pyro-sequencing. The sequences were analyzed in QIIME and measures of beta-diversity, defined as species diversity differences between habitats, were correlated with water quality parameters. At least one frog from each site tested was positive for *Bd*, but no frogs were positive for ranavirus. Water quality among sites varied with regard to pH (4.10 to 8.66), conductivity (137.5 μ S/cm to 3.51 mS/cm), ionic content, and dissolved organic carbon (0.13 mg/L to 11.7 mg/L). Our study identified over 300 different genera of microbes representing 68 orders present on frogs on this site. The most abundant genera isolated were Moraxellaceae and Sphingobacteriaceae. Site was found to drive the beta-diversity of the cutaneous microbiomes of the *Lithobates* species between sites. Dissolved oxygen content, pH, conductivity, cations (Na^+ , K^+ , Ca^{2+} , Mg^{2+}), phosphorous and arsenic levels were primary drivers of beta diversity of microbiomes.

Keywords: Amphibians, microbiomes, 454-pyrosequencing, environmental quality

EFFECT OF FLAVOMYCIN (FLAVOPHOSPHOLIPOL) ON THE ACQUISITION AND LOSS OF ANTIMICROBIAL RESISTANCE IN MULTIDRUG-RESISTANT *SALMONELLA ENTERICA* SEROVAR ENTERITIDIS IN BROILER CHICKENS

K. Lim, M. El-Gazzar, I. Nana, W. Gebreyes of the Department of Veterinary Preventive Medicine

Objective: Nontyphoidal *Salmonella* is a significant foodborne, zoonotic pathogen worldwide. The spread of plasmid-mediated antimicrobial resistance in multidrug-resistant (MDR) *Salmonella* Enteritidis is an important problem to the food animal industry and public health. In this study, we hypothesize that the feed additive, flavophospholipol has a positive effect in reducing the incidence of antimicrobial resistant bacteria in the guts of broilers by 1) reducing transfer of conjugative plasmids and 2) curing of plasmids.

A randomized controlled challenge study was designed with 270 day-old broiler chicks allocated to eight treatment groups with control (basal diet with no additive), 10ppm or 64ppm of flavophospholipol in feed. Isolation of *Salmonella* using the RV/XLT4 conventional method and antimicrobial susceptibility testing using the Kirby Bauer method were conducted to determine the acquisition or loss of antimicrobial resistance. Pulsed Field Gel Electrophoresis (PFGE) was used to confirm the absence of contamination by extrinsic *Salmonella* strains.

Results: The odds of isolating ampicillin-resistant transconjugant salmonellae from the control group to that of treatment group (flavophospholipol 64 ppm) ranged from 1.22 – 10.31 at four sampling time points. Ampicillin-resistant transconjugant salmonellae in the control group were 2.53 – 6.03 times as likely to be streptomycin-resistant and 2.00 – 7.02 times as likely to be tetracycline-resistant compared to treatment group (flavophospholipol 64ppm) in the same period. The odds that transconjugant salmonellae isolated from the control group acquired resistance against third generation cephalosporins compared to the treatment group (flavophospholipol 64ppm) ranged from 15.17 – 24.58 in the same period. Loss of resistance associated with plasmid curing was not observed. PFGE genotyping showed that recovered *Salmonella* isolates were derivatives of challenge strains. The results demonstrated that flavophospholipol 64ppm given in feed have an anti-conjugative effect but no plasmid curing effect on MDR bacteria and aid in minimizing the impact of antimicrobial resistance.

Keywords: Flavomycin; Flavophospholipol; *Salmonella*; Antimicrobial Resistance; Chickens

ENTEROBACTERIACEAE PRODUCING EXTENDED SPECTRUM BETA-LACTAMASES FROM WILD BIRDS ON OHIO DAIRIES.

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Objective: Extended-spectrum beta lactamases confer bacterial resistance to critically important antimicrobials. Livestock are an important reservoir for the zoonotic food-borne transmission of resistant enteric bacteria. Our aim is to describe the potential role of migratory and resident wild birds in the epidemiology of transmissible extended-spectrum beta lactamase-mediated bacterial resistance on dairy farms.

Methods: Using mist nets, we captured and sampled 285 wild migratory and resident birds either immediately outside of or 600 feet away from freestall barns on three Ohio dairy farms. Individual swabs were used to obtain both a cloacal and external surface swab of feathers and feet from each bird. Samples were inoculated into MacConkey broth containing 2 ug/ml cefotaxime and inoculated onto MacConkey Agar with 8 ug/ml of ceftaxime, 4 ug/ml of cefepime, or 2 ug/ml of meropenem to identify the *bla*_{CMY}, *bla*_{CTX-M}, and carbapenemase phenotypes.

Results: Fifty-eight birds (21.40%) produced ceftaxime-resistant isolates, representing the expected phenotype of *bla*_{CMY}, and eight birds (3.0%) produced cefepime-resistant isolates, representing the expected phenotype of *bla*_{CTX-M}, from either their cloacal swab/fecal sample or from their external feather/feet swab. There was no difference in the prevalence of either gene between migratory and resident birds or between the prevalence of *bla*_{CMY-2} phenotype and distance from the barn. However, prevalence of the *bla*_{CTX-M} phenotype was higher among birds sampled immediately outside the dairy barns compared to those sampled 600 feet away.

Discussion: Our results suggest that wild birds can serve as mechanical and/or biological vectors for Enterobacteriaceae with resistance to extended spectrum beta-lactamases. Birds live in close contact with dairy cows and their feed, therefore transmission locally from farm to farm is possible. Finding a similar prevalence in non-migratory birds and those migrating from the Southern US, Central and South America, suggests the potential for regional and intercontinental movement of these genes via birds.

Keywords: Antibiotic resistance, wildlife, vector, livestock

CHARACTERIZATION AND DYNAMICS OF THE RUMEN AND FECAL MICROBIOME OF THE NORTH AMERICAN MOOSE (*Alces alces*) C. McCullough, L. Solden, K. Wrighton, and B. Wolfe. The Ohio State University, College of Veterinary Medicine, Department of Microbiology, and The Columbus Zoo & Aquarium

North American moose, *Alces alces*, have been difficult to maintain in human care due to unique dietary needs and associated health problems, including weight loss, gastroenteritis and diarrhea. In the wild, these signs have been associated with a diagnosis of 'Moose Wasting Syndrome'. The goals of this study were to investigate the value of fecal samples in assessing gastrointestinal health, and to evaluate the success of transfaunation in restructuring the moose rumen microbiome. Our specific objectives were to: a) characterize the microbial populations of the rumen and feces of the moose; b) determine whether fecal microbial populations are reflective of rumen flora; and c) ascertain the fate of cow rumen microbes introduced into the moose rumen.

Baseline rumen (n=6) and fecal (n=3) samples were collected from three juvenile female moose housed at the Columbus Zoo & Aquarium, and the animals were subsequently transfaunated with domestic cow rumen fluid. Weekly recipient rumen (orogastric gavage or rumenocentesis) and biweekly fecal (direct) samples were collected from each moose for five weeks. Genomic DNA was extracted from donor rumen and recipient samples (n=72) and the microbiome was analyzed via Illumina amplicon sequencing of 16s rRNA tags (V4 region).

Our results suggest that fecal microbial communities do not specifically reflect rumen flora. Additionally, the incorporation of transfaunated microbial populations into the rumen flora varies between recipients. The specific fate of transfaunated heterospecific rumen microbes are described and related to predictions of the effectiveness of transfaunation in improving overall moose health.

Key words: *Alces alces*, microbiome, North American moose, rumen, transfaunation

AMYLOIDOSIS IN CHEETAHS, TRANSMISSIBLE?

K. McLean and R. Garabed. Dept. of Preventive Medicine

Amyloidosis is a chronic, protein misfolding disease that causes pathology through the accumulation of misfolded amyloid A protein in visceral organs, often leading to death of the animal. The continued increase of amyloidosis in captive cheetahs (*Acinonyx jubatus*) is of grave concern for the species, yet nothing is definitively known about its mechanism of transmission. One hypothesis is that amyloidosis is transmissible, similar to prion diseases. Transmission models from other prion diseases such as scrapie and chronic wasting disease were used in conjunction with cheetah demographic and disease data collected from the cheetah species survival plan (SSP) pathologist and cheetah stud book, in order to determine the likelihood that amyloidosis is infectious based on past captive transfers and historical amyloidosis infections. The likelihood of infection transmitting between cheetahs housed together was quantified using odds ratios. Odds ratio for mid-level exposure was 0.603 ($p=0.362$, CI: 0.199-1.787) and for low-level exposure was 0.695 ($p=0.555$, CI: 0.203-2.320), so there was no significant effect of exposure to infected cheetahs on development of amyloidosis. To refine the comparison, metapopulation models were designed, populated with demographic and transfer data, and compared with infection data. While our analysis does not disprove the infectious transmission route, transmission is not supported based on our initial findings. Having more participation in the study from zoos around the United States by providing access to post-mortem specimens and results could significantly improve our study and better elucidate the mode by which amyloidosis is transmitted in cheetahs.

Keywords: Zoo, Metapopulation, Prion Disease Model, Odds Ratio

DOG POPULATION SURVEY AND DEVELOPMENT OF A RABIES DISEASE MODEL IN GONDAR, ETHIOPIA. A Medley and J. O'Quin. College of Veterinary

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The prevalence of human rabies cases in Ethiopia is second only to India and yearly incidence is increasing. The Ohio State University in partnership with the University of Gondar, Ethiopia, is developing a program to prevent and control rabies virus in the human and animal populations of the North Gondar Zone. A reduction in human rabies cases can be achieved by decreasing canine rabies through vaccination and population control. To determine the best vaccination rate and effect or synergy of population control, a rabies disease model will be created, enabling the region to focus limited resources more effectively. To create a disease model for Gondar, the dog population was required, and a transect survey was employed in June 2014. In addition to the population estimate, demographics such as sex, reproductive status, age, and information about the human/canine relationship were collected. Thirteen transects representing urban, semi urban, residential, and rural areas of Gondar were walked and the number of dogs recorded. Each transect was walked twice, and every dog photographed as a 'capture-recapture' method which was employed to avoid underestimating the population. Dogs seen on both passes of a transect were counted only once. Using proportions of the representative transects, the dog population was estimated to be 6,500 in the non-rural areas, and 1-2 dogs per household present in rural areas. There were 2.7 males for every female, and 91.6% of the population were young, intact adults, indicating that population control would require a substantial investment. 15.7% of females were pregnant or lactating with 1-3 surviving pups per litter. The number of dogs was found to be most closely associated with human housing, indicating that vaccination efforts should initially be focused in the suburbs. The rabies disease model is currently in process.

Keywords: population survey, rabies, disease model, vaccination, population control, transect survey, capture recapture

EXTENDED-SPECTRUM β -LACTAMASE AND CARBAPENEMASE-PRODUCING ENTERIC BACTERIA RECOVERED FROM PATIENTS AT THE OSU MEDICAL CENTER, 2013

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Today the dissemination of multi-drug resistant bacteria in healthcare environments is of critical concern. The prevalence of Enterobacteriaceae expressing extended spectrum β -lactamase (ESBL) and carbapenemase (CRE) mediated resistance threatens the efficacy of our most vital antimicrobial arsenal. OSUMC patient samples collected for the hospital *Clostridium difficile* surveillance program were screened to estimate the frequency of carriage of ESBL and CRE resistance genes in patient enteric flora. Inpatient diarrheic stool submissions (n=692) received for bacterial culture at the Clinical Microbiology Laboratory at OSUMC between October and December, 2013 were screened. Each submission was aliquoted to a transport swab and couriered to the veterinary sciences laboratory for culture. Each swab was inoculated to MacConkey broth with 2 μ g/ml cefotaxime and incubated overnight at 37°C. This broth was subsequently inoculated to 3 MacConkey agars supplemented with cefepime 4 μ g/ml, cefoxitin 4 μ g/ml, and meropenem 2 μ g/ml to select for the ESBL and CRE phenotypes. These selective cultures yielded 196 isolates (28 %) with reduced susceptibility to cefotaxime that were further characterized biochemically and genetically. Seventeen (2.5%) samples harbored *E. coli* isolates carrying the AmpC *bla*_{CMY-2}. Another 21 (3.0%) samples produced isolates harboring the ESBL *bla*_{CTX-M}: 19 carrying CTX-M-15 and 2 with CTX-M-27. Two (0.3%) samples produced *Klebsiella pneumoniae* isolates expressing carbapenem resistance. Of these, 1 *K. pneumoniae* carried the plasmid mediated carbapenemase NDM-1 while the second isolate harbored KPC-2. Our results show that while the prevalence of CRE is rare in the fecal flora of hospitalized patients, the threat of nosocomial CRE infections disseminated from an enteric flora reservoir exists. The detection of carbapenemase-producing enteric bacteria in this population emphasizes the need for CRE surveillance and patient risk assessment in order to prevent the dissemination of this important resistance genotype.

Keywords: Carbapenemase, Extended spectrum β -lactamase, Enterobacteriaceae, Healthcare

VALIDATING TESTS FOR *NEOSPORA CANINUM* EXPOSURE IN WILDLIFE POPULATIONS WITHOUT A GOLD STANDARD K. Moreno-Torres, B. Wolfe, M. Moritz and R. Garabed. Depts of Veterinary Preventive Medicine and Anthropology

Surveillance of wildlife diseases is crucial to understand and control infectious diseases in human, wildlife and domestic animals. However, validation of laboratory tests that are used to screen diseases in wildlife populations are mainly based on studies in domestic animals. Consequently, the use of these tests on wildlife populations may lead to uncertainty in the estimated population parameters. Fortunately, Bayesian latent class analysis can be used to estimate the prevalence of pathogens and specificity and sensitivity of tests in populations in the absence of a gold standard. We will demonstrate this method by evaluating the accuracy of cELISA kit (VMRD®) used in estimating prevalence in three wildlife populations. The ability to accurately evaluate disease status and prevalence in a population allows us to understand and determine the effectiveness of control programs, especially, in multi-host pathogen systems where integrated management is essential for the coexistence of human, livestock and wildlife populations.

Keywords: Multi-host pathogen systems, *Neospora caninum*, test validation, Bayesian latent class analysis, wildlife populations.

THE IMPACT OF STOCKING DENSITY AT THE FEED BUNK IN DAIRY CATTLE ON FECAL CONCENTRATIONS OF *SALMONELLA* SPP. L.M. Muñoz-Vargas¹, J. Pempek², K. Proudfoot¹, M. Eastridge², and G. Habing¹. ¹Dept. of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, USA, ²Dept. of Animal Sciences, The Ohio State University, Columbus, OH, USA.

Livestock are important reservoirs of non typhoidal *Salmonella* (NTS). However, no research to date has examined the association among stress, stocking density, and NTS shedding around calving. The objective of this study was to quantitatively determine the impact of stress caused by higher stocking density at the feed bunk on the NTS shedding through the calving period in dairy cattle. This longitudinal study included 120 cows from a commercial dairy farm in Ohio. Block randomization was used to assign dry cows into one of four groups with different stocking density conditions at the feed bunk. Study groups included: A) overstocking from 60 to 1, B) 60 to 20, C) 20 to 1, and D) understocked from 60 to 1 d prior to calving. In total, 360 fecal and blood samples were collected at 3 time points relative to calving (-60, -15, and +7 d). Cultures of fecal samples were used to determine the NTS prevalence, and qPCR was used to estimate NTS concentration. Indicators of stress were measured using serum concentrations of non-esterified fatty acids (NEFA), and fecal concentrations of cortisol metabolites. Preliminary results demonstrate that a substantial proportion of cows began shedding NTS closer to the calving day. Overall, 75.0% and 80.6% of cows were culture positive at 60 and 15 d prior to calving, respectively. The prevalence of NTS at -60 and -15 d for each group was, respectively: A) 66.7%, 73.3%, B) 70%, 81.8%, C) 73.3%, 72.2%, and D) 90.0%, 96.7%. The association between fecal concentrations of *Salmonella* and stress measurements will be presented. This study offers an opportunity to understand how stress associated with stocking density influences the ecology of NTS in dairy farms. Data generated from this study could lead to specific farm management strategies to decrease the prevalence of NTS on dairy farms.

Keywords: Non-typhoidal *Salmonella*, dairy cattle, stress, qPCR, stocking density

THE TRANSMISSION OF INFLUENZA A VIRUS IN SWINE AT AN AGRICULTURAL FAIR, 2014. S. Nelson, J. Nolting, J. Edwards, N. Bliss, M. Zentkovich, A. Edmunson, A. Bowman. Department of Veterinary Preventive Medicine

Influenza A virus (IAV) infections in exhibition swine are a public health risk, as evidenced by the 309 cases of swine lineage IAV detected in humans during 2012. Approximately 25% of Midwestern agricultural fairs have IAV infected pigs, but little is known about IAV transmission among the pigs during the course of these fairs. In the present study, all pigs were sampled every day of an eight day fair to study the epidemiology of IAV in this setting. Upon entry to the fair, each pig was tagged, weighed, and evaluated for illness. This was accomplished by corralling the pigs individually through a set of gates during which, a snout wipe was collected from each pig. The wipe was put into 5ml of brain heart infusion broth and frozen at -70°C until rRT-PCR could be performed. During the course of the fair, 2,743 samples were collected from 421 pigs. IAV was detected in 186/419 (44.39%) pigs on day one of the fair, while on day two IAV was detected in only 16/421 (3.79%) pigs. IAV was detected in 44/420 (10.47%) pigs on day three, in 99/420 (23.57%) pigs on day four, in 201/416 (48.31%) pigs on day five, 242/341 (71.81%) pigs on day six, 124/160 (78.48%) pigs on day seven, and 149/151 (98.67%) pigs on day eight. These data show the rapidity with which IAV can spread within a population. We hypothesize the decrease in IAV prevalence between day one and two was due to pig snout contact with the gates during weigh-in. This likely resulted in contamination and an increased number of pigs testing IAV positive on day one. These preliminary data support the reduction of exhibition time as a method of decreasing IAV prevalence in the exhibition swine, which would reduce the risk of human exposure to IAV.

Keywords: pigs, influenza A virus, transmission

THE EFFECT OF EXERCISE ON BEHAVIORAL AND PHYSIOLOGICAL MEASURES OF STRESS IN CHEETAHS (*Acinonyx jubatus*) IN HUMAN CARE. E. Puthoff, 1 A. Osowski, 1 B. Baird, 2,3 M. Schook, PhD,2,3 L. Amendolagine, 2 and B. Wolfe, DVM, PhD, Dipl ACZM1,4,5.1Dept. of Veterinary Preventive Medicine, The Ohio State University, College of Veterinary Medicine, Columbus, OH; 2The Cleveland Metroparks Zoo, Cleveland, OH; 3Dept. of Biology, Case Western Reserve University, Cleveland, OH; 4The Columbus Zoo & Aquarium, Powell, OH; 5The Wilds, Cumberland, OH

In human care, the cheetah (*Acinonyx jubatus*) experiences low reproductive success and a high prevalence of certain diseases, challenges which have both been attributed to high stress levels.¹ Studies have suggested that running and hunting behaviors may reduce stress, and zoological facilities are beginning to provide an outlet for these behaviors through exercise programs, in which cheetahs chase a moving lure on a designated course.² The purpose of this study was to analyze the impact of this lure course exercise on physiological and behavioral measures of stress in cheetahs from the Columbus Zoo and Aquarium (CZA), the Wilds, and Cleveland Metroparks Zoo (CMZ). Cheetahs were divided into treatment (exercised on a lure course; n=4) and control (not exercised; n=10) groups. Fecal samples were collected every other day from June through August, and fecal glucocorticoid metabolites (FGM) were measured by enzyme-immunoassay. Thirty 30-minute focal observations, balanced between morning, midday, and afternoon time periods, were conducted for each cheetah when the animal was not participating in formal exercise. Behavioral observations indicated that cheetahs involved in exercise programs displayed significantly more mobile behaviors (p=0.02) and non-stereotypic locomotion (p=0.05) than did non-exercised animals. FGM enzyme-immunoassays are complete, and results from the analyses are pending, expected late March 2015. These data, in conjunction with associated studies, lend insight into the impacts of exercise on the welfare of cheetahs in human care.

Key words: *Acinonyx jubatus*, behavior, cheetah, glucocorticoid, exercise, stress

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POPULATION STUDY OF PLASMA LEVELS OF GAMMA AMINO-BUTYRIC ACID (GABA) IN NON-RACING PERFORMANCE HORSES. S Schumacher DVM, M

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The primary inhibitory neurotransmitter in mammals is gamma amino-butyric acid (GABA) which exerts its primary action in the central nervous system (CNS). In the racehorse industry, exogenous GABA has been used with the intention to minimize a loss of energy through nervous behavior in the hours leading up a race. In the last several years, the use of exogenous GABA has been documented in show horses participating in breed shows and disciplines outside of racing. The administration of this inhibitory neurotransmitter violates the rules of the United States Equestrian Federation (USEF) and the Fédération Equestre Internationale (FEI). As for all endogenous substances, the regulatory challenge is to develop a threshold value for which any excessive amount is detected is definitive for an exogenous administration. Previous work has been published describing the circulating plasma levels of GABA in thoroughbred racehorses using pre-race blood samples. The goal of this population survey was to describe the normal circulating plasma levels of endogenous GABA in non-racing horses. Blood samples were obtained by private veterinary practitioners from 130 horses. Age of the horses ranged from 3 to 30 and included mares, geldings and stallions. A gas chromatography-mass spectrometry (GC/MS) method was used to analyze plasma GABA concentrations. The mean value \pm SD for GABA was 22.37 ± 8.1 ng/ml. Normal distribution could be obtained following log transformation or following outlier identification and removal. One outlier was removed using Tukey's method. The mean value \pm SD for GABA following outlier removal was 22.1 ± 7.48 ng/ml. There was no control over individual time of day for sample collection so the impact of diurnal variations was not assessed. As a result of the work done on racing thoroughbreds, the current recommended plasma threshold for GABA is 190ng/ml. A more suitable threshold for non-racing performance horses may be determined from the results of this study.

Keywords:

PREVALENCE OF STAPHYLOCOCCUS AUREUS IN SWINE EXHIBITED AT AGRICULTURAL FAIRS IN OHIO. A.V. Sharova¹, J. van Balen², A.S. Bowman², A.E. Hoet^{1,3}.

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Worldwide reports of methicillin-resistant *Staphylococcus aureus* (MRSA) indicate a novel livestock-associated strain (LA-MRSA) is becoming widely prevalent in commercial swine, particularly at slaughter. Possible risk factors for swine colonization at slaughter include transport stress and commingling of pigs from different herds during lairage. Exhibition swine in the USA experience similar conditions when appearing at agricultural fairs: farm to fair transportation stress, commingling with multisource swine, and high human-animal contact. This study's objectives were to determine the prevalence and characteristics of *S. aureus* in fair exhibition swine in Ohio over two years, and to identify potential risk factors of fairs for MRSA-positive pigs. We expected the exhibition swine risk factors to result in high prevalence of MRSA. A total of 200 pigs were enrolled in the active surveillance program, divided equally between 2013 and 2014. Twenty randomly selected swine were screened for *S. aureus* at each of five fairs. In addition, an epidemiologic survey was completed by each fair's manager. *S. aureus* was isolated and identified by pre-enrichment culture and standard microbiology procedures. Of 160 pigs sampled at four county fairs, 0.006% were *S. aureus*-positive overall. Of 40 pigs sampled at slaughter from the State Fair, 72.5% were *S. aureus*-positive overall. All isolates were susceptible to methicillin. Additional antibiotic susceptibility testing identified high rates of resistance to tetracycline (96.7%), erythromycin (90%), clindamycin (90%), and ampicillin (76.7%). Furthermore, 90% of *S. aureus* isolates were multidrug resistant to three or more classes of antibiotics. The majority (29/30) of *S. aureus* isolates originated from pigs exhibited at the largest fair sampled. Results from this study indicate high prevalence of *S. aureus* at the State Fair. With 95 agricultural fairs held annually in Ohio alone, more research is needed to determine whether *S. aureus* is a concern at large fairs nationwide.

Keywords: *Staphylococcus aureus*, agricultural fairs, exhibition swine.

**PHENOTYPING AND GENOTYPING METHICILLIN-RESISTANT
STAPHYLOCOCCUS PSEUDINTERMEDIUS IN A VETERINARY TEACHING**

HOSPITAL. R.C. Soltys, T. Yeates, J. Van Balen Rubio, A.E. Hoet, Dept. of Veterinary Preventive Medicine

Staphylococcus pseudintermedius is part of the normal flora of cats and dogs. Over the past decade, an increasing number of isolates have been found to be resistant to not only beta-lactams but also other classes of antimicrobials. Methicillin-Resistant *S. pseudintermedius* (MRSP) carries the *mecA* gene, conferring resistance to all beta-lactam antibiotics, and also possibly multidrug resistance. Therefore, nosocomial MRSP infections represent a significant threat to the health of hospitalized animals. Due to close contact between patients, veterinary personnel, and the environment, cross-contamination and circulation of MRSP is highly likely within veterinary hospitals. However, little research has been done to understand the epidemiology of MRSP in hospitals, especially in regards to the role veterinary personnel and the environment play in introducing and spreading this pathogen. This study is a retrospective analysis of MRSP isolates collected from canine patients at The Ohio State University Veterinary Teaching Hospital (OSU-VTH) between 2007 and 2013. It is part of a larger, ongoing study to characterize the epidemiology and evolution of MRSP over time at the OSU-VTH, including prevalence of MRSP on environmental surfaces and in hospital personnel. It is hypothesized that MRSP strains with shared antimicrobial susceptibility profiles will be detected in both hospital personnel and the environment, as well as in the retrospective isolates. The retrospective isolates were first characterized using a disc diffusion phenotyping protocol to determine antimicrobial susceptibility profiles. They were then analyzed using pulsed field gel electrophoresis (PFGE) to determine genotype, including *mecA* confirmation. Final results are pending.

Keywords: *Staphylococcus pseudintermedius*, PFGE, genotyping, phenotyping, retrospective analysis, epidemiology

WILD BIRDS AS VECTORS FOR *SALMONELLA* ON OHIO DAIRIES

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Wild birds are known to be reservoirs for transmission of *Salmonella* spp. Previous studies have found that *Salmonella* is present sporadically in the intestinal flora of wild birds. Birds are attracted to farms due to the presence of feedstuffs, and the resulting close contact with livestock provides the potential for pathogen transmission. Enteric pathogens such as *Salmonella* can colonize a wide variety of species and thus could easily be transmitted between livestock and wild birds. The symptoms of salmonellosis are potentially severe and can impact animal health as well as profitability of the farm, and so prevention is important.

The goal of this study was to determine if wild birds play a role in the transmission of *Salmonella* as either a mechanical vector by transmitting salmonella on their feet, or as a biological vector by transmitting salmonella through their excrement on Ohio dairy farms. External and cloacal swabs were taken from 346 live wild birds captured with mist nets in close proximity to 3 dairy farms in Ohio. Environmental fecal samples were also collected from the barns at the dairies. Swabs were placed in nutrient broth and incubated for 24 hours at 37°C and then moved to Rappaport Vassiliadis (RV) broth. The RV broth was incubated for 24 hours at 42°C and then streaked for isolation onto XLT4 agar. Identity of bacteria as salmonella was confirmed using standard biochemicals. The cow fecal samples were screened for *Salmonella* in a similar manner.

Of the 346 wild birds sampled, *Salmonella* was isolated from four. *Salmonella* was isolated from wild birds at two of the three locations. Although the dairy cows had a high prevalence of *Salmonella*, this did not directly correlate with the prevalence of *Salmonella* recovery in wild birds that were within 500 yards of the barn. Based on our findings, wild birds likely do not play an important role in the transmission of *Salmonella* on Ohio dairy farms.

Keywords: *Salmonella*; wild birds; cattle; transmission; prevalence

PEDIATRIC EXPOSURES TO VETERINARY PHARMACEUTICALS (1999-2013).

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Introduction: Pet ownership is common in households with children, with more than 50% of these households in the US having pets. Pet contact is associated with a number of health benefits however there are also risks. One minimally studied risk is the inadvertent exposure of children to veterinary pharmaceuticals intended for pets. The goal of this project is to describe the epidemiology of injuries associated with veterinary pharmaceuticals in children ≤ 19 years.

Methods: A retrospective review was conducted on cases managed by the Central Ohio Poison Control Center (COPCC) from 1999 to 2013. Using specified search terms, cases related to exposure of veterinary pharmaceutical products were extracted from the COPC database. Identified case narratives were reviewed. Descriptive statistics were calculated for all variables.

Results: Out of 5298 cases reviewed, 2954 met our inclusion criteria with 1446 (48.9%) involving children ≤ 19 years of age. Nearly half (42.5%) of these exposures involved young children (≤ 5 years). The most common circumstance for exposure was exploratory behavior (e.g., "child got ahold of medication"; 61%). The most common route of exposure was ingestion (92.9%). The majority of exposures were expected to result in no long-term/lasting health effects, although 86 (0.06%) of the cases were managed by a health care facility and three of the cases resulted in moderate health effects.

Discussion: Children were frequently exposed to veterinary pharmaceuticals, with the greatest risk to children ≤ 5 years. Although most cases were not associated with a serious health effects, the chance for significant health risks and financial and emotional stress for families exists when pet pharmaceuticals are available in the homes of young children. Additional attention to veterinary pharmaceutical practices such as providing pet medications in child-resistant containers and including instructions with the medications for use in homes with young children is needed.

Keywords: veterinary medications, pediatrics, poison center, poisoning

RAPID AND ACCURATE DETECTION OF A NOVEL METHICILLIN RESISTANCE GENE, *mecC*, IN *STAPHYLOCOCCUS AUREUS*. A. Wong, J. van Balen, and A.E. Hoet, Department of Veterinary Preventive Medicine

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of skin and soft-tissue infections and nosocomial infections. This significant pathogen is characterized by its resistance to antibiotics commonly used against staphylococcal infections, which limits therapeutic options. As a result, MRSA infections are difficult to control and expensive to treat. It has been known for decades that MRSA drug resistance is mediated by the *mecA* gene, which encodes a variant penicillin-binding protein that is resistant to all beta-lactam antibiotics. Recently, a novel homologue of this gene, *mecC*, was identified in an MRSA isolate from a dairy cattle facility in England. The recent report of the novel *mecC* gene is a public health concern and requires surveillance to determine its prevalence and potential spread in the USA. The goal of this project is to develop a multiplex-PCR assay that is capable of screening *S. aureus* isolates from different sources for the presence of *mecA* and *mecC* genes, while concurrently confirming the bacterial species. We hypothesize that currently circulating *S. aureus* isolates in the USA, which lack the *mecA* gene but are phenotypically resistant to beta-lactams antibiotics, may harbor the novel *mecC* gene. The conditions for the multiplex-PCR reaction will be optimized with several known control isolates, including the original *mecC* MRSA strain. This protocol will allow to simultaneously identify *mecA*, *mecC* and the *Staphylococcus*-genus specific 16S rRNA. The evaluation of isolates, using the standardized and validated multiplex-PCR, from bovine mastitis infections and from milk bulk tanks collected across Ohio will serve as the initial step in the screening MRSA from multiple sources. These pilot studies will aid in understanding the potential presence and prevalence of *mecC* in the USA. Furthermore, the analysis of epidemiological data related to *mecC* carrier isolates will help us to understand the spread of this novel resistance gene.

Keywords: MRSA, multiplex PCR, *mecC*, beta-lactam antibiotics

**IMMUNOLOGY
AND
INFECTIOUS DISEASES**

IMID - 1

THE EFFECT OF SAMHD1 ON HIV-1 GAG PROTEIN SYNTHESIS AND VIRION RELEASE. J. Antonucci Johnson, S. de Silva, C. St. Gelais and L. Wu
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Background: Restriction factors are cellular proteins defined by their ability to inhibit viral infection. Sterile alpha motif and HD domain-containing protein 1 (SAMHD1) is a mammalian protein identified as an HIV-1 restriction factor. Through the hydrolysis of deoxynucleotide triphosphates (dNTPs), SAMHD1 reduces the amount of intracellular dNTPs required for efficient HIV-1 reverse transcription. Recent studies have identified SAMHD1 as a 3' to 5' exonuclease, capable of degrading single-stranded RNAs (ssRNA) including *in vitro* synthesized transcripts of HIV-1 RNA, and blocking HIV-1 infection through its ribonuclease activity. Based on these studies, we hypothesize that SAMHD1 may restrict HIV-1 protein synthesis and virion release through cleavage of viral mRNA.

Methods and Results: We transfected HIV-1 proviral DNA into HEK293T cells alongside a plasmid expressing SAMHD1 or an empty vector control. To determine HIV-1 protein production in the cell and virion release into the supernatant, we measured the levels of HIV-1 Gag and p24 proteins by ELISA and immunoblotting. Our results show that SAMHD1 expression has no effect on intracellular Gag production and viral release from virus producing cells. To test the effect of SAMHD1 expression on the infectivity of newly produced HIV-1, we infected an HIV-1 reporter cell line with viruses generated from HEK293T cells in the presence or absence of SAMHD1. Our results show SAMHD1 expression does not alter the infectivity of newly produced HIV-1.

Conclusions: Overall, our results show that SAMHD1 does not reduce HIV-1 protein synthesis and virion release or affect the infectivity of HIV-1 produced in the presence of SAMHD1. Our data suggest that the exonuclease activity of SAMHD1 may not be responsible for HIV-1 restriction. These results will extend the knowledge of the mechanism by which SAMHD1 restricts HIV-1 infection.

KEYWORDS: SAMHD1, HIV-1, Viral Restriction

IMID - 2

THE EFFECT OF RESPIRATORY SYNCYTIAL VIRUS INFECTION ON EOSINOPHIL LEVELS IN THE LUNG G. Green¹, N. Petroff¹, D. Huey¹, K. La Perle^{1,2}, and S. Niewiesk¹

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The effect of respiratory syncytial virus (RSV) infection on eosinophil levels in the lung remains poorly understood. Eosinophils are of interest during RSV infection for three reasons. First, an anti-viral role has been suggested for eosinophils during RSV infection. Secondly, pulmonary eosinophilia is considered to be a hallmark of “vaccine-enhanced disease” in experimental animal models. Lastly, there is evidence to suggest that RSV infection may be linked to the development of allergic asthma, a hallmark of which is also eosinophilia.

Infection of cotton rats with RSV, measles virus, and influenza virus did not increase eosinophil levels in the lung. These results are consistent with the “textbook” expectation for virus infections. In contrast and again consistent with expectations, *Staphylococcus aureus* infection leads to a marked increase in neutrophils. To determine the effect of RSV infection on pulmonary eosinophil levels in an allergy model, cotton rats were treated with house dust mite antigen and challenged with different combinations of allergen and RSV. Following treatment with house dust mite antigen, pulmonary eosinophil levels were significantly increased. Infection with RSV, however, did not alter the percentage of pulmonary eosinophils. After immunization with formalin-inactivated vaccine and subsequent challenge, a high percentage of eosinophils was induced. However, a part of that increase was due to the presence of cellular proteins in both the vaccine and challenge virus preparation. In summary, RSV infection did not lead to an increase in eosinophils after primary infection and in conjunction with an allergen, but did in a model of vaccine-enhanced disease.

Keywords: Respiratory Syncytial Virus, eosinophils, respiratory infection

IMID - 3

CHRONIC INGESTION OF LOW DOSES OF CADMIUM ALTERS THE GUT MICROBIOME AND IMMUNE HOMEOSTASIS TO ENHANCE ALLERGIC SENSITIZATION. E Kim¹, X Xu¹, SO Opiyo², M Lambert¹, M Lin¹, HE Steiner¹, J Jee¹, E Cormet-Boyaka¹, PN Boyaka^{1,3}

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Low doses of cadmium can be ingested due to its presence in contaminated water and its accumulation in leafy vegetables, fish and grains. Pollutants are believed to contribute to the increased incidence of allergy diseases. We addressed whether chronic ingestion of low doses of cadmium could impact allergic sensitization and thus, favor the development of allergic diseases. Conventional C57BL/6 mice given low doses of cadmium in the drinking water for 28 days exhibited a significant reduction of bacterial diversity, and an alteration of the Firmicutes to Bacteroidetes ratio. This treatment also activated both the canonical and the non-canonical NF- κ B pathway and promoted proinflammatory cytokine and antimicrobial responses in the gut. The effects of cadmium were at least partially independent of the gut microbiome since germ-free C57BL/6 mice subjected to the same treatment developed the same profile of responses although at a lower degree. Finally, conventional mice chronically treated with low doses of cadmium developed higher antigen-specific IgE responses upon oral sensitization with OVA and cholera toxin as adjuvant. Furthermore, upon nasal antigen, cadmium-treated mice developed higher airway allergic response, which were characterized by the increased levels of IL-17 and Th1 responses control mice. In summary, the environmental pollutant cadmium can be a major regulator of gut immune homeostasis and a cause for increased allergic responses at distant mucosal sites.

IMID - 4

SHORT AND LONG TERM EFFECTS OF ANTIBIOTIC TREATMENT ON THE EQUINE FECAL MICROBIOME R. Liepman, J. Swink, G. Habing, P. Boyaka and R. Toribio

Acute colitis is the most common and devastating complication of antibiotic therapy in horses. Fecal culture often fails to identify etiologic agents and has low sensitivity in detecting organisms that are difficult to cultivate. Metagenomics can generate genetic diversity profiles from microbial communities. There are limited data to explain how antimicrobials affect the equine fecal microbiome over time.

Our objectives were to provide a description of the fecal bacterial structures (microbiome) of normal horses, to determine how commonly used antibiotics affect these communities over five days of treatment, and if these changes persisted over 30 days.

Sixteen healthy horses with no history of underlying disease were used. They were randomly assigned to one of four treatment groups (n=4 horses per group). Each group was treated intravenously with antibiotics (ceftiofur, enrofloxacin, oxytetracycline) or saline (placebo) for five consecutive days. Fecal samples were collected daily during treatment. Bacterial DNA was extracted, PCR amplified and analyzed by 454 pyrosequencing of the 16S rRNA gene from baseline, 3, 5, and 30 days post-treatment. Linear mixed models were used to analyze differences between treatment groups and time points.

Overall, 18 phyla were identified in all horses and 19.9% of 16S sequences remained unclassified. Alpha diversity was not significantly altered by treatment or over time, although samples were highly diverse. Community structure between samples changed with most differences observed in *Proteobacteria*, *Firmicutes*, *Bacteroidetes* and *Verrucomicrobia* (P<0.05). Five days of treatment was sufficient to identify changes in fecal microbiota, and these changes were frequently specific to the antibiotic. Ceftiofur and enrofloxacin treatment resulted in a >90% reduction in *Proteobacteria* compared to baseline and other treatments. Ceftiofur and enrofloxacin treatment led to the most significant changes in major phyla over time. Within treatment groups, several changes in phylum structure persisted at 30 days, however, some returned to near baseline.

Keywords: Horse, microbiome, antibiotic associated diarrhea

IMID - 5

IDENTIFICATION OF CGAMP AS AN EFFECTIVE ADJUVANT FOR INDUCTION OF MUCOSAL IGA VIA SUBLINGUAL IMMUNIZATION. T. Martin, J. Jee, H.E. Steiner, and P.N. Boyaka. Dept. of Veterinary Biosciences

Mucosal immunization provides protective immunity both locally through the induction of secretory IgA antibodies (SIgA) and systemically. Cholera toxin (CT) and heat-labile toxin (LT) from *Escherichia coli* have been used as mucosal adjuvants to increase SIgA in animal models. However, adverse effects such as facial paralysis and intestinal fluid accumulation are associated with intranasal and oral administration with these adjuvants. Mucosal immunization via the sublingual route could avoid these adverse effects while still effectively inducing SIgA. However, neither alum nor most of the experimental adjuvants are effective for promoting systemic and SIgA responses by sublingual vaccines. Cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) is a cyclic dinucleotide capable of activating innate immunity by binding to stimulator of interferon gamma genes (STING) on the endoplasmic reticulum. To examine the effectiveness of cGAMP as an adjuvant for sublingual immunization, C57BL/6 mice were immunized with recombinant *Bacillus anthracis* protective antigen (PA) in the presence of cGAMP. Sublingual co-administration of cGAMP elevated antigen-specific serum isotype IgG antibodies, at levels comparable to those achieved by cholera toxin (CT) or CpG oligodeoxynucleotide (CpG ODN) as adjuvants. The later adjuvant also promoted broad SIgA responses in multiple mucosal secretions. In contrast, cGAMP only promoted SIgA antibody responses, which were restricted to the respiratory tract (i.e., only salivary and bronchoalveolar lavages). Finally, analysis of immune cell subsets in oral tissues 2 hours after immunization showed that cGAMP-treated sublingual tissues had higher numbers of B lymphocytes.

Keywords: sublingual, mucosal, immunization, adjuvant, Immunoglobulin A , IgA, cGAMP

IMID - 6

DEFINING THE GUT-MAMMARY-SECRETORY IGA AXIS DURING PORCINE EPIDEMIC DIARRHEA VIRUS (PEDV) INFECTION IN PIGS S.M. Neal^a, F. Chimelo Paim^a, A. Rauf^a, T. Annamalai^a, A.N. Vlasova^a, K. Lager^b, Q. Wang^a, L.J. Saif^a

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Due to the immature immune system of neonatal piglets, passive lactogenic immunity to PEDV, a deadly diarrheal disease of newborn piglets, is critical for protection via the gut-mammary gland-secretory IgA (sIgA) axis. However, gestational age and dose required to stimulate immunity in lactogenic secretions remains elusive. We hypothesize that frequencies of lymphocytes and antibody secreting cells (ASC) trafficking from the gut to the mammary gland in response to PEDV will correlate with sIgA in lactogenic secretions and subsequent passive protection in suckling piglets. At 96 days of gestation, gilts were inoculated with PEDV or media as a control. At parturition, all piglets were PEDV-challenged 3 days post-partum. PEDV-specific IgA and IgG memory ASC were detected in blood of the PEDV-inoculated gilts post-inoculation, but only in the mock-inoculated gilts after piglet challenge (post-contact day 14 and 17, respectively). In colostrum and milk, PEDV-inoculated gilts had higher frequencies of IgA⁺ as well as CD21⁺CD79 β ⁺ (primed and/or activated) B lymphocytes when compared to mock-inoculated gilts. PEDV-specific IgA and IgG ASC as well as B lymphocytes in colostrum and milk were associated with PEDV inoculation of gilts. Mortality rate of PEDV-challenged piglets of the PEDV-inoculated gilt was 0%, but was 87.5% for piglets of the mock-inoculated gilt. These data will be used in future experiments to determine the impact of gestational age on the gut-mammary-sIgA axis and subsequent immune protection in neonatal suckling piglets.

Keywords: porcine epidemic diarrhea virus, lactogenic immunity, gut-mammary gland secretory IgA axis

**SPECIFIC CHANGES IN MURINE ALVEOLAR TYPE II CELL MICRORNA
EXPRESSION RESULT FROM INFECTION WITH INFLUENZA A VIRUS**

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As the 8th leading cause of attributable annual mortality in the USA, influenza A viruses are a significant public health concern. Severe primary influenza can result in acute lung injury (ALI), which is characterized by hypoxemia, pulmonary edema, and impaired lung function. Currently, there are few therapeutic options for patients with influenza A virus-induced ALI. MicroRNAs (miRs) are short, endogenous, non-coding RNA's that regulate expression of multiple genes simultaneously at the post-transcriptional level. miRs play roles in host immunity and cell survival and altered miR expression may contribute to the pathogenesis of other forms of ALI. Influenza A virus infection has been shown to alter miR expression at the whole lung level. However, impact of infection on miR expression in alveolar type II (ATII) cells, which are a primary site of viral replication, has not previously been determined. We identified more relevant miR targets for potential therapeutic intervention by specific analysis of ATII cell miR expression profiles. We infected C57BL/6 mice intranasally with a mouse-adapted strain of H1N1 influenza (A/WSN/33; 10,000 pfu/mouse in 50 µl saline) and confirmed that infection resulted in progressive hypoxemia and lung dysfunction. At 0, 2, and 6 days post-infection, we collected lungs for ATII cell isolation. Lungs were digested in dispase, and the resulting cell suspension was filtered then panned to remove contaminating leukocytes. Microarray analysis indicated that influenza A virus infection of C57BL/6 mice results in a progressive rise in expression of *miR-155* in ATII cells. Inoculation with higher doses of virus resulted in a greater increase in ATII cell *miR-155* expression. Future studies will 1) test the ability of ATII cell targeted lipoplex vehicles to deliver synthetic antagomiR's to inhibit *miR-155* and reduce ALI severity in mice, and 2) determine effects of *miR-155* blockade on the miRnome of ATII cells through expression profiling.

Keywords: Influenza, microRNA, acute lung injury, ATII cell, expression profiling

THE EFFECT OF STRESS ON ECOLOGY OF *NEOSPORA CANINUM* IN BISON

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Neospora caninum is a major cause of abortion in cattle worldwide. Its reactivation has been associated with a predominant Th1 to Th2 cytokine shift. Stress also has been associated with immunosuppression and a Th1/Th2 shift. We hypothesized that chronic stress would cause a Th1 to Th2 shift in cytokines, leading to reactivation causing increased antibody titers. Thirty females were divided into a control and treatment group. The treatment group was subjected to high stock density and herding once a week for 8 weeks. Control group animals had limited human interaction and free-grazing pasture settings. Serum samples were collected before and after the 8-week trial period to evaluate *Neospora* antibody titers and immunologic function. Fecal samples were collected throughout the 8-week study to determine corticosterone and progesterone concentrations. Both control and treatment groups demonstrated a stress response. Both groups also demonstrated a decrease in *Neospora* antibody titers when compared to samples taken two years previous, as well as a decrease in estimated white blood cell counts from the start to the end of the study suggesting immune suppression due to chronic stress. When evaluating individual immune responses, a significant positive correlation between week 8 corticosterone and week 8 antibody titers was calculated ($r= 0.495$, $p=0.0063$), suggesting a Th2 bias due to acute stress from staging and chute manipulation for final sample collection. These findings suggest that chronic and acute stress may cause different effects on the immune function, which could make it more difficult to understand *N. caninum*'s complex pathogenesis.

Keywords: *Neospora caninum*, *Bison bison*, Th1/Th2

THE EFFECT OF PHOSPHORYLATION OF MOUSE SAMHD1 ON RESTRICTION OF HIV-1 AND MURINE LEUKEMIA VIRUS INFECTIONS

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Human SAMHD1 (hSAMHD1) functions as a retroviral restriction factor in non-dividing cells mainly by limiting intracellular dNTP levels to complete viral reverse transcription. Phosphorylation of hSAMHD1 at T592 by cyclin dependent kinase 1 (CDK1) and CDK2 impairs its restriction of HIV-1 infection. As mouse SAMHD1 (mSAMHD1) and hSAMHD1 are highly conserved proteins, it is conceivable that similar mechanisms may regulate their anti-retroviral functions in cells. Our previous work identified that both hSAMHD1 and mSAMHD1 interact with CDK1/2 and cyclin A2. However, it remains unknown whether phosphorylation of mSAMHD1 regulates its restriction of retroviral infection. In this study, we identified phosphorylation of residue T634 in mSAMHD1 by mass spectrometry, and confirmed the phosphorylation in dividing cells using a phospho-specific SAMHD1 antibody. Using dominant-negative mutants, siRNA-mediated knockdowns, or specific inhibitors of CDK1 and CDK2 to treat mSAMHD1-expressing cells, we found decreased levels of T634 phosphorylated mSAMHD1 in cells. Furthermore, we examined the effect of T634 phosphorylation on mSAMHD1-mediated restriction of HIV-1 in differentiated human monocytic U937 cells and murine leukemia virus (MLV) in dividing mouse fibroblast NIH3T3 cells. These cell lines were transduced with lenti-viral vectors to stably express similar levels of mSAMHD1 wild-type (WT), phospho-ablative (T634A) or phospho-mimetic (T634D) mutants. We are confirming the effect of mSAMHD1 T634 phosphorylation on HIV-1 restriction. Interestingly, we found that MLV infection was reduced in NIH3T3 cells expressing mSAMHD1 WT or the mutants (T634A/D) compared to control cells. Our results indicate that phosphorylation of mSAMHD1 at T634 is regulated by CDK1 and CDK2 in cells. MLV infection is restricted by mSAMHD1 in dividing NIH3T3 cells independent of T634 phosphorylation of mSAMHD1, suggesting a novel mechanism of mSAMHD1-mediated MLV restriction.

Keywords: HIV-1, MLV, mouse SAMHD1, phospho-regulation

HETEROZYGOSITY FOR THE F508DEL MUTATION IN CFTR PROMOTES ALTERNATIVE MACROPHAGE ACTIVATION IN MICE FOLLOWING INFLUENZA CHALLENGE. P.S. Woods and I.C. Davis. Department of Veterinary Biosciences and The Ohio State College of Medicine.

Influenza A virus is a readily transmissible respiratory pathogen that remains a significant threat to human health. Severe primary influenza infection can result in development of pulmonary edema and hypoxemia: key features of acute lung injury (ALI). We have shown that influenza-induced ALI in C57BL/6 (WT) mice is associated with increased bronchoalveolar epithelial cell Cl⁻ secretion via the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel. C57BL/6-congenic mice that are heterozygous for the F508del mutation in CFTR (HET mice) exhibit a 50% reduction in both CFTR expression and CFTR-mediated Cl⁻ transport. We subsequently showed that HET mice did not develop ALI following influenza A virus infection. Consequently, we hypothesized that: 1) HET AMs undergo “alternative” activation (M2 polarization) in response to infection; and 2) this M2 polarized AM response is critical to amelioration of influenza-induced ALI in this mouse strain. Compared to WT AMs, Arginase 1 (Arg1) protein expression was higher in HET AMs at 6 d.p.i. HET AMs also expressed less inducible nitric oxide synthase (iNOS) protein at this timepoint, which is characteristic of a M2 phenotype. Additionally, Arg1 enzymatic activity was higher in HET AM lysates. BALF from HET mice contained higher levels of urea and lower levels of nitrate/nitrite (end-products of Arg1 and iNOS metabolism, respectively) than WT BALF at 6 d.p.i. Lower levels of oxidized protein were also evident in HET BALF suggesting minimal production of reactive oxygen species by HET AMs. Interestingly, reciprocal bone marrow transplantation revealed that the HET epithelium is driving M2 polarization. These findings suggest that the anti-inflammatory nature of HET AMs contributes to attenuation of influenza-induced ALI in HET mice. Hence, we propose that, by promoting M2 polarization of AMs, short-term inhibition with CFTR inhibitors may be a novel approach to preventing influenza-induced ALI.

Key Words: influenza, CFTR, macrophage, lung injury

**MOLECULAR
AND
CELLULAR BIOLOGY**

MCB - 1

The Role of Tax-1 and the Alternative NF- κ B and Akt Signaling Pathways in HTLV Transformation. J Al-Saleem¹, M Cherian², H Baydoun², M Kvaratskhelia¹, L Ratner², and PL.Green¹

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Human T-cell Leukemia Virus Type-1 (HTLV-1) is a complex retrovirus infecting 15-25 million people worldwide, and is the etiological agent of a malignancy of CD4+ T cells termed Adult T-Cell Leukemia. By contrast, HTLV-2 is non-pathogenic in humans. Both HTLV-1 and HTLV-2 express related Tax proteins termed Tax-1 and Tax-2, respectively. Studies have revealed that Tax-1 contains a C-terminal PDZ (post synaptic density protein) domain binding motif (PBM) and a central leucine zipper region (LZR), which are absent in Tax-2. Previous studies indicated that these two domains are important for the ability of Tax-1 to activate alternative the NF- κ B signaling pathway. Since Tax-2 is incapable of activating alternative NF- κ B signaling we proposed that Tax-1 activation of the alternative NF- κ B pathway is important for the HTLV-1 pathogenesis. We set out to identify binding partners of Tax-1 that are important for activation of alternative NF- κ B. Using Tax-1 mutants that do not possess the PBM or LZR we identified several potential candidates via a proteomic screen. We plan to utilize siRNA knockdowns to screen these candidates for importance in Tax-1 driven alternative NF- κ B activation. During our analyses we found that deletion of the PBM from Tax-1 did not cause a deficiency, but resulted in an enhancement of alternative NF- κ B activation. With further analysis, we found that Tax-1 PBM is important for the ability of Tax-1 to activate Akt. Tax-1 diminishes the function of PTEN (Phosphatase and Tensin homologue), which inhibits the PI3K-Akt-mTOR pathway. We found that Tax-1, but not PBM deleted Tax-1, competes with PTEN for binding to DLG-1 (Drosophila disk large tumor suppressor), which leads to an increase in Akt activation. These studies suggest that alternative NF- κ B and Akt signaling pathways may explain the differences in HTLV-1 and HTLV-2 pathogenesis. Moreover, these findings suggest a new approach to therapeutics for HTLV-1 diseases.

Keywords: HTLV, Tax, NF- κ B, AKT

MOLECULAR CLONING OF OUTER MEMBRANE PROTEINS OF *NEORICKETTSIA HELMINTHOECA*, THE CAUSATIVE AGENT OF SALMON POISONING DISEASE. K. Bachman, M. Lin, Z. Cheng, Y. Rikihisa. Department of Veterinary Biosciences.

Neorickettsia helminthoeca, the causative agent of Salmon Poisoning Disease (SPD), is an obligate helminth-borne intracellular bacterium that infects reticuloendothelial cells of canids. The disease is acquired through ingestion of salmonid fish infested with the metacercariae stage of the trematode vector that harbors *N. helminthoeca*. The geographic distribution of the snail required to complete the digenetic life cycle of this trematode makes SPD endemic to the Pacific Coast of the United States. Bacteria released from the trematode infect intestinal macrophages and spread throughout the reticuloendothelial system, where they become enclosed in membrane-bound vacuoles. Clinical signs of the disease include fever, anorexia, lethargy, vomiting, ocular discharge, and diarrhea that may contain blood. Current diagnostic techniques include PCR and serology with either immunofluorescence or Western blotting. The high morbidity and mortality rates of the disease signified the need for a more rapid diagnostic test. The purpose of this research is to identify the surface protein antigens of *N. helminthoeca* that elicits an immune response in dogs affected with SPD. Previous research has shown that P51, SSA, NSP1, NSP2, and NSP3 are the immunodominant surface protein antigens of *Neorickettsia risticii*, the causative agent of Potomac Horse Fever that is closely related to *N. helminthoeca* phylogenetically. Using molecular cloning techniques, the orthologs of these surface proteins have been cloned from *N. helminthoeca* genomic DNA, expressed, and purified to homogeneity. The immunoreactivity of these surface proteins will be analyzed using defined SPD immune sera collected from experimentally *N. helminthoeca*-infected dogs by Western blot analysis. Applications of this research will facilitate the development of rapid and specific immunodiagnosis and potential vaccine candidates against *N. helminthoeca*-infection using defined immunogenic proteins.

Keywords: *Neorickettsia helminthoeca*, Salmon Poisoning Disease, Diagnosis, Molecule cloning, Bacterial Outer Membrane Proteins, Recombinant Proteins, Trematode, Western Blotting

CHARACTERIZATION OF STAT3 IN FELINE ORAL SQUAMOUS CELL

CARCINOMA CELL LINES AND TUMOR SAMPLES. M. Brown, M. Bear, T. Rosol, W. Kisseberth, C. London. Dept. of Veterinary Clinical Sciences

Introduction: Signal transducer and activator of transcription 3 (STAT3) plays a critical role in tumorigenesis due to regulation of pathways involved in cell proliferation, survival and angiogenesis. STAT3 has been shown to be dysregulated in many cancers, including head and neck squamous cell carcinoma (HNSCC). Feline oral squamous cell carcinoma (OSCC) is similar to advanced or recurrent HNSCC in that it is poorly responsive to traditional therapies and carries a guarded prognosis. Therefore, investigation into mechanisms of carcinogenesis and development of novel treatments for OSCC is needed. The purpose of this study was to characterize expression and activation of STAT3 in feline OSCC cell lines and tumor samples and to investigate the biologic activity of a STAT3 inhibitor, LLL12, in feline OSCC cell lines.

Results: We evaluated 3 feline OSCC cell lines and one (SCCF2) exhibited high levels of STAT3 phosphorylation and this correlated to sensitivity to LLL12 treatment. Exposure of SCCF2 cells to LLL12 resulted in decreased expression of pSTAT3 and STAT3, apoptosis, inhibition of colony formation and reduced expression of the STAT3 transcriptional target, survivin. In contrast, other STAT3 transcriptional targets, including VEGF and MCL-1, increased after treatment. This was likely due to an LLL12 mediated upregulation of HIF-1 α , which is also drives VEGF and MCL-1 expression. Other feline OSCC cell lines with low basal STAT3 phosphorylation did not exhibit these effects, suggesting that STAT3 inhibition was responsible for the observed findings. Lastly, pSTAT3 immunohistochemistry was performed using a feline OSCC tissue-microarray and demonstrated expression in 48% of samples.

Conclusions: These data demonstrate that LLL12 has biologic activity against a feline OSCC cell line expressing pSTAT3 and that STAT3 represents a therapeutic target in this disease. However, given the upregulation of several STAT3 transcriptional targets following treatment, further investigation into the role of STAT3 in OSCC is warranted.

Keywords: STAT3, oral squamous cell carcinoma, feline

STA-1474, A HEAT SHOCK PROTEIN 90 INHIBITOR, HAS POTENT ANTITUMOR ACTIVITY IN *IN VITRO* MODELS OF CANINE LUNG CANCER

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The prognosis for canine advanced lung cancer remains poor and new treatments are needed. Heat shock protein 90 (HSP90) is an ATPase dependent molecular chaperone that is essential for posttranslational maturation and stability of client proteins, many of which are important for the proliferation of cancer cells. We investigated the activity of STA-1474, a HSP90 inhibitor, in two canine lung cancer cell lines. Relative viability, proapoptotic effects and the half-maximal inhibitory concentrations (IC₅₀) were determined after exposure to three chemotherapeutic agents and three small molecule inhibitors (STA-1474, crizotinib and torceranib phosphate). With respect to currently used cytotoxic chemotherapeutics, treatment of the BACA cell line with vinorelbine achieved the lowest IC₅₀ (0.729 μM). However, when the CLAC cell line was treated with increasing concentrations of vinorelbine for 72h, an IC₅₀ was never reached. STA-1474, inhibited growth and induced apoptosis of both cell lines in a dose-dependent manner. The IC₅₀ for 72h treatment with STA-1474 was 0.08 and 0.11 μM for BACA and CLAC, respectively. STA-1474 used at a biologically achievable dose downregulated protein expression of IGF-Irβ, EGFR, HER2, p-Akt, p-mTOR, and p-MAPK proteins in the CLAC line and HER2, p-Akt and p-MAPK in the BACA line. Both cell lines not only grow as adherent monolayers but also formed *in vitro* spheroids within three days. The IC₅₀ of STA-1474 for the BACA spheroid model was higher than the monolayer culture (0.348 μM vs. 0.168 μM) and the IC₅₀ for the CLAC spheroids could not be calculated. Seventy-two hour treatment of cancer-associated fibroblasts with STA-1474 resulted in a dose dependent decrease in relative viability with a low IC₅₀ of 0.28 μM. The activity profile of STA-1474 provides preliminary evidence that this compound is superior to the currently available treatments and may offer an effective therapeutic opportunity to manage the canine lung cancer patient.

Keywords: Canine, Lung cancer, HSP90, STA-1474, Ganetespib, Torceranib phosphate, Chemotherapy, Small Molecule Inhibitors

THE ROLE OF GASTRIN-RELEASING PEPTIDE RECEPTOR (GRPR) SIGNALING IN PROSTATE CANCER. S. M. Elshafae¹, H. Ding², W. P. Dirksen¹, M. Tweedle² and T. J. Rosol¹. Dept. of Veterinary Biosciences¹, The Ohio State University, Columbus, OH, USA and Dept. of Radiology², Wexner Medical Center, The Ohio State University, Columbus, OH, USA.

The gastrin-releasing peptide receptor (GRPR) is a cancer-associated antigen that is upregulated in prostate cancer (PCa) and other solid tumors of the mammary gland, lung, head and neck, colon, uterus, ovary and kidney. GRPR is highly expressed in both prostate intraepithelial neoplasia (PIN) and invasive prostate carcinoma. Also, it has been found to be upregulated in the majority of lymph node metastases and 50-70% of bone metastases in prostate cancer. However, little is known about its role in prostate cancer. This study explored the effects of the GRP analogue, bombesin (BB), on the growth of the canine prostate cancer cell line (Ace-1) and whether GRPR signaling has an important role in prostate cancer metastasis. The effect of BB on cell proliferation, migration and epithelial-mesenchymal transition (EMT) gene expression was examined using a cell proliferation assay, a wound healing assay and real-time RT-PCR. Our *in vitro* study revealed that GRPR signaling promoted cell growth and enhanced the migration and invasion of the tumor cells. In addition, activation of GRPR receptors upregulated epithelial-to-mesenchymal transition (EMT) markers (Twist, Snail and Slug) and downregulated other epithelial markers (E-cadherin and B-catenin). Blocking of GRPR signaling induced the mesenchymal-to-epithelial transition (MET) phenotype through upregulation of E-cadherin and downregulation of Vim and Snail. These data suggest that GRPR signaling is important in prostate cancer metastasis and targeting GRPR signaling may be a promising strategy for treatment of prostate cancer. Future studies will identify the downstream molecules of GRPR signaling pathway in prostate cancer.

Keywords; Prostate cancer, GRPR, EMT, Metastasis

THE DEXH-BOX HELICASE RHA/DHX9 SELECTIVELY REGULATES PATHOGENIC RETROVIRAL GENE TRANSLATION BY A NOVEL CAP-DEPENDENT

MECHANISM. S. Fritz and K. Boris-Lawrie. Department of Veterinary Biosciences.

Retroviruses are known to exploit complex posttranscriptional mechanisms to facilitate their infectivity and pathogenicity. We identified the DEXH-box helicase RNA helicase A (RHA/DHX9) as a critical host factor important for the translation control of pathogenic retroviruses that infect animal and human hosts, including avian, simian, and the human pathogenic retroviruses HIV-1 and HTLV-1. This targeted translation control results from the select recognition and binding of RHA to a distinct RNA element, termed the posttranscriptional control element (PCE), in the 5' leader of these viral mRNAs. Together RHA-PCE association facilitates robust ribosome loading and viral protein production. An outstanding question is the mechanistic role of RHA in this process: how is RHA interacting with the translation machinery to facilitate efficient viral protein production?

Our preliminary data supported a role for RHA in cap-dependent initiation, the first and rate-limiting step of translation. Cap-dependent initiation is a highly choreographed process dependent upon cap-associated protein factors, either the predominant eIF4F or the non-canonical CBP80/20 protein complexes, to recruit the ribosome and stimulate translation. We hypothesized that the association of RHA with these cap-associated initiation factors was important for its mechanistic role in this process. Through the use of extensive cellular co-immunoprecipitation assays we identified a novel cellular interaction between RHA and the non-canonical CBP80/20 cap initiation complex. Polysome profiling validated a functional role for RHA-CBP80/20 association in targeted translation control. Further translation initiation analysis revealed significance for RHA-CBP80/20 association in engaging RHA within the mechanistic process of translation initiation. Our identification of a RHA-CBP80/20 translation complex distinguishes RHA from related DEXH-box helicases DHX29 and DDX3, which function in translation initiation of other viral families via interaction with eIF4F. Together the results from this study have identified a novel RHA-CBP80/20 cap translation complex that is selectively co-opted by pathogenic retroviruses to facilitate their protein production.

Keywords: RNA helicase A (RHA/DHX9), translation regulation, retrovirology, cancer biology

GROWTH FACTOR SIGNALING AND MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) ACTIVATION AS POTENTIAL UPSTREAM CELLULAR EVENTS IN A SEPSIS-RELATED LAMINITIS MODEL. Authors: A.K. Gardner¹, C.S. Kelly¹; A.W. van

Eps²; M.R. Watts¹, J.K. Belknap^{1*}

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Sepsis-related laminitis (SRL), an often fatal sequela in critical equine patients secondary to endotoxemia and sepsis-potentiating diseases, appears to be due to aberrant laminar cell signaling reportedly involving inflammatory and possibly other signaling pathways. The only documented effective treatment for sepsis-related laminitis is regional deep hypothermia (RDH, foot submerged in ice water). Laminitis occurs when there is disruption of the laminar basement membrane and hemidesmosome dissolution, resulting in cell to cell dysadhesion. Mitogen-activated protein kinases (MAPKs), activated in inflammation or downstream of growth factor signaling are therapeutic targets for many disease processes. Our objectives were to assess MAPK signaling in laminar tissue in a model of sepsis-related laminitis, and to determine the effect of RDH on MAPK signaling. Laminar concentrations of MAPKs were assessed from two groups of horses receiving a carbohydrate-overload with samples collected at different time points versus a control. Another set was taken from a carbohydrate-overload model where one front limb was treated with RDH while the other remained at ambient temperature. Laminar concentrations and cellular localization of the MAPKs and growth factor-associated signaling proteins were assessed. Whereas no change in laminar p38 MAPK was found in the CHO models, laminar concentrations of growth factor-related signaling molecules including the phosphorylated/activated MAPK, extracellular signal-regulated kinase (ERK) 1/2 and its downstream effector ribosomal protein S6 (RPS6) were increased ($p < 0.05$) at the onset of laminitis. Hypothermia did not inhibit ERK 1/2 or RPS6 activation. Signaling related to growth factor-related pathways should be further investigated in SRL, especially since it has been implicated in cell-cell dysadhesion and upregulation of the ERK 1/2 pathway.

Keywords: equine, laminitis, sepsis, growth factor signaling, mitogen-activated protein kinase (MAPK)

FELINE MAMMARY CANCER, NOVEL NUDE MOUSE MODELS AND MOLECULAR CHARACTERIZATION OF VASCULAR INVASION. B. B. Hassan^{a,b}, J. K. Simmons^a, W. P. Dirksen^a, S. M. Elshafae^a, S. M. Sokkar^b and T. J. Rosol^a. ^a Depart. of Veterinary Biosciences, The Ohio State University, Columbus, OH, USA and ^b Depart. of Pathology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

Feline mammary carcinoma is similar to human breast cancer in the age of onset, incidence, histopathological features, biologic behavior, and pattern of metastasis. The annual incidence of feline mammary neoplasia was estimated at 13-25 per 100,000 female cats. About 85%- 93% of feline mammary tumors are malignant and Siamese cats appear to have a 2-fold increased risk. Molecular characterization of invasion and metastasis genes in feline mammary cancer was conducted using six fresh-frozen primary feline mammary carcinomas, two subcutaneous feline mammary cancer xenografts and six feline mammary cancer cell lines namely; FMC-m, FON-p, FON-m, FKN-p, FYM-p and FNN-m. The primary cancer tissues were derived from cats of different breeds. The feline mammary tumor cell lines were established from primary and metastatic lesions in feline patients with spontaneous mammary cancers. The goal of this study was to evaluate the effects of injection site on tumor growth and metastasis in vivo and to characterize the molecular features of feline mammary cancer by the expression of genes responsible for lymphangiogenesis, angiogenesis, tumor progression and lymph node metastasis. Two feline mammary cancers were injected subcutaneously, while feline mammary tumor cell lines were injected by 3 different routes (subcutaneous, intratibial and tail vein injection) into nude mice. Tumors were monitored and confirmed with gross necropsy, histopathology and quantitative real time RT-PCR (qRT-PCR). The histologic appearance of the subcutaneous xenografts resembled the primary tumors. The invasion and metastasis genes that were highly expressed in all samples, included PDGFA, PDGFB, PDGFC, FGF2, EGFR, ERBB2, ERBB3, VEGFD, VEGFR3 and MYOF.

Key words: Mammary cancer, Metastasis, Lymphangiogenesis, Cat.

THE HISTONE DEACETYLASE INHIBITOR AR-42 SUPPRESSES TUMOR GROWTH IN MOUSE MODELS OF PANCREATIC CANCER

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Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer death in the United States. Prognosis remains dismal, with a 5-year survival of less than 6% for all stages. However, the median survival still remains at approximately 6 months due to inherent or acquired drug resistance to cytotoxic agents. This study was aimed at evaluating the efficacy of AR-42, a novel histone deacetylase (HDAC) inhibitor developed in our laboratory and currently in clinical trials, in suppressing tumor growth in PDAC. HDAC inhibitors have been shown to induce growth arrest, differentiation, and apoptosis in multiple types of human cancer cells. AR-42 demonstrated potent anti-proliferative effects on the growth of six human pancreatic cell lines (AsPC-1, COLO-357, Panc-1, MiaPaCa-2, BxPC-3, and SW1990) as measured by MTT assay. Growth suppression in AR-42 treated cells was associated with dose-dependent modulation of proliferation and apoptotic markers, including reduced levels of the apoptotic regulators phospho-AKT and BCL-X_L, as well as increases in the pro-apoptotic markers BAK and PARP cleavage. Hallmark features of histone deacetylase inhibition, including up-regulation of cyclin-dependent kinase p21 and hyperacetylation of histone H3 were also observed. The *in vivo* efficacy of AR-42 was demonstrated in a subcutaneous AsPC-1 tumor xenograft mouse model and a transgenic (KrasLSL.G12D/+ P53 flox/flox Pdx-cre(+)) mouse model of pancreatic cancer. Mice were treated with AR-42 at 50mg/kg by oral gavage every other day, resulting in suppression of tumor burden in the xenograft and transgenic models by 57% and 42%, respectively, versus vehicle control. Tumor suppression in the AsPC-1 xenograft tumors was associated with HDAC inhibition, increased apoptosis, and inhibition of proliferation. These results suggest that the use of AR-42 represents a therapeutically promising strategy for the suppression of tumor growth in pancreatic cancer.

Keywords: pancreatic cancer, PDAC, histone deacetylase inhibition

SUPPRESSION OF BREAST CANCER STEM CELLS IN THE MMTV-HER2/NEU MOUSE MODEL BY THE HDAC INHIBITOR AR-42

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In many types of cancer, a self-renewing subset of the tumor population described as cancer stem cells is linked to tumor recurrence, drug resistance, and metastasis. Our preliminary data have shown that treatment of human triple-negative breast cancer cell lines MDA-MB-231 and SUM 159 with histone deacetylase (HDAC) inhibitors AR-42 and vorinostat caused a dose-dependent downregulation of cancer stem cell biomarkers. Experiments using pan-HDAC inhibitors, class-specific HDAC inhibitors, the HDAC8-selective inhibitor PCI-34051, and individual HDAC isoform knockdown suggest that HDAC8 is responsible for this phenomenon. Thus far, there is no established mouse model for investigating the response of breast cancer stem cells in chemotherapeutic and chemopreventive studies. Mounting evidence suggests that a breast cancer stem cell subpopulation exists in the MMTV-Her2/neu mouse, and that these cells may be most similar genetically to luminal progenitor cells of humans. However, to date, this model has not been used in a prospective study for therapies targeted at the cancer stem cell subset. Advantageously, the MMTV-Her2/neu mouse has been well-characterized in the literature as a model of human breast cancer, and is superior to other xenograft or syngeneic models in that it is immunocompetent and that tumors arise in a stochastic manner, similar to human cancers. We have utilized NT5 mammary tumor cells derived from the MMTV-Her2/neu mouse to predict the *in vivo* effects of HDAC inhibition on breast cancer stem cells in this model. *In vitro* experiments demonstrated downregulation of stem cell biomarkers and decreased mammosphere formation. Notch1 downregulation by AR-42 and PCI-34051 was reversed by proteasome inhibition. An allograft study in mice treated with dietary AR-42 showed marked tumor suppression. Our data support the hypothesis that histone deacetylases, specifically HDAC8, are directly involved in regulating the breast cancer stem cell population and suggest translational potential for new therapeutic strategies.

Key words: breast cancer stem cells, Notch, triple-negative, MMTV-Her2/neu, mouse models, HDAC inhibitor, AR-42, chemoprevention

SAMHD1: A PUTATIVE TUMOR SUPPRESSOR IN CUTANEOUS T CELL LYMPHOMA. R. Kohnken¹, S. de Silva¹, A. Mishra², P. Porcu³, and L. Wu^{1,2}.

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The mechanisms underlying initiation and progression of cutaneous T cell lymphoma (CTCL) is poorly understood. A critical step in transformation from a normal cell to a neoplastic cell is uncontrolled cellular proliferation via dysregulation of the cell cycle. The availability and balance of intracellular deoxynucleoside triphosphates (dNTP) is critical to the maintenance of genomic stability and regulated cell cycle progression. Intracellular dNTPs are degraded by a novel mammalian hydrolase, sterile alpha-motif and HD-domain containing protein 1 (SAMHD1) that functions as a critical host restriction factor against HIV-1 and other viruses. SAMHD1 has been implicated to play a critical role in the attenuation of cell growth by limiting the dNTP pool available for DNA replication and DNA damage repair. Loss of SAMHD1 function through mutation or transcriptional regulation may lead to excess and imbalanced dNTP pool, resulting in genomic instability, enhanced mutagenesis, and aberrant progression through the cell cycle. Reduced SAMHD1 protein expression is apparent in several types of cancer, including CTCL. In a subset of CTCL patients with Sezary syndrome, the tumor spreads in the blood causing a leukemic-like disease of CD4+ T-cells. In these patients' malignant T-cells, SAMHD1 protein expression is significantly reduced compared to CD4+ T-cells from healthy donors. The mechanisms by which SAMHD1 is down-regulated in cancer cells are not fully understood. DNA hypermethylation of the *SAMHD1* promoter has been shown to reduce SAMHD1 protein expression in CTCL and lung cancer. In this study, the role of microRNA-181 repression of SAMHD1 expression in CD4+ T-cell lines was evaluated. Cell growth, apoptosis, and efficiency of cellular transformation were also analyzed using over-expression or knockdown of SAMHD1 protein in human and mouse cells. These studies demonstrate that SAMHD1 down-regulation is apparent in cancer cells through epigenetic mechanisms and contributes to cellular proliferation and development of CTCL.

Keywords: cancer, cell cycle, cutaneous T cell lymphoma (CTCL), deoxynucleoside triphosphates (dNTP), SAMHD1, tumor suppressor

EFFECTS OF BONE MARROW DERIVED FIBROBLASTS, MACROPHAGES, AND DENDRITIC CELLS ON STAT3 PHOSPHORYLATION IN THE OSA8

OSTEOSARCOMA CELL LINE. M. Leonard, M. Bear, W. Kisseberth, and C. London, Depts. of Veterinary Biosciences and Veterinary Clinical Sciences

Osteosarcoma (OSA) is the most common bone tumor of dogs and humans and despite aggressive therapy, 90% of dogs and 30-40% of children still die of disease. Both canine and human OSA cell lines and fresh tissues exhibit constitutive phosphorylation of STAT3, a transcription factor known to promote metastasis and chemotherapy resistance. OSA cell lines express receptors for cytokines IL-6 and oncostatin M (OSM), which promote STAT3 phosphorylation. As OSA typically develops on the endosteal surface, tumor cells are in contact with bone marrow derived cells that could serve as a source of cytokines inducing STAT3 phosphorylation. The purpose of this project is to identify which cells in the bone marrow microenvironment contribute to STAT3 phosphorylation in OSA cells. Bone marrow was collected from healthy dogs undergoing spay or neuter at OSU VMC. Following red cell lysis and Ficoll separation, cells were cultured with GM-CSF/IL-4/IL-12 to generate dendritic cells, M-CSF to generate macrophages, or complete medium alone to generate fibroblasts. Immunophenotyping demonstrated that macrophages expressed CD45, MHCII, and CD14 while dendritic cells expressed CD45, MHCII, and CD4. Conditioned medium was created by culturing bone marrow derived cells in Stemline serum free medium for 24 hours and adding this to serum starved OSA8 cells. Stemline alone or Stemline supplemented with OSM served as negative and positive controls, respectively. After 4 or 17 hours of culture, OSA8 cells were collected and pSTAT3/total STAT3 was detected by Western blotting. After 17 hours of culture with conditioned medium from macrophages, STAT3 phosphorylation was increased. This was also observed when the macrophages were stimulated with IL-6 prior to generation of the conditioned medium. These data suggest that cytokines produced by macrophages may contribute to the observed STAT3 phosphorylation in OSA cells. Studies are ongoing to more fully characterize the role of macrophages in OSA biology.

Keywords: osteosarcoma, STAT3, macrophages, IL-6, oncostatin M

ROLE OF PROTEIN ARGININE METHYLTRANSFERASE 5 OVER-EXPRESSION IN HTLV-1-DRIVEN CELLULAR TRANSFORMATION AND LEUKEMIA

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Human T-cell leukemia virus-1 (HTLV-1) is a retrovirus that infects an estimated 15-25 million people worldwide. HTLV-1 is the causative infectious agent of adult T-cell leukemia/lymphoma (ATL) and a neurodegenerative disease (HAM/TSP). While the probability of presenting any symptoms related to HTLV-1 infection is relatively low (5-10% for the lifetime of an infected individual), the disease progression and prognosis of those infected individuals who develop ATL is fatal, with a median survival range of 8-10 months. Unfortunately, ATL is highly chemotherapy resistant and while many current therapies improve ATL patient survival, the patients consistently relapse. Therefore, a need exists to develop treatments that improve ATL outcome. We have recently identified PRMT5 (protein arginine methyltransferase 5) as a potential target to modulate HTLV-1 gene expression. We find that PRMT5 levels are elevated in T-cell leukemia/lymphoma cell lines compared to freshly isolated naïve T-cells. Likewise, PRMT5 levels are elevated in total PBMCs isolated from ATL patients. However, PRMT5 RNA levels do not correlate to PRMT5 protein levels, suggesting a possible post-transcriptional method of regulation. Furthermore, we also show that PRMT5 levels are elevated during T-cell transformation by using HTLV-1 short-term immortalization assays. Utilizing shRNA vectors, we demonstrate that knockdown of endogenous PRMT5 results in decreased cellular proliferation and increased HTLV-1 viral gene expression. In addition, we observe a decrease in cell proliferation and an increase in viral gene expression when HTLV-1-infected/-transformed cells are treated with a novel small molecule inhibitor of PRMT5 (PRMT5i). Conversely, PRMT5i does not induce reactivation from HIV-1 chronically infected cells. We previously reported a protein-protein interaction between the HTLV-1 accessory protein, p30, and PRMT5. We further show PRMT5 enhances the ability of p30 to inhibit viral gene expression. In conclusion, we find PRMT5 to be a negative regulator of HTLV-1 and a potential target in HTLV-1-mediated disease.

Keywords: HTLV-1, PRMT5, p30, ATL treatment

GROWTH FACTOR SIGNALING: THERAPEUTIC TARGETS IN EQUINE METABOLIC SYNDROME-ASSOCIATED LAMINITIS

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Equine laminitis is a separation of laminar basal epithelial cells (LBEC) from the underlying basement membrane leading to structural failure of the laminae and a crippling displacement of the third phalanx. In equine metabolic syndrome-associated laminitis (EMSAL), hyperinsulinemia is purported to cause LBEC dysregulation through activation of the IGF-1 receptor (IGF1R). IGF1R activation impacts epithelial cell regulation through the ERK1/2 and PI3K/AKT signaling pathways which converge at mTORC1 to activate multiple growth-related proteins including RPS6. RPS6 activation, which is documented to play a central role in epithelial cell dysregulation in human disease states, has recently been found to occur in laminae in EMSAL. The purpose of this study was to use an equine epithelial cell culture model to assess signaling pathways downstream of IGF1R that lead to RPS6 activation and to identify potential therapeutic targets for EMSAL. Equine skin keratinocytes (primary culture) were serum starved, pretreated with inhibitors for mTORC1 (10nM Rapamycin), ERK1/2 (10 μ M U0126), or PI3K (0.1 μ M, 0.5 μ M, 1.0 μ M Wortmannin), stimulated by insulin and protein harvested for immunoblotting. Rapamycin effectively inhibited RPS6 phosphorylation in insulin-stimulated epithelial cells. Wortmannin decreased RPS6 activation in a dose dependent manner (1.0 μ M caused complete inhibition), whereas U0126 only partially inhibited RPS6. U0126 and 0.5 μ M Wortmannin together decreased RPS6 phosphorylation more effectively than either one alone, suggesting the involvement of both ERK1/2 and PI3K/AKT pathways in equine epithelial RPS6 activation. As mTORC1 is further "downstream" of the other proteins in the signaling cascades assessed (e.g. PI3K and ERK1/2), its value as a therapeutic target is greater as its inhibition is less likely to cause side effects due to unwanted inhibition of multiple homeostatic signaling events. Therefore, the mTORC1 protein complex should be further investigated as a potential therapeutic target for horses at risk of or suffering from EMSAL.

Keywords: laminitis, equine metabolic syndrome, growth factor receptor signaling, epithelial cell

DECELLULARIZATION TO PRODUCE BIOLOGICAL SYNOVIAL EXTRACELLULAR MATRIX SCAFFOLDS. Reisbig, N.A., Hussein, H.A.G.H, Pinnell, E., Bertone, A.L
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Cartilage debilitation is estimated to cost industrialized countries 1-2.5% of total gross domestic production. Present treatments focus on symptom relief and there are no therapies that can reverse the loss of chondrocyte function. Regenerative cells in combination with scaffolds for targeted delivery, is a treatment that could promote cartilage repair. The objective of this study was to find a method to produce decellularized synovium (synECM) that could serve as a living scaffold when seeded with viable cells.

Villous synovium harvested from the equine stifle was randomly assigned to control (no processing) or 1 of 4 decellularization methods in triplicate: 1) 0.1% peracetic acid solution (1X); 2), same as method 1 but repeated (2X); 3) 1% Triton X/DNAse, and; 4) same as method 3 with 2M sodium chloride solution replacing the Triton X. Control and decellularized tissue was cut with a biopsy punch and tested for morphology (histologic, scanning electron microscopy) and efficiency of decellularization (cell content, DNA content, DNA fragmentation and histology). All decellularization methods resulted in non-viable synovium and loss of cells. The PAA procedure (1X) had no significant loss of villous matrix integrity but had significantly greater retention of cells, cellular DNA ($P=6.5^{-18}$), and DNA size ($> 200\text{bp}$) than all other methods. The 2X PAA procedure also had no significant loss of matrix integrity, but had fewer cells than 1X, significantly ($p=4.9^{-7}$) lower retention of cellular DNA compared to the control and 1X, and similar low DNA retention (101ng/mg) and small DNA size ($<200\text{bp}$) as methods 3 and 4. TritonX/DNAase and 2MNaCl/DNAase damaged villous structure leaving little to no discernable synovium, no cells identifiable, low (140-143ng/mg) and small ($<200\text{bp}$) residual DNA content. The 0.1% PAA, performed twice, was considered to have the best scaffold potential due to the low cellularity, low DNA content and retained villous architecture.

Keywords: Decellulaization, Extracellular Matrix Scaffold, Synovium, Horse

OSTEOPROTEGERIN IN BONE-INVASIVE FELINE ORAL SQUAMOUS CELL

CARCINOMA CELL LINE. W. Supsavhad, W. Dirksen and T.J.Rosol. Department of Veterinary Biosciences, College of Veterinary Medicine, Ohio State University

Feline oral squamous cell carcinoma (FOSCC) is an aggressive head and neck cancer that has a strong propensity to invade maxillary and mandibular bone, which leads to serious morbidity. Inhibition of the ability of FOSCC to invade oral bone has the potential to improve the quality of life of cats with oral cancer and serve as important adjunct therapy with surgery, chemotherapy and radiation therapy. The ratio of Receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG) in bone determines the local activity of osteoclasts. The more OPG, the less bone resorption occurs. We found that SCCF2 (bone-invasive FOSCC cell line) had significantly less feline OPG compared to the less invasive FOSCC cell line (SCCF3). A full-length feline OPG (OPGv.1) clone as well as an alternative splice variant clone (OPGv.2) was sequenced. Two feline OPG variant clones along with control vector were stably transfected to SCCF2 cells. Feline OPG, RANKL, PTHrP and EGFR mRNA levels were compared between transfected SCCF2 cells (F2-OPGv.1, F2-OPGv.2 and F2-vector) and parent SCCF2 using quantitative real time PCR. Significantly greater feline OPG mRNA levels in F2-OPGv.1 and F2-OPGv.2 compared to control vector and parent SCCF2 were found. No different RANKL, PTHrP and EGFR mRNA levels between transfected cells and parent SCCF2 were observed. Feline OPG protein measurement by feline-specific solid-phase sandwich ELISA and ex vivo bone resorption experiment using calvaria co-culture are ongoing. In conclusion, we successfully cloned full-length and one splice-variant feline OPG as well as transfected them into SCCF2 cells. These cell lines can be useful for study the effect of OPG in bone-invasive feline oral squamous cell carcinoma in vitro.

Keywords: Feline, oral squamous cell carcinoma, osteoprotegerin (OPG)

STRUCTURE/FUNCTION

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DELAYED CRYOTHERAPY AND INFLAMMATORY SIGNALING IN SEPSIS-RELATED LAMINITIS. K. Dern, M. Watts, B. Werle, A. van Eps, J. Belknap. Dept of Veterinary Clinical Sciences

In recent studies investigating sepsis induced laminitis, a marked increase of molecules associated with inflammatory signaling was detected in laminitic lamellar tissue, and a dramatic decrease in these molecules was observed in limbs which were treated with profound digital hypothermia. Concurrently, it has been established that profound digital hypothermia limits the progression of equine laminitis both in clinical patients and experimentally induced laminitis. A recent study found that hypothermia initiated at the onset of lameness provided similar protection against lamellar failure as when hypothermia was initiated prior to the onset of clinical signs. The aim of the present study was to determine if initiation of hypothermia at the onset of lameness results in a similar inhibition of inflammatory signaling as described when hypothermia was initiated prior to the onset of lameness. Gene expression of molecules associated with inflammatory signaling were compared between lamellar samples from limbs which were iced at the onset of lameness (CRYO), limbs which were untreated (NON-RX), and limbs in which laminitis was not induced. Although multiple inflammatory mediators were increased in untreated limbs when compared to non-septic controls, digital hypothermia didn't cause a significant decrease in the gene expression of any of the molecules associated with inflammatory signaling. The lack of inflammatory inhibition in lamellar tissue samples in the current study, when evaluated in conjunction with the degree of lamellar protection provided by cryotherapy, indicates that the protective effects of digital hypothermia might not be through inflammatory pathways.

Keywords: Laminitis, Cryotherapy, Inflammation, Sepsis

EFFECTS OF DECREASING DIETARY STARCH AND INCREASING DIETARY FIBER ON STOOL CONSISTENCY AND PARASITE LOADS IN CAPTIVE LOWLAND GORILLAS (*Gorilla gorilla gorilla*)

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In recent decades, health concerns of lowland gorillas have led to re-evaluation of diets fed to great apes in human care. These studies suggest increased fiber diets, more closely mimicking the diet in the wild, may decrease the incidence of obesity, cardiac disease, and abnormal behavior associated with eating. This retrospective study investigated gastrointestinal health in lowland gorillas in response to a change in diet from high starch/low fiber, to high fiber/low starch. Hypothesizing that a change in diet from high starch formulated biscuits to primarily vegetables and browse would be associated with increased stool firmness and decreased incidence of parasites, we utilized medical and diet records of 17 adult lowland gorillas at the Columbus Zoo and Aquarium over a period of 19 years. We analyzed records among adult gorillas between 1996 and 1998, and compared these data to similar-aged adult gorillas post-diet change, between 2008 and 2014. We found that there was a trend towards a decrease in the occurrence of abnormal stool with the low starch/high fiber diet ($p=0.072$), that abnormal stool overall was more likely to have parasites ($p=0.00$) and there was a significant decrease ($p=0.03$) in *Neobalantidium coli* infection. *N. coli* is a protozoan gastrointestinal parasite of human and non-human primates, which can be carried asymptotically as well as associated with diarrheal disease. While it is rarely found as a commensal gastrointestinal parasite in wild African great apes, it is found in up to 50% of tested African apes in zoos. Recently, *N. coli* infection prevalence has been recognized in a variety of species to be associated with high dietary starch levels. Our research suggests that increasing fiber content and decreasing starch and sugar in the diet of gorillas is associated with improved stool consistency and a decrease in shedding of *N. coli*.

Keywords: western lowland gorilla; diet; neobalanatidium coli; fiber; starch

NANO-ENCAPSULATED BILIRUBIN PROTECTS MURINE PANCREATIC ISLET CELLS EXPOSED TO HYPOXIC STRESS. B. Fullagar¹, W. Rao², X. He², C. Gilor¹, F. Xu¹ and C. Adin³. ¹Department of Veterinary Clinical Sciences and ²Department of Biomedical Engineering, The Ohio State University; ³Department of Veterinary Clinical Sciences, North Carolina State University.

Pancreatic islet transplantation is the only curative, non-invasive treatment for type 1 diabetes mellitus. Our group is pursuing islet transplantation in diabetic dogs. However, isolation stress and hypoxia cause loss of up to 70% of islet mass prior to any immune insult. Bilirubin, an endogenous antioxidant, can improve survival of murine pancreatic allografts during hypoxic stress, but has poor bioavailability. We hypothesized that nano-encapsulation of bilirubin in Pluronic 127-chitosan nanoparticles (nBR) would improve uptake by murine pancreatic islet cells and improve their viability following hypoxic stress. Nano-bilirubin was synthesized and its release characteristics were studied in vitro. Cellular uptake of nBR was compared to free bilirubin (fBR) in an insulinoma cell line (INS-R3) model using confocal-like structured illumination microscopy. Then, islets were isolated from C57BL/6 mice and treated with concentrations of 0-20 μ M of nBR, fBR or empty NP (eNP), prior to incubation under standard or hypoxic conditions. Cell viability was assessed via Hoescht and Propidium Iodide staining and epifluorescent microscopy. Release of bilirubin was greatest from nBR suspended in protein-rich solution. Increased, selective uptake of nBR by INS-R3 cells was demonstrated. Survival of murine islets exposed to hypoxia was significantly improved by the administration of nBR at 5-10 μ M or eNP at 5-20 μ M. Administration of fBR had dose-dependent cytotoxic effects. Delivery of bilirubin using Pluronic F127-chitosan NP improves uptake by murine islets compared to fBR and offers dose-dependent protective effects following hypoxic stress. Immunomodulatory effects of chitosan have been previously reported and their mechanism of action in NP warrants further investigation.

Keywords: diabetes, islet, pancreas, transplant, bilirubin, nanoparticle, nanotechnology, immunology.

EFFECTS OF MEGAVOLTAGE IRRADIATION ON THE FORMATION AND RESORPTIVE ACTIVITY OF OSTEOCLASTS IN VITRO ER Helffrich, MR Williams, ER Green and MJ Allen, Department of Veterinary Clinical Sciences

Radiation therapy is commonly used as an effective means to treat bone cancer, however, radiation has also been shown to dramatically increase the chance of fracture following treatment. Animal studies in our laboratory indicate that there is an early decrease in osteoclast numbers after radiation, followed by a rebound increase that likely explains the loss of bone and increase in bone fragility after radiation. We hypothesized that osteoclast precursor cells, derivatives of bone marrow macrophages, are directly sensitive to radiation injury. Osteoclast precursors were isolated as mouse bone marrow macrophages (BMM ϕ) and irradiated with doses of 0, 0.1, 0.2, 0.5, 1.0, 2.0 Gy. Cells were then seeded onto either plain or calcium phosphate-coated 24-well plates and supplemented with M-CSF and RANK ligand to stimulate osteoclastogenesis. Osteoclast formation was quantified by counting the number of TRAP-positive multinucleate cells. Osteoclastic bone resorption was quantified by measuring pit formation on the coated plates. Radiation produced a dose-dependent decrease in osteoclast formation, with statistically significant inhibition at all doses above 0.2 Gy. The IC₅₀ for radiation was approximately 0.5 Gy. Similar results were seen with the pit resorption assay, although statistically significant inhibition was only seen at 1 and 2 Gy. These results support the hypothesis that osteoclast precursors are intrinsically sensitive to radiation injury in vitro. The negative effects were more evident on osteoclast formation than on pit resorption, suggesting that even a limited number of osteoclasts are capable of significant resorptive activity in this cell culture system.

Keywords: bone biology, bone, osteoclast, radiation, megavoltage, osteoclastogenesis

EFFECT OF EXPOSURE TO DIM LIGHT AT NIGHT AND PARTICULATE MATTER ON C3H/HeNHsd MICE. Hogan, MK; Qinghua, S; Nelson, R.J. Department of Neuroscience. Bergdall, V, Department of Veterinary Preventive Medicine.

Air and light pollution contribute to altered fetal development, breast and prostate cancers, metabolic and cardiorespiratory diseases, and central nervous system (CNS) disorders. A component of air pollution, particulate matter, and the phenomenon of dim light at night (dLAN) result in neuroinflammation, which has been implicated in CNS disorders such as impaired cognition and anxiety, depression, and other mood disorders.

In this study, male C3H/HeNHsd mice, with intact melatonin production, were used to model humans exposed to circadian disruptions and contaminated environmental air.

We hypothesized exposure to 2.5 μ m of particulate matter (PM_{2.5}) and dLAN (5 lux) combines to upregulate neuroinflammatory cytokine expression and alter hippocampal morphology compared to mice exposed to filtered air (FA) and housed under a standard light-dark cycle (LD). Secondly, we hypothesized that exposure to PM_{2.5} and dLAN provoke anxiety-like and depressive-like responses.

For four weeks, four groups of mice were simultaneously exposed to PM_{2.5} or FA and/or dLAN or LD utilizing the Versatile Aerosol Concentration Enrichment System (VACES). Following exposure, anxiety and depressive-like behaviors were assessed utilizing several behavioral assays (open field assay, elevated plus maze, forced swim test, sucrose anhedonia). At termination of the study, hippocampi were collected for qPCR and morphological analyses.

Results of behavioral assay, qPCR, and hippocampal morphological analysis are comparable to previous PM_{2.5} and dLAN studies conducted on mice and implicate PM_{2.5} and dLAN as potential factors contributing to depression and anxiety. Short-term exposure to particulate matter and light pollution resulted in upregulation of neuroinflammatory cytokines and CA1 hippocampal structural changes as well as depressive-like responses in these rodents (sucrose-preference). However, when combined, PM_{2.5} and dLAN exposure did not have additive effects, as hypothesized.

Keywords:

COMPREHENSIVE KNEE OSTEOARTHRITIS ASSESSMENT IN A DOG

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The stifle is the most commonly diseased joint in the dog. The commonest cause of canine stifle disease is cranial cruciate ligament injury, which is then associated with subsequent damage to the medial meniscus and Osteoarthritis (OA) progression. Positron emission tomography (PET) using 2-deoxy-2-[¹⁸F]-fluoro-D-glucose (FDG) reflects glucose metabolism and can detect inflammation. Recent development in MRI technology used in the field of musculoskeletal research have provided reliable information on the joint structure as well as changes over time.

The aim of the present study was to use several imaging diagnostic techniques, including: Radiography, MRI, PET-CT and micro PET-CT to assess a surgically created model of OA in the dog knee.

Materials: Five skeletally mature Beagles underwent ACLT in one knee via arthroscopy, the contralateral knee served as control. Before, 3, 6 and 12 weeks after ACLT, under general anesthesia, the dogs underwent radiography, PET/CT and MRI. MRI was performed using a 3 Tesla MRI body system with an 8-channel coil. PET/CT was performed using a Philips Gemini TF 64 PET-CT system. Inveon micro PET and micro CT system was used for the harvested femur and tibia.

Results: The sequences used allowed qualitative and quantitative assessment of the dog knee. Specifically articular cartilage, bone, meniscus and cruciate ligaments.

Discussion: This study showed that several imaging diagnostic modalities were suitable and provided innovative sequences to assess a surgically model of OA in the dog knee.

Conclusion: This study demonstrated that MRI and PET-CT are useful technologies to provide serially and reliable *in vivo* dog knee assessment. Providing promising tools to evaluate joint changes serially during the development of osteoarthritis.

Keywords: Canine knee, High-Field MRI, PET-CT, Osteoarthritis.

USING URINARY HORMONE ANALYSIS TO PREDICT GENDER AND ASSESS FETAL VIABILITY IN THE INDIAN RHINOCEROS (*Rhinoceros unicornis*). K.

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The high incidence of stillbirth (16% of all births)³ within the captive population of Indian rhinos has been an impediment to achieving sustainability with the species. The majority (67%) of stillbirths have been associated with male calves.³

As fetal gender may be a contributing factor in stillbirth in the Indian rhino, knowledge of the sex of calves in utero could help identify those dams at a potentially higher risk.

For this study, we examined if urinary hormone analysis of maternal testosterone (T), cortisol (C) and corticosterone (C₁) could be used to establish physiological markers to predict gender, parturition date and assess fetal viability during pregnancy. Urinary C₁ had not yet been validated for the species, so samples collected during a previously conducted ACTH stimulation test¹ were utilized.

Longitudinal urine samples were collected starting on day of insemination and continued throughout gestation and for one week post-parturition. Of the six pregnancies monitored, three resulted in live births of female, two resulted in live births of male calves, and one resulted in birth of a male calf that exhibited signs of respiratory distress and died ~12 hours post-parturition.

Significantly higher concentrations of urinary T were excreted from dams carrying male versus female calves during all months of gestation. Similarly, glucocorticoids C and C₁ were excreted in higher concentrations during all months of gestation in dams pregnant with male calves when compared to those pregnant with female calves (P<0.05). As urinary C₁ has the potential to signal independently of C, and because in some species the fetus preferentially secretes C₁ versus C, we sought to validate urinary C₁ in this species and determine its utility as a prognostic tool for fetal viability. Pharmacological validation was shown via a 12-fold increase in urinary C₁ concentrations measured 16-hours after ACTH injection in an Indian rhino bull.

Key words: fetal gender, glucocorticoids, pregnancy, *Rhinoceros unicornis*, testosterone, urinary hormones

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EDUCATIONAL

STUDENT DRIVEN EDUCATION: A MODEL FOR SUCCESS IN SURGICAL

TRAINING. J. Abbruzzese, B. Dent, M. Andres, A. Deak, L. Diangelo, L. Timperman, T. Motta. Department of Veterinary Clinical Sciences

The Veterinary Surgery Club (VSC) at The Ohio State University strives to further students' exposure to the discipline of veterinary surgery and to enhance their engagement in the learning process throughout all stages of their veterinary education.

Utilizing small group peer-assisted learning, low fidelity surgical models, and faculty-led cadaveric simulations, the VSC is able to supplement the already rigorous surgical instruction offered in the pre-clinical core curriculum.

Members have access to online training modules and receive hands-on instruction in basic surgical instrumentation and techniques in their first semester, providing skills which are then applied in simulation and cadaveric labs. As students progress through the curriculum, they can receive additional training from faculty to become peer mentors to incoming students. The experience of peer teaching ensures that student mentors have much deeper understanding of fundamental surgical skills.

The dedication and enthusiasm of the advanced students allows the VSC to provide a mentor to student ratio of 1:4. This fosters a more intimate, personalized learning environment than that of the core curriculum which students find both highly attractive and effective. Since instituting this educational model, the VSC has nearly doubled in membership and now supports 70% of the first year students and over 50% of the 484 students enrolled in the pre-clinical curriculum.

The VSC is funded entirely through membership dues and fundraising. Yearly, thirty hours of hands-on instruction and six hours of lecture are donated by approximately 10 faculty members. Overall, students are eager to invest in supplemental training and report improved skill retention.

Keywords: veterinary surgery, veterinary surgery club, veterinary education, peer-assisted learning, peer teaching, student mentor, fundamental surgical skills

RESEARCH PATHOLOGY SUPPORT FOR EXPERIMENTAL ANIMAL MODELS PROVIDED BY THE COMPARATIVE PATHOLOGY & MOUSE PHENOTYPING SHARED RESOURCE. K. La Perle. Department of Veterinary Biosciences.

The Comparative Pathology & Mouse Phenotyping Shared Resource (CPMPSR) at The Ohio State University supplies readily available, affordable, expert experimental pathology support to investigators utilizing animal models of human and veterinary disease. The CPMPSR comparative pathologists are familiar with the normal anatomy, physiology, and pathology of many animal species, including the potential impact of confounding factors such as age- and strain-related background lesions, pathogens, and husbandry practices on study outcomes. Primary research interests for the CPMPSR pathologists encompass cancer biology, developmental pathology, endocrine disease, immune-mediated conditions, neurobiology, and toxicologic pathology. However, translational research based on any animal model is supported. The CPMPSR offers a full array of pathology services, and can tailor its support to the needs of a client. Routine procedures include comprehensive macroscopic and microscopic examinations with an emphasis on phenotype characterization of newly produced lines of genetically engineered mice as well as pre-clinical efficacy and toxicity studies. Other common methods include clinical chemistry, hematology, radiography, whole slide digitization (Aperio) and quantification, frozen and paraffin slide preparation, tissue microarray preparation, and many special histochemical and immunohistochemical staining techniques. The CPMPSR pathologists are valuable collaborators for all facets of animal model development including study design, optimal sample collection, data analysis and interpretation, and communication. The CPMPSR was created to serve the experimental pathology needs of investigators at The Ohio State University, especially those in the seven health-related schools and the Comprehensive Cancer Center. However, the CPMPSR also functions as a referral service for experienced biomedical scientists at many other institutions (academic, government, and industrial).

Keywords: animal model, genetically engineered mice, histology, pathology, pre-clinical