THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

ADVANCES IN VETERINARY MEDICINE RESEARCH

17 APRIL 2014

BOOK OF ABSTRACTS

PROGRAM

April 17, 2014

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 PLATFORM PRESENTATIONS

Dr. Lance Visser
Dr. Joelle Fenger
Ms. Sarah Fritz

KEYNOTE SPEAKER

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

Dr. Carol Reinero
Associate Professor and Director,
Comparative Medicine Laboratory, University of Missouri-Columbia

"What Spontaneous and Experimental Feline Asthma Can Teach Us About Human Asthma"

POSTER SESSION

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

PROGRAM CHAIR

Dr. Cheryl London

ORGANIZED BY

Michele Morscher

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THE OHIO STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

Office of Research and Graduate Studies

ADVANCES IN VETERINARY MEDICINE
RESEARCH DAY

Awards Presentation, Graduate Student Platforms, and Keynote Address

Thursday, April 17th
12:00-2:00 pm
Veterinary Medical Center Auditorium
Corner of Coffey Road and Tharp Street
Enter from Coffey road and go up the stairs

Featuring:

"What Spontaneous and Experimental Feline Asthma Can Teach Us About Human Asthma"

Dr. Carol Reinero
associate professor and the
Director of the Comparative Medicine Laboratory,
University of Missouri-Columbia

all posters will be on display on 1st and 2nd floors
Veterinary Medicine Academic Building

Poster Judging Schedule:
April 16th: 2p.m. - 5p.m. for Professional Students
April 17th: 8p.m. - 10:30p.m. for Graduate Students

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POSTER JUDGING SESSIONS

Wednesday, April 16, 2014
2:00 – 5:00 pm
Veterinary Student Poster Judging

Thursday, April 17, 2014
8:00 – 10:30 am
Graduate Student Poster Judging

Thank you to the following faculty, graduate students, post-docs and guests for taking time out of their busy schedules to judge 81 posters.

Joelle Fenger	Matthew Allen
Tatiana Motta	Stan Micek
Kate Hayes-Ozello	Kris Hennessy
Eason Hildreth	Tom Rosol
Greg Sumner	Chander Celly
Andrew Bowman	Mellissa Hicks
Stefan Niewiesk	Phillip Lerche
Mike Oglesbee	Judith Radin
Karen Tefft	Jim DeWille
Antoinette Marsh	Steve DiBartola
Paivi Rajala-Schultz	Ioano Boeris
Kathy Boris-Lawrie	Gati Singh
Armando Hoet	Tom Wittum

GRADUATE STUDENT
PODIUM PRESENTATION

ECHOCARDIOGRAPHIC ASSESSMENT OF RIGHT VENTRICULAR SYSTOLIC FUNCTION IN CONSCIOUS HEALTHY DOGS. L.C. Visser,[1] B.A. Scansen,[1] N.V. Brown,[2] K.E. Schober,[1] and J.D. Bonagura.[1] Dept. of Veterinary Clinical Sciences[1] and Center for Biostatistics[2], The Ohio State University.

The echocardiographic assessment of right ventricular (RV) function is severely underdeveloped in dogs. We sought to generate reference intervals and to determine the feasibility, repeatability and inter- and intraobersever variability for several echocardiographic indices of RV function in healthy dogs including tricuspid annular plane systolic excursion (TAPSE), RV fractional area change (FAC), pulsed wave tissue Doppler-derived systolic myocardial velocity of the lateral tricuspid annulus (S'), and speckle-tracking echocardiography-derived global longitudinal RV free wall strain and strain rate. We also sought to determine if these indices could track changes in RV function following a single oral dose of pimobendan and atenolol. A prospective, blinded, randomized, crossover study of 80 healthy, unsedated dogs was performed. All dogs underwent 4 echocardiograms – twice for baseline, once 3-hours post-pimobendan and once 3-hours post-atenolol. All RV systolic function indices were feasible to acquire with adequate repeatability; inter- and intraobserver variability was low with all average coefficients of variation <10%. All indices exhibited a significant ($P \leq 0.001$) correlation to body weight and weight-specific reference values were generated via allometric scaling. Gender had no effect on any of the RV function indices. All RV function indices showed a significant ($P<0.0001$) increase and decrease from baseline following pimobendan and atenolol, respectively. Mixed model analysis for the percent change in RV function showed that treatment for all RV function indices had a significant ($P<0.0001$) effect, whereas the other covariates did not. Further study is warranted to determine the value of these RV function indices in dogs with cardiovascular disease.

Keywords: Echocardiography, Right Ventricle, Canine, Reference values

GRADUATE STUDENT
PODIUM PRESENTATION

DISSECTING THE ROLE OF MIR-9 IN NORMAL AND MALIGNANT MAST CELL BIOLOGY.

J.M. Fenger[1], M.D. Bear[2], S.J. Galli[3], V. Coppola[4], W.C. Kisseberth[1], C.A. London[1,2]
Department of [1]Veterinary Clinical Sciences, [2]Veterinary Biosciences, The Ohio State University College of Veterinary Medicine, [3]Department of Pathology, Stanford University School of Medicine, [4]Department of Molecular Virology, Immunology, and Medical Genetics, The Ohio State University Comprehensive Cancer Center

Mast cells (MC) are key effectors in a variety of physiological and pathological processes, including allergic disorders, chronic inflammatory diseases, and tumor progression. MicroRNAs (miRs) are small non-coding RNAs that regulate gene expression and their dysregulation is implicated in numerous pathologic conditions. To study the role of miR-9 in MC biology, we generated a transgenic mouse model that expresses miR-9 in a tissue-specific manner in the presence of the Cre recombinase. A 625bp mmu-miR-9-1 sequence was cloned into the Rosa26 locus and the targeting vector was linearized and electroporated into S1B6a mouse embryonic stem (ES) cells. G418-resistant clones were screened by southern blotting and correctly targeted ES cell clones were used to create chimeras for transmission. F1 miR-9-Tg mice were propagated and crossed with carboxypeptidase A3-Cre(Cpa3-Cre) mice that restricts expression of miR-9 to mast cells and basophils. Using qRT-PCR we have shown that bone marrow-derived MCs(BMMC) from Cpa3-Cre/miR-9-Tg mice express significantly higher levels of miR-9 compared to single transgenic mice demonstrating that our miR-9-Tg mice function in a tissue-specific manner. Enforced expression of miR-9 significantly enhanced Cpa3-Cre/miR-9 BMMC invasion compared to Cpa3-Cre or miR-9 BMMCs. Cpa3-Cre/miR-9 BMMCs expressed high levels of CMA1, a MC chymase that plays a key role in activating pro-matrix metalloproteases, and demonstrated increased MMP-2 activity as compared to Cpa3-Cre or miR-9 BMMCs. These findings support a role for miR-9 in enhancing invasion in normal MCs and suggest a mechanism by which miR-9 contributes to invasion through the activation of CMA1. Future studies utilizing our transgenic mouse model will identify the molecular mechanisms responsible for miR-9-induced CMA1 expression and enhanced invasion in normal MCs, assess the effects of miR-9 on normal MC biology *in vivo*, an investigate the contribution of miR-9 in promoting MC invasion, metastasis, and tumor progression in mouse models of malignant mast cell disease.

Keywords: Mast cell, miR-9

GRADUATE STUDENT
PODIUM PRESENTATION

RHA/DHX9 REGULATES ONCOGENIC RETROVIRAL AND CELLULAR GENE EXPRESSION THROUGH A DISTINCT POSTTRANSCRIPTIONAL MECHANISM. S. Fritz and K. Boris-Lawrie. Department of Veterinary Biosciences.

RNA helicase A (RHA/DHX9) is an essential gene necessary for the regulated expression of the cellular proto-oncogenes junD and huR and the oncogenic retrovirus HTLV-1. Dysregulation of RHA is associated with breast, prostate, and lung cancer and affects pathogenic retrovirus gene expression. We identified RHA as a critical translation regulator of junD and huR as well as avian, simian and human species retroviruses, including HTLV-1 and HIV-1. Here RHA associates with a distinct RNA element, termed the posttranscriptional control element (PCE), located in the 5' termini of these target transcripts. The association of RHA with the PCE forms a RNA-protein complex that facilitates cap-dependent translation. Dysregulation of RHA-PCE biology impairs retroviral replication and elicits a cellular stress response.

The objective of the study conducted here was to elucidate the molecular basis by which RHA facilitates polyribosome loading. Preliminary biochemical data led us to hypothesize that the conserved N- and C-termini of RHA recruit essential translation cofactors necessary for ribosome recruitment and protein production. Results of extensive co-immunoprecipitation and GST pull-down studies revealed the following: (1) RHA associates with the non-canonical translation cofactors CBC and CTIF along with translation-associated PABP1 and (2) the N- and C- termini of RHA are both necessary and sufficient for its interaction with CBC, CTIF, and PABP1. Support for a functional role of these associations in RHA-dependent translation came from mutagenesis studies in which terminal amino acid mutations eliminated RHA-cofactor interaction and corresponded with a loss in RHA-PCE translation activity. These results suggest that the association of RHA with non-canonical translation cofactors is part of the molecular basis by which this posttranscriptional regulator governs the translation of oncogenic cellular and viral mRNAs. Together, our results provide mechanistic insight into RHA's association with breast, prostrate, and lung cancer as well as lymphocytic infections by HIV-1 and HTLV-1.

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

EDUCATIONAL

ED - 1

DEVELOPMENT AND PILOT OF THE CASE MANAGER: A VIRTUAL PATIENT EXPERIENCE FOR VETERINARY STUDENTS. JK Byron[1], SE Johnson[1], LCV Allen[2], C Brilmyer[3], R Griffiths[4]. [1]Dept. of Veterinary Clinical Sciences, [2]Educational Design Systems, [3]The Health Sciences Library Digital Solutions, and [4]Office of Distance Education and eLearning

There is an increasing demand in veterinary education to engage students, teach and reinforce clinical reasoning, and provide access anytime/anywhere to quality learning opportunities. In addition, accrediting bodies are asking for more concrete documentation of essential clinical skills outcomes. Unfortunately, during the clinical year in a referral hospital setting, students are at the mercy of chance regarding the types of cases they will encounter and have an opportunity in which to participate. Patient and case simulation technology is becoming more popular as a way to achieve these objectives in human and veterinary medical education. Many of the current options available to the veterinary medical education community to develop virtual patient cases are too time-consuming, cost-prohibitive, or difficult for the instructor or learner to use. In response, we developed a learning tool, Case Manager (CM), which is low-cost and user-friendly. The CM was designed to meet the demands of veterinary education by providing students with an opportunity to cultivate clinical reasoning skills and allowing for real-time student feedback. We launched a pilot test with 37 senior veterinary medical students as part of their Small Animal Internal Medicine clinical rotation. Students reported CM increased their engagement with the material, improved diagnostic and problem-solving skills, and broadened their exposure to a variety of cases. In addition, students felt CM was superior to a more traditional, less interactive, case presentation format.

Keywords: Case Simulation, Virtual Patient, Educational Technology

CLINICAL RESEARCH

CR - 1

DIFFUSION TENSOR IMAGING IN DOGS WITH AND WITHOUT CERVICAL SPONDYLOMYELOPATHY. J. Armstrong[1], R.C. da Costa[1], S. Choi[2], P. Martin-Vaquero[1], B. Dewey[3], S. Smith[3] From the [1]Dept of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH [2]Dept of Radiology, The Ohio State Medical Center, Columbus, OH, and [3]Institute of Imaging Science, School of Medicine, Vanderbilt University, Nashville, TN

Cervical spondylomyelopathy (CSM) is the most common disease of the cervical spine in large breed dogs. Despite a large variety of treatment options, there is a limited understanding of disease mechanisms and lack of clearly defined prognostic indicators. Diffusion tensor imaging (DTI), an advanced magnetic resonance imaging (MRI) technique recently applied to humans, is sensitive to changes in tissue microstructure and could yield prognostic information. This study evaluated changes in fractional anisotropy (FA), median diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AD) between dogs with and without CSM. We hypothesized that DTI differences would exist in CSM-affected dogs compared to unaffected dogs, furthering the understanding of disease mechanisms.

Fourteen control and 14 CSM-affected Great Danes were enrolled for analysis with a novel DTI protocol optimized for the canine cervical vertebral column. All dogs underwent a 3.0 T MRI protocol of T1 and T2 weighted images in the sagittal and transverse planes. Post-processing using proprietary software generated tensor estimation and metric maps. FA, MD, RD, and AD were measured from 3 transverse DTI sections over each disc region (C2-C7) using DTI Studio's ROI Editor software. MRIs of CSM-affected dogs were also evaluated for spinal cord signal changes.

Random-effects linear regression analysis identified significant differences in FA and RD ($P < 0.05$) between dogs with and without CSM. Differences in MD and AD were not significant ($P > 0.05$). In CSM-affected dogs, significant differences were identified for FA, RD, and AD when comparing the sites with and without signal changes at the C5-C6 intervertebral space.

FA and RD differences suggest that demyelination and loss of axonal integrity are present in spinal cords of CSM-affected dogs. Additionally, results indicate that evaluation of spinal cord microstructure with DTI yields information that could assist in the treatment and prognostication of CSM-affected dogs.

Keywords: cervical spine, diffusion tensor imaging, dog, wobbler syndrome

CR - 2

INFLUENCE OF RED BLOOD CELL TRANSFUSION ON TEG TRACINGS IN NATURALLY ANEMIC DOGS. A. Brooks, E. Cooper, J. Guillaumin, G Couto. Department of Veterinary Clinical Sciences

Previous work demonstrated that canine blood manipulated to be anemic *in vitro* had "hypercoagulable" thromboelastography (TEG) tracings compared to non-anemic blood. However, the impact of hematocrit (Hct) on TEG in naturally anemic dogs is unknown, and is the population in which this effect is most clinically relevant. Therefore we hypothesized that following transfusion, TEG values would become less "hypercoagulable", despite minimal change in underlying disease status in a short time period.

Pre and post packed red blood cell transfusion samples were taken from 22 dogs and assessed by TEG at 30 minutes from phlebotomy. Viscosity was measured on a cone-and-plate viscometer. Signalment, weight, underlying disease process, volume of transfused blood, and CBC data were also collected. Patient data was further subdivided into 3 broad categories of anemia: bleeding (10), IMHA (6), and non-regenerative (6).

Median Hct pre-transfusion was 11% (range 6-26) and median Hct post-transfusion was 19% (range 12-31). Mean volume of transfused pRBC was 11(±2) mL/kg, and mean Hct increased by 7%(±3) after transfusion. Post-transfusion samples had significantly higher Hct and viscosity, and significantly lower platelets, white blood cells (WBCs), MA and G than pre-transfusion samples. Increasing delta MA (pre minus post) was significantly ($r=0.5$, $p=0.03$) correlated with increasing Hct (post minus pre). Based on the slope of the best-fit line, approximately every 1% increase in Hct causes a decrease in MA of 0.5mm (±0.2). Differences in anemia severity, WBC count, and TEG variables were noted between disease categories, with IMHA dogs having the highest WBC counts and lowest pre-transfusion Hct.

Increasing Hct is correlated with decreasing MA and G TEG variables in anemic dogs. Within the range of anemia and diseases studied, these changes are small and unlikely to significantly impact clinical decision making, but do suggest an impact of red cell mass on measurement with TEG.

Keywords: Anemia, thromboelastography, hematocrit, transfusion

CR - 3

COMPARING URINE CREATININE AND SPECIFIC GRAVITY OVER FREEZE/THAW CYCLES IN THE INDIAN RHINOCEROS (*Rhinoceros unicornis*) S. Cantara[1], K. McKinnon[2], P. Hermes[2], C. Pinto[1] and M. Stoops[2] [1]The Ohio State University College of Veterinary Medicine; [2]Cincinnati Zoo & Botanical Garden, Center for Conservation and Research of Endangered Wildlife (CREW)

To increase the population of the critically endangered Indian rhino, the reproductive cycles of captive, female Indian rhinos is being studied. To study these cycles, hormone levels are measured to determine critical events such as estrous, ovulation, and pregnancy. The simplest and least invasive way to collect a sample of body fluid, in an animal like rhino, is urine. Urine is collected on a daily basis and analyzed to determine estrogen and progesterone levels which provide information about the rhino's reproductive cycle. This information allows for timing of breeding or artificial insemination. In order to get an accurate measurement of hormones levels in the urine, the concentration of the urine must be determined. Currently, creatinine is used as an index of urine concentration; but, another method is measuring specific gravity. Repeated freeze/thaw cycles of samples could result in degradation of creatinine and inaccurate measurements of hormones. This project involved measuring creatinine and specific gravity of urine over repeated freeze/thaw cycles to determine if there are differences over these cycles. This study found that over the three freeze/thaw cycles the amount of creatinine in each sample decreased. There was variation in specific gravity over the two freeze/thaw cycles, but to a lesser extent. This data suggests that creatinine decreases over the freeze/thaw cycles possibly due to degradation with temperature fluctuation and changes in physical state of the urine. Specific gravity shows less variation as this is a measure of the density of the liquid. This study concludes that creatinine can be used a reliable indicator of urine concentration if the sample is collected, frozen and thawed once before hormone analysis. However, often, samples are shipped to distant institutions for analysis, so samples could undergo multiple freeze/thaw cycles. In these cases, specific gravity could be used as an indicator of urine concentration.

Keywords: Indian Rhinoceros, urine creatinine, urine specific gravity

CR - 4

DEVELOPMENT AND VALIDATION OF A LOW-FIDELITY, LOW-COST SURGICAL SIMULATION MODEL TO TEACH CANINE ORCHIECTOMY. B. Carter, T. Motta, E. Sweazy, L. Hill, M. McLoughlin. Department of Veterinary Clinical Sciences

At The Ohio State University College of Veterinary Medicine, minimal hands-on experience during the first two years leads to lack of experience and high stress levels for many students during the third year. In this study, we aimed to test the hypothesis that low-fidelity surgical simulation models can help to increase skill level and confidence, while decreasing students' perceived stress and anxiety.

To investigate this, a low-fidelity, low-cost surgical simulator for orchiectomy was created. This model allows students to practice a number of fundamental surgical skills performed during an orchiectomy. Twenty-four inexperienced first and second year students volunteered to participate in this study. All were instructed on performing an orchiectomy using lecture, videos and handouts. Twelve students were randomly chosen to receive the model and 30 minutes of training. A quiz and questionnaire were utilized to evaluate the perceived benefits of the model. All students performed an orchiectomy on a cadaver and were graded by two expert faculty and two surgery technicians using recorded video and a detailed rubric.

All students indicated that the use of the model improved perceived performance, increased confidence, and decreased stress. Knowledge retention was improved by the use of the model ($p=0.05$). Skill level was also improved, as evidenced by rubric scores that were significantly higher for students given the model in the areas of spermatic fascia disruption ($p=0.041$), and clamping and ligation of both the first (score, $p=0.0032$; time, $p=0.0086$) and second ($p=0.024$) testicles. Our findings strongly indicate that the use of a low-fidelity model is highly beneficial in developing fundamental skills during early stages of surgical training. Utilization of the model also benefits animal health and well-being by providing an alternative to the use of animals for early surgical education, while contributing to the formation of new graduates with improved basic skill sets.

Keywords: Simulation, Orchiectomy, Surgical Skills, Surgical Training, Low-fidelity

CR - 5

ADRENAL STEROIDS AND STEROID PRECURSORS IN HOSPITALIZED NEWBORN FOALS K.A. Dembek[1]; S.D. Hurcombe[1]; N.M. Slovis[2]; S. M. Reed[3]; R.E. Toribio[1]. [1]The Ohio State University, College of Veterinary Medicine; Columbus, OH, USA; [2]Hagyard Equine Medical Institute, Lexington, KY, USA; [3]Rood and Riddle Equine Hospital, Lexington, KY, USA.

Sepsis is the leading cause of newborn foal mortality. In response to sepsis there is activation of the hypothalamic-pituitary-adrenal axis (HPAA), which is characterized by increased release of corticotropin-releasing hormone (CRH), adrenocorticotropin (ACTH) and cortisol. Dysfunction of the HPAA or relative adrenal insufficiency (RAI) has been associated with sepsis and mortality in newborn foals. Although, diagnosing and treating RAI is a major target of investigation in foals, comprehensive information on the adrenal response to sepsis is limited. Most studies have been focused on cortisol, while other essential adrenocortical steroids have been overlooked. We hypothesize that adrenal steroids and steroid precursors will be higher in septic compared to healthy foals on admission, and their blood levels will increase over 24h in septic foals. Hormones concentrations will be associated with severity of disease and mortality.

Blood samples were collected on admission (time 0) and 24h later (time 1) from 15 septic (sepsis score ≥12), 15 sick-non-septic (SNS; sepsis score <12) and 4 healthy foals of <5 days of age. Blood concentrations of CRH, ACTH, cortisol, aldosterone, pregnenolone, progesterone, 17α-OH-progesterone, androstenedione, and dehydroepiandrosterone (DHEA) were determined by immunoassays.

CRH and cortisol concentrations were higher in septic than in healthy and SNS foals at both time points ($P<0.05$). Septic foals had higher aldosterone, pregnenolone, progesterone, 17α-OH-progesterone, androstenedione, DHEA concentrations compared to healthy and SNS foals at time 1. Non-surviving hospitalized foals had higher CRH, ACTH, cortisol, pregnenolone and progesterone concentrations at time 1 compared to surviving foals. Aldosterone and 17α-OH-progesterone concentrations were higher in foals that died at both time points ($P<0.05$).

The adrenal gland response to sepsis in foals is characterized by persistently increased concentrations of steroids and steroid precursors over the first 24h of hospitalization. Aldosterone and 17α-OH-progesterone concentrations are potent predictors of non-survival at admission and 24h later in hospitalized foals.

Keywords: sepsis, steroids, adrenal insufficiency, foal, equine

CR - 6

MINIMALLY INVASIVE APPROACH TO LUMBOSACRAL DECOMPRESSION IN A CADAVERIC CANINE MODEL. BT Dent, BF Hettlich. Department of Veterinary Clinical Sciences

Aims
The aim of this study was to determine feasibility of a minimally invasive surgical (MIS) approach using a human MIS lumbar retractor for canine lumbosacral dorsal laminectomy and partial diskectomy and to compare this technique to the standard open surgical (OS) approach. Our hypothesis was that MIS would significantly decrease soft tissue dissection, while producing laminectomy and diskectomy dimensions comparable to the OS approach.

Methods
Sixteen large breed canine cadavers underwent lumbosacral dorsal laminectomy and partial diskectomy through either a standard OS (n=8) or MIS approach (n=8). Skin and fascial incision length, procedure time, and intraoperative complications were recorded. Postoperatively explanted specimens were evaluated for laminectomy and diskectomy dimensions and visible damage to cauda equina and exiting nerve roots.

Results
Mean length of skin and fascial incisions in the OS group were significantly longer than the MIS group ($p<0.001$ for both). Mean laminectomy length was similar between both approaches but width was significantly wider in the MIS group ($p=0.17$ and $p<0.001$, respectively). Both approaches achieved similar partial diskectomy dimensions. Surgical time was significantly longer for MIS approaches compared to OS ($p<0.001$), with a mean of 17.0 minutes for MIS compared to 11.7 minutes for OS.

Conclusion
Results indicate reduced incision lengths with comparable laminectomy and diskectomy dimensions using the MIS approach. While in this in vitro model, the MIS approach required more time to complete, this difference may diminish in clinical cases.

Clinical relevance
Dogs undergoing lumbosacral dorsal laminectomy are usually large breed dogs. The traditional open approach requires a large skin incision and soft tissue dissection, especially in an overweight dog. A minimally invasive approach accomplishing the same surgical result while minimizing soft tissue trauma could reduce post-operative pain and recovery time, and may lower wound related complications. Clinical studies are needed to confirm benefit and assess operating times in vivo.

Key words
Dog, lumbosacral, dorsal laminectomy, minimally invasive surgery, pipeline retractor

CR - 7

EFFECTS OF PERI-OPERATIVE ANALGESIA AND ANESTHESIA ON POST-OPERATIVE PAIN FOLLOWING AN EXCISIONAL WOUNDING PROCEDURE IN C57BL/6 MICE. C. Doerning, M. Hogan, A. Das, S. Roy and V. Bergdall. Veterinary Preventive Medicine, The Ohio State University

Experimental research procedures utilizing animal models have the potential to cause pain, and it is imperative this be alleviated when possible to ensure humane use of animals in research studies. Previously, analgesics were not used for wound healing studies due to their association with immunomodulation, potentially obscuring research studies at the molecular level. However, current animal research approaches require that analgesia be utilized unless proven unnecessary or detrimental to obtaining data. The goal of this project was to evaluate the effects of perioperative analgesia in the mouse used in an excisional dermal wound healing study.

Four groups of 8-week-old C57BL/6 male mice underwent isoflurane anesthesia and received perioperative subcutaneous treatments (0.003mg buprenorphine, 0.125mg bupivacaine, 0.003mg buprenorphine + 0.125mg bupivacaine [B+B], or 0.15mls saline) prior to receiving two 6.0mm full-thickness excisional wounds. Behavioral assessments including nest building, exploratory activity, and hyper-analgesia were used to assess well-being at 4, 6, 8 and 24hrs, followed by euthanasia and tissue collection for histopathological analysis.

Nest complexity scoring (NCS) revealed a significant decrease in nesting behavior for all treatment groups. Saline-treated and bupivacaine groups had significantly higher NCS than buprenorphine and B+B treated mice.

Exploratory behavior was assessed by open field testing; mice receiving buprenorphine and B+B had increased centerfield passes compared to other treatment groups. All analgesic-treated mice had significantly increased rearing behavior compared to saline-control mice.

Hyperalgesia developing in response to pain is assessed using vonFrey hairs. There was no difference between treatment groups at any of the timepoints.

Buprenorphine and B+B treated mice displayed more exploratory behavior compared to other groups, although both had impaired nesting behavior, potentially due to sedative effects of the drug. Bupivacaine-alone treatment did not alter behavior assessments compared to the saline-control. Postmortem analysis of effects of these compounds on the inflammatory response of wound healing is ongoing.

Keywords: Analgesia, surgery, C57Bl/6 mice, nociception, open field, nestlet complexity, vonFrey

CR - 8

COAGULATION DEFECTS IN DOGS WITH NATURALLY OCCURRING CHRONIC KIDNEY DISEASE AS ASSESSED BY PFA-100 AND THROMBOELASTOGRAPHY.

A. Dudley, J. Byron, E. Warry, and M.J. Burkhard. Departments of Veterinary Clinical Sciences and Veterinary Biosciences.

Bleeding is a complication of chronic kidney disease (CKD) in humans and can occur despite normal coagulation times and platelet counts. These abnormalities are caused, in part, by platelet dysfunction and abnormal platelet-vessel wall interactions. Dogs with experimentally induced CKD have prolonged mucosal bleeding times, however this test is variable and error prone. In dogs with naturally occurring CKD, coagulation defects and platelet dysfunction have not been evaluated using new point-of-care tests of platelet function and global coagulation. Our objective was to compare platelet function between healthy dogs and dogs with CKD using the PFA-100® and thromboelastography (TEG). Blood samples from 11 dogs with naturally occurring CKD and 10 healthy control dogs were collected. Platelet function was assessed by measuring PFA-100® closure times (CT) using collagen and epinephrine (Col+Epi) and Col+ADP agonists. Reaction time (R), clot formation time (K), α-angle (α), maximal amplitude (MA) and global clot strength (G) TEG variables were analyzed. Dogs with CKD had significantly prolonged PFA-100® Col+ADP CT compared to healthy dogs ($p = 0.01$). No significant difference in Col+Epi CT was found between healthy and CKD dogs. There was a significant increase in TEG MA ($p < 0.01$) and G ($p = 0.01$) in dogs with CKD compared to healthy controls. The remaining TEG variables (R, K, and angle) were not significantly different between CKD and healthy dogs. Dogs with CKD appear to have platelet dysfunction despite normal platelet counts. However, despite platelet dysfunction, these patients are hypercoagulable based on their significantly elevated MA and G TEG variables. Additional studies are needed to identify the underlying platelet defect(s) in these patients and what role they play in whole body coagulation, as well as their clinical significance.

Keywords: Platelet, Coagulopathy, Hypercoagulable, Renal

CR - 9

COMPARISON OF UNILATERAL, STAGED BILATERAL OR SINGLE-SESSION BILATERAL SURGERY FOR THE TREATMENT OF BILATERAL MEDIAL PATELLAR LUXATION IN DOGS. BA Fullagar and BF Hettlich. Department of Veterinary Clinical Sciences

Medial patellar luxation (MPL) occurs bilaterally in approximately 50% of affected dogs. Single-session bilateral surgical MPL correction in dogs has not been reported, but could shorten overall recovery and reduce owner expense. This retrospective study compared complication rates in dogs undergoing single-session bilateral MPL surgery to unilateral and staged bilateral surgery.

Dogs with bilateral MPL that underwent unilateral, staged bilateral or single-session bilateral surgery between 1999-2012 were studied. Clinical characteristics and complication rates were compared between groups and risk factors for major complications were explored. Preliminary statistical analyses were performed using Kruskal-Wallis test, Chi-square analysis and logistic regression.

40 dogs had unilateral, 16 dogs staged bilateral and 12 dogs single-session bilateral surgery (96 stifles). Median bodyweight of the single-session bilateral group was significantly ($p=0.012$) lower than the two other groups. Complications occurred in 22/96 stifles (22.9%), of which 11/96 (11.5%) required revision surgery. Timing of surgery was not significantly associated with a major complication ($\chi^2=0.205$; $p=0.925$). Tibial tuberosity transposition (TTT) was a significant predictor of major complications (Wald $\chi^2=3222.113$; $p<0.001$). No other significant predictors of complications were identified.

Increasing bodyweight and MPL grade were not risk factors for complications in this study. Although TTT has been shown to reduce reluxation rates, the procedure may increase the frequency of major, implant-associated complications. Single-session bilateral surgery for MPL is a feasible treatment option, with a complication rate comparable to unilateral or staged bilateral MPL surgery.

Keywords: medial patellar luxation, dog, complication, bilateral, unilateral

CR - 10

ACP-196: A second generation Btk inhibitor demonstrates biologic activity in a canine model of B-cell non-Hodgkin lymphoma. H. Gardner*[1], B. Harrington*[1], R. Izumi[2], A. Hamdy[2], A. Kaptein[3], B. Van Lith[3], C. London[1], J. Byrd[4], A. Johnson[4], W. Kisseberth[1].

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Bruton tyrosine kinase (Btk) signaling is known to be a critical factor in the progression of B-cell malignancies, including non-Hodgkin lymphoma (NHL), resulting in the development of targeted therapeutics that inhibit this kinase. Ibrutinib, a small molecule inhibitor of Btk, has demonstrated significant clinical activity in chronic lymphocytic leukemia and other B-cell malignancies. ACP-196 is a second generation Btk inhibitor with increased target selectivity and enhanced *in vivo* potency compared to ibrutinib. To better define the clinical potential of ACP-196 prior to the initiation of human studies, we evaluated its activity in canine B-cell NHL (cBCL) *in vitro* and *in vivo*. Using two immunophenotypically confirmed cBCL cell lines, we demonstrated potent inhibition of Btk and its downstream effectors ERK 1/2 and PLCγ2 following treatment with ACP-196 at concentrations as low as 10nM. Seventeen client-owned dogs with BCL were enrolled in a phase I dose escalation clinical trial of ACP-196. Btk occupancy was assessed using a biotin-tagged probe derived from ACP-196. At 24 hours after dosing at 2.5 mg/kg, 83-99% Btk target occupancy was observed. Partial response, as assessed by a modified RECIST scheme, was achieved in 4 dogs. Stable disease lasting over 14 days was noted in 7 dogs. Both dogs receiving 15 mg/kg twice daily were removed from the study prior to development of progressive disease due to development of adverse events, one of which was likely study related. ACP-196 was well tolerated at doses ranging from 2.5-10 mg/kg with mild gastrointestinal side effects. These data demonstrate that ACP-196 has single agent biologic activity in a spontaneous companion animal model of human NHL. Studies are ongoing in dogs with BCL to further define both regimen and dose prior to evaluation of ACP-196 in humans.

Keywords: Dog, Lymphoma, B-cell, BTK, non-Hodgkin lymphoma, BCR

CR - 11

EVALUATION OF HUMORAL AND CELLULAR IMMUNOISOLATION BY DUAL NANOPOROUS ENCAPSULATION OF MURINE ISLETS*, IN VITRO*

B Gogluizza, C Adin, F Xu, and J Ma. Dept. of Veterinary Clinical Sciences

Transplantation of islet cells provides a potential curative therapy for Type 1 Diabetes Mellitus (T1DM); however, current transplant protocols require lifelong immunosuppressive drugs. As this obligation is not clinically favorable, our laboratory has developed a dual nanoporous encapsulation technique that is intended to provide an immunoisolatory barrier for the transplantation of islet cells. The device allows for exchange of small, necessary materials such as glucose and insulin, but has been developed to exclude larger molecules and cells. We are using indirect fluorescent antibody staining and a mixed lymphocyte-islet response test to evaluate resistance to the humoral and cellular immune responses, in vitro. Our hypothesis is that dual nanoporous coating will provide a protective effect from both types of immune responses.

Keywords: Islets of Langerhans, Type 1 Diabetes Mellitus, immunoisolation

CR - 12

CAN SERUM AMYLOID A PREDICT REPRODUCTIVE EFFICIENCY IN THE POSTPARTUM OF MARES? DG Howell[a], CF Scoggin[b], and MA Coutinho da Silva[a]. [a]Department of Veterinary Clinical Sciences; [b]Claiborne Farm, Paris, KY.

Mares are often bred on the first estrus post-partum, termed foal heat (FH), and fertility is usually lower due to multiple factors including poor uterine involution and chronic inflammation/infection. Serum amyoid A (SAA) is an acute phase protein that has been shown to be a sensitive marker of inflammation in horses. We hypothesized that concentrations of SAA would remain elevated beyond Day 3 post-partum in mares with uterine inflammation/infection and that elevated SAA around the time of breeding would correlate with impaired uterine involution and decreased fertility. Thoroughbred mares were bred on FH (n = 8) or on their second estrus post-partum (Control; n = 13). All mares were evaluated on Day 4 post-partum and again on the day before breeding. Evaluations consisted of uterine evaluation via palpation/ultrasonography per rectum and endometrial culture and cytology as well as blood collection for SAA determination. Data were analyzed using Fisher's Exact Test with significance set at $P<0.05$. Overall, SAA concentrations were not correlated with the degree of uterine involution or fertility. On Day 4 post-partum, SAA was elevated (i.e. >50 mg/L) in 3/21 (14%) mares; however, only one mare (5%) had poor uterine involution. Moreover, on the same day, 15/21 (72%) mares presented moderate to severe cytological evidence of uterine inflammation. Growth of pathogenic bacteria was observed in 16/21 (76%) mares on Day 4 post-partum. On the day before breeding, SAA was below baseline in all mares in both groups. On the same day, two FH mares had cytological evidence of inflammation and two FH mares had cultures yielding pathogenic bacteria. On Day 15 post-ovulation, 2/8 (25%) FH mares and 10/13 (77%) control mares were pregnant. In conclusion, our results indicate that SAA does not appear to be a reliable indicator of uterine involution or predictor of fertility in mares.

Keywords: SAA, mare, foal heat, fertility, horse

CR - 13

EFFECT OF SERUM CALCIUM STATUS AT CALVING ON SURVIVAL, HEALTH, AND PERFORMANCE OF POST-PARTUM DAIRY COWS AND CALVES. A. Hunter[1], M. Maquivar[2], S. Bas[1], T.A. Brick[1], W.P. Weiss[3], H. Bothe[4], J. Velez[4], and G.M. Schuenemann[1]
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The objective was to assess the effect of clinical and subclinical HYPO (≤8 mg/dL) at calving on survival, health, and performance of lactating dairy cows and calves. Prepartum dairy cows (primiparous, n = 450; multiparous, n = 334) from one dairy herd were monitored for imminent signs of birth (appearance of amniotic sac outside the vulva) until birth. Calving ease, time of birth, single or multiple calves, calf sex, and stillbirth (born dead or died within 24 h after birth), BCS immediately after calving, and hygiene score of the perineum were recorded. Total serum Ca (HYPO) of cows was determined within 2 h after calving. The effect of HYPO on survival (died or culled within 30 DIM), metritis, and pregnancy per AI (P/AI) for first services of lactating cows were assessed using GLIMMIX. The effect of HYPO on calf survival, failure of passive transfer (FPT; serum total proteins ≤5.5 mg/dL), and diarrhea within 10 d of age were assessed using GLIMMIX. Diarrhea was defined as a calf presenting fluid or bloody feces (scores 2-3; 0-3 scale) and >5% dehydration or fever (≥39.5 °C). The overall prevalence of HYPO was 15%. Cows experiencing HYPO at calving had greater proportion ($P < 0.05$) of metritis (29.4%) and culling within 30 DIM (23.5%) compared to non-hypocalcemic cows (17.3% and 6.9%, respectively). The proportion of P/AI at first service was not different between HYPO (30%) and non-HYPO cows (37%; $P > 0.05$). The proportion of stillbirth and FPT was not different ($P > 0.05$) between calves born from HYPO or non-HYPO cows. However, calves born from HYPO cows had greater (49%; $P < 0.05$) proportion of diarrhea than those calves born (33.3%) from non-HYPO cows. Findings from the present study showed that HYPO at calving had significant health implications for both dams and calves.

Keywords: Cow and Calf Health, Dairy and Hypocalcemia

Note: The abstract was submitted for the 2014 Joint ASAS/ADSA meeting.

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THE EFFECT OF ANGLE SLICE ACQUISITION ON COMPUTED TOMOGRAPHIC CERVICAL VERTEBRAL COLUMN MORPHOMETRY IN GREAT DANES. A. Jurkoshek, R.C. da Costa, P. Martin-Vaquero. Department of Veterinary Clinical Sciences

Computed tomography (CT) is a diagnostic modality that is routinely used for evaluation of dogs with neurologic conditions, including cervical spondylomyelopathy (CSM). CT scans can be acquired with the transverse images aligned either parallel to the endplates or perpendicular to the vertebral canal. The purpose of this study was to determine the effect of angle acquisition on morphometric evaluation of the cervical vertebral column of Great Danes with and without CSM.

Twenty-eight Great Danes, 15 CSM-affected and 13 control dogs, underwent CT imaging. A set of images was obtained with the transverse slices aligned parallel to the endplates and another one with the transverse images aligned perpendicular to the vertebral canal. For each different set, transverse slices from the cranial, middle, and caudal aspect of the vertebral bodies from C2-C7 were evaluated. At each vertebral location the following measurements were made: height, width, transverse area, left dorsal to right ventral height (LDRV), and right dorsal to left ventral height (RDLV). Measurements were analyzed using random-effects linear regression models.

Significant differences between the measurements obtained from the two sets of transverse images acquired at different angles were found only at the cranial locations ($P<0.05$). The middle and caudal vertebral locations did not show significant differences.

The funnel-shape morphology of the vertebral canal with stenosis of its cranial aspect may be responsible for the significant differences found. Considering that the morphometric parameters showed significant differences, it is important to define and follow a standardized scanning protocol when morphometric evaluations using CT are planned.

Keywords: computed tomography, vertebral canal, angle, morphometry, cervical spondylomyelopathy

CR - 15

BLOOD CONCENTRATIONS OF GHRELIN, LEPTIN, MOTILIN AND CORTISOL IN EQUINE COLIC S. D. Hurcombe, M. Larberg, M.C. Mudge, K. Dembek and R.E. Toribio. Department of Veterinary Clinical Sciences

Purpose: In mammals, ghrelin, leptin, and motilin are believed to regulate hunger and gastrointestinal motility. However, little is known about the endogenous dynamics of these hormones in horses with colic. Our objective was to determine the concentration of these hormones and cortisol in horses with colic at admission and discharge or death and relate these to clinical findings.
Methods: Whole blood from adult horses (n=20) presenting with colic was collected at admission and discharge or death during June and July 2013. Clinical findings and a gut sound score (GSS) were recorded for all horses. Control horses (n=16) without colic were examined and blood drawn for assays. EDTA-aprotinin plasma and serum were used to determine hormone concentrations by validated assays (ELISA or RIA). Analysis of different groups of horses with colic (medical versus surgical disease, large versus small intestinal lesion, and survival versus non-survival) and admission versus discharge were performed using non-parametric testing. $P<0.05$ was significant.
Results: $Cortisol_{admission}$ was higher in colic horses compared to $cortisol_{discharge}$ (P=0.004) including medical (P=0.015) and surgical lesions (P=0.009). Horses with LI lesions had lower cortisol concentrations at discharge compared to admission (P=0.0024) but was not observed for SI lesions (P=0.25). Non-survivors had higher $motilin_{admission}$ concentrations compared with survivors (P=0.037). No other differences in hormone concentrations between various groups were observed or between admission and discharge.
Horses with a GSS >4 had lower $motilin_{admission}$ concentrations than horses with a GSS ≤ 4 (P=0.04). Significance was even greater when a GSS cut-off of ≤ 1 was used indicating a virtual absence of borborygmi. $Motilin_{admission}$ concentrations were highest in horses with poorer borborygmi (P=0.01).
Conclusions: Ghrelin and leptin concentrations did not change in horses with colic. Increased motilin was associated with decreased gut sounds and non-survival. Hypercortisolemia at admission than resolved at discharge was associated with survival.

Keywords: colic, equine, motilin, cortisol, leptin, ghrelin

CR - 16

LAPAROSCOPIC PLACEMENT AND URODYNAMIC EFFECTS OF AN ARTIFICIAL URETHRAL SPHINCTER IN CADAVERIC DOGS E. Luckring, K. Ham, C. Adin, M. McLoughlin, and J. Stull. Depts. of Veterinary Clinical Sciences and Veterinary Preventative Medicine

Urethral sphincter mechanism incompetence (USMI) resulting in urinary incontinence occurs in dogs following surgical correction of ureteral ectopia, up to 1 out of 5 female dogs following ovariohysterectomy, and is an acquired condition in male dogs. Through laparotomy, placement of a percutaneously controlled artificial urethral sphincter (AUS) has been shown effective in treating incontinence. The aim of this project was to describe laparoscopic application of the AUS and evaluate the efficacy of the AUS using urethral pressure profilometry (UPP) and uroendoscopy. We hypothesized that laparoscopic placement of the AUS would result in increased pressure across the urethral lumen.

An AUS was implanted in 10 adult female cadaveric dogs utilizing a laparoscopic technique. Maximum urethral closure pressure (MUCP) and cystourethral leak point pressure (CLPP) values, as well as cystoscopic urethral lumen occlusion data, were obtained at 0%, 25%, 50%, and 75% AUS inflation. Necropsy was performed following the procedure to assess for complications. Median±SD MUCP was 48.9±5.1 cm H_2O at 0% AUS fill, which increased to 243.5±109.0 cm H_2O following 75% inflation. Median±SD CLPP was 5.0±4.9 cm H_2O at 0%, which increased to 23.2±15.6 cm H_2O at 75% inflation. Uroendoscopic evaluation showed progressively decreasing urethral luminal area. Significant changes in UPP values as well as uroendoscopic urethral luminal area were associated with increasing inflation of the AUS ($p<0.05$). No complications were noted in any of the 10 cadavers at necropsy.

Laparoscopic placement of an AUS can be successfully performed and this approach increased UPP parameters and demonstrated visible occlusion of urethral lumen in cadaver dogs. These findings were consistent with our hypothesis and previous urodynamic studies placing the AUS device laparotomically, which would suggest a similar urodynamic outcome in cases treated with either an open or laparoscopic approach. Further studies of laparoscopic placement in clinical cases affected by USMI are warranted.

Keywords: laparoscopy, USMI, AUS, incontinence

CR - 17

CYTOKINE CONCENTRATIONS IN THE CEREBROSPINAL FLUID OF GREAT DANES WITH CERVICAL SPONDYLOMYELOPATHY. P. Martin-Vaquero[1], R.C. da Costa[1], S.A. Moore[1], A.C. Gross[2], T.D. Eubank[2]. 1. College of Veterinary Medicine, The Ohio State University, Columbus, OH. 2. Davis Heart & Lung Research Institute, The Ohio State University Medical Center, Columbus, OH.

Chronic neuroinflammation is involved in the pathogenesis of human cervical spondylotic myelopathy and may also play a role in canine cervical spondylomyelopathy (CSM). Our goal was to compare the cerebrospinal fluid (CSF) cytokine concentrations of clinically normal (control) and CSM-affected Great Danes (GDs). We hypothesized that the CSF from CSM-affected GDs would have higher concentrations of neuroinflammatory cytokines when compared to control GDs. Thirty client-owned GDs (15 control, and 15 CSM-affected) were prospectively enrolled. Dogs underwent cervical vertebral column MRI and cerebellomedullary cistern CSF collection. Routine CSF analysis (total protein, cell counts) was performed. A commercially available canine multiplex immunoassay was used to measure concentrations of granulocyte macrophage colony-stimulating factor (GM-CSF), interferon-gamma (IFN-γ), inducible protein-10 (CXCL10), interleukin (IL)-2, IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, keratinocyte-derived chemokine (CXCL1), monocyte chemoattractant protein-1/chemokine ligand 2 (MCP-1/CCL2), and tumor necrosis factor-α (TNF-α). Cytokine concentrations were compared between control and affected GDs. Associations with the administration of anti-inflammatory medications, disease duration and severity, severity of spinal cord (SC) compression, and SC signal changes were investigated in affected GDs. There were no differences between groups for IL-6, CXCL1, and IL-10. Affected GDs had significantly lower MCP-1/CCL2 than control GDs (P = 0.028). Affected GDs with severe (P = 0.002) and moderate (P = 0.022) SC compression had lower MCP-1/CCL2 than those with mild compression. There were no associations with administration of anti-inflammatory drugs, disease duration, or disease severity. IL-6 concentrations were significantly higher (P < 0.001) in GDs with SC signal changes. The remainder cytokines were below detectable limits. Lower MCP-1/CCL2 in CSM-affected GDs may compromise monocyte chemotaxis and interfere with clearance of axonal and myelin debris, delay axon regeneration, and affect recovery. Higher IL-6 in CSM-affected GDs with SC signal changes suggests more severe neuroinflammation in this subgroup of affected GDs.

Keywords: biomarker, dog, spinal cord disease, Wobbler syndrome

CR - 18

VALIDATION OF A NEW METHOD FOR EVALUATION OF MARGINS OF TUMOR RESECTION IN DOGS. K. McHenry, W.C. Kisseberth, DVM, MS, PhD, Diplomate ACVIM, D. Russell, BVMS (Hons), Diplomate ACVP and V. Subramaniam, PhD. College of Veterinary Medicine, The Ohio State University, Columbus, OH.

A new, non-invasive approach for assessing tumor margins in dogs has been developed based on the induction of eddy currents in necropsy tissue. Eddy currents are miniscule, circular currents that are produced in tissue as a result of the application of a varying electrical current through a conducting probe. By measuring eddy currents and comparing the conductivity between normal tissue and tumor, we hypothesize that margins of tumor resection can be assessed. In this study, eddy currents were measured in grossly normal tissues from dogs presented to the OSU-VMC necropsy service. A panel of normal tissues consisting of lung, liver, spleen, skin, and lymph node, was evaluated in each dog in order to characterize potential variation between individual dogs as well as different tissue types. Eddy currents were also measured in dogs with primary and metastatic mast cell tumors and surrounding normal tissue. Eddy current measurements were correlated with the histopathological assessment of the tumors and surrounding normal tissue. It is expected that eddy current measurements will vary dog to dog and in different tissues types. However, it is expected that each dog has a characteristic eddy current measurement in normal tissue and that these measurements will vary significantly between tumor and surrounding normal tissue. Complete excision of a tumor can be difficult to definitively determine both surgically and histopathologically. Ultimately, the goal of this project is to develop a hand-held probe that can be used by surgeons intra-operatively to assess tumor margins to ensure complete removal of cancerous tissue. Successful completion of this study will provide the necessary data for conducting a clinical trial using eddy currents to determine tumor-free margins intra-operatively in client owned dogs. The outcome could revolutionize the field of surgical oncology in both veterinary and human medicine.

Keywords: Eddy currents, tumor margins, surgical oncology

CR - 19

A SIMPLIFIED METHOD OF WALKING TRACK ANALYSIS FOR DOGS WITH SPINAL CORD INJURY M. Oldach, R. Song, T. Rathburn, W. Levings, S. Moore. College of Veterinary Medicine, Department of Veterinary Clinical Sciences, The Ohio State University, Columbus Ohio

Methods to objectively measure recovery in dogs with spinal cord injury (SCI) are limited. Our goal is to develop a simple, reliable method to quantify recovery in these dogs for clinical trial use. We aim to determine whether coefficient of variance in stride length (CoV-SL) and base of support (CoV-BS) calculated using a simplified walking track differs between normal and SCI-affected dogs, and whether CoV-SL correlates with the Basso, Beattie, Bresnahan locomotor scale (BBB). 20 neurologically and orthopedically normal small-breed dogs and 28 small-breed dogs with SCI from intervertebral disc extrusion (IVDE) were recruited from a veterinary teaching hospital. Walking tracks were acquired by applying colored paint to the dogs' paws and walking them down 10' of paper. 3 trials were performed in each control to establish normal values for CoV-SL and CoV-BS. SCI-affected dogs were evaluated using the same method when consistently able to step independently. A single investigator (MSO) measured stride length for each limb to calculate CoV-SL. Two investigators (WL,TR) measured distances between forelimbs and hind limbs for CoV-BS. A t-test comparing CoV-SL in normal dogs (n=20) and SCI-affected dogs (n=28) identified significant differences in all four limbs (left thoracic $p = 0.001$, right thoracic $p = 0.008$, left pelvic $p = 0.004$, right pelvic $p = 0.008$). A t-test comparison of CoV-BS in normal dogs (n=20) and SCI-affected dogs (n=26) identified significant differences in thoracic and pelvic limbs (thoracic $p = 0.01$, pelvic $p = 0.02$). CoV-SL in SCI-affected dogs showed moderate correlation with BBB scores in the left pelvic limb ($r = -0.4$, $p = 0.03$) and a trend toward significant correlation in the right pelvic limb ($r = -0.4$, $p = 0.05$). These findings support the utility of our simplified walking track to measure CoV-SL in dogs with SCI caused by IVDE.

Keywords: spinal cord injury, canine, outcome measures

CR - 20

SURGICAL NAVIGATION IMPROVES THE PRECISION AND ACCURACY OF COMPONENT ALIGNMENT IN CANINE TOTAL KNEE REPLACEMENT. Peters KM, Hutter EE, Siston RA, Allen MJ. Departments of Veterinary Clinical Sciences and Mechanical & Aerospace Engineering.

Long-term success in total joint replacement depends on accurate implant alignment. In humans, errors in positioning of the tibial and/or femoral components are associated with an increased risk of poor outcomes, including implant failure. The goal of the current study was to determine whether computer-assisted surgical navigation improves the accuracy of tibial component alignment in canine total knee replacement (TKR). Seventeen sets of TKR radiographs were reviewed to determine the incidence and magnitude of tibial component misalignment. Misalignment of greater than 3° in the frontal and sagittal planes was identified in 12% and 24% of radiographs respectively. A cadaveric study was then performed in order to compare tibial component alignment in the frontal and sagittal planes following either standard ("surgeon-guided") component placement or computer-assisted ("navigation-guided") placement. Results were compared against the current gold standard recommendations of a neutral (0° varus-valgus) cut in the frontal plane and 6° of caudal slope in the sagittal plane. Errors in frontal plane alignment were significantly reduced in navigated stifles (mean 0.41°, range -0.71° to +0.81°) than in non-navigated stifles (mean 1.24°, range -2.35° to +1.93°) ($p<0.01$). Sagittal plane alignment was also better in the navigated group (mean error 0.46°, range -0.87° to +1.59° versus a mean error of 1.08°, range -3.77° to +4.81° for the navigated and non-navigated group respectively) ($p<0.05$). These results support the hypothesis that surgical navigation significantly improves accuracy and decreases variability in tibial component alignment. Clinical trials are now needed to determine whether these improvements in surgical accuracy lead to better clinical outcomes in terms of joint function and a reduction in long-term mechanical wear of the implant.

Keywords: Computer-assisted surgery; cadaver; dog; total knee replacement

CR - 21

EFFECT OF CHOLESTEROL-LOADED CYCLODEXTRINS ON MEMBRANE CHOLESTEROL LEVELS, CAPACITATION STATUS AND POST-THAW FUNCTION OF FELID SPERM. I. A. Plourde[1], H. L. Bateman, MS[2], and W. F. Swanson, DVM, PhD[2]
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Propagation of genetically diverse felid populations would benefit from enhanced sperm cryopreservation. In felids, substituting soy-lecithin for egg-yolk in cryomedium has improved sperm cryopreservation.[3] In species such as elephants[1] and cattle[2], the addition of cholesterol-loaded cyclodextrins (CLCs) to sperm prior to freezing has been shown to benefit cryopreservation. In this study, we set out to test the effects of combining methods.

Domestic cat sperm (n=2 males, 3-4 ejaculates/male) were incubated with CLCs (0, 1.5 or 3.0 mg/ml), and cholesterol levels were measured using an Amplex Red Cholesterol Assay. Sperm from each CLC group were treated with calcium ionophore and evaluated for induced acrosomal loss. Membrane cholesterol levels were increased ($P < 0.05$) after exposure to both 1.5 and 3.0 mg/ml CLC. Pre-freeze motility was reduced and capacitation was delayed ($P < 0.05$) with 3.0 mg/ml CLC relative to treatment with 0 or 1.5 mg/ml CLC. To assess post-thaw parameters, cat sperm treated with CLCs were frozen using soy-lecithin cryomedium, thawed and then cultured in vitro. Both post-thaw motility and percentage of acrosome intact sperm were reduced ($P < 0.05$) with 3.0 mg/ml CLC, but results were similar ($P > 0.05$) between the lower concentrations. Oocytes were collected laparoscopically from domestic cats (n = 7 females, 147 oocytes total) and inseminated with frozen-thawed sperm pre-treated with 0 or 1.5 mg/ml CLC. Fertilization percentages did not differ ($P > 0.05$) between treatment groups (0 CLC, 33.3%, 25/75; 1.5 mg/ml CLC, 26.4%, 19/72). Preliminary results from a single cheetah (*Acinonyx jubatus*) and single fishing cat (*Prionailurus viverrinus*) suggest that sperm membrane cholesterol may be lower than the domestic cat. Cholesterol content appeared to increase in both species after exposure to 1.5 mg/ml CLC. In summary, our findings suggest that CLC treatment increased cholesterol content of felid sperm membranes. The higher CLC concentration was detrimental to sperm motility, capacitation and post-thaw sperm traits whereas the lower CLC concentration did not improve post-thaw sperm function in domestic cats.

LITERATURE CITED

1. Kiso, W. K., A. Asano, A. J. Travis, D. L. Schmitt, J. L. Brown, and B. S. Pukazhenthi. 2012. Pretreatment of Asian elephant (*Elephas maximus*) spermatozoa with cholesterol-loaded cyclodextrins and glycerol addition at 4°C improves cryosurvival. Reproduction, Fertility and Development 24: 1134-1142.
2. Purdy, P.H., and J.K. Graham. 2004. Effect of cholesterol-loaded cyclodextrin on the cryosurvival of bull sperm. Cryobiology 48: 36-45.
3. Vick, M.M., H.L. Bateman, C.A. Lambo, and W.F. Swanson. 2012. Improved cryopreservation of domestic cat sperm in a chemically defined medium. Theriogenology 78: 2120-2128.

Keywords: Felid, Cryopreservation, Cholesterol-loaded cyclodextrins, sperm

CR - 22

COMPUTED TOMOGRAPHIC ASSESSMENT OF PROXIMAL FEMORAL MORPHOLOGY IN DOGS. L.C. Pugliese[1], M.J. Allen[1], J. Dyce[1] [1]Veterinary Clinical Sciences

Introduction: Proximal femoral morphology is important for implant stability. Various morphologies have been proposed including stovepipe (SP) and normal (C) based upon a cylindrical or more conical shape, respectively. We hypothesized that SP femora would have a larger medullary volume, thinner cortical bone, and less dense cancellous bone than N dogs.

Materials and methods: Dogs undergoing total hip replacement (THR) were CT scanned to create three-dimensional models of the femora using computer software. Calculations of bone volume of cortical and cancellous bone within the proximal femur were made. Statistical analysis was performed using $p<0.05$ as significant.

Results: Six dogs with 3 SP and 3 C were used. The mean cortical volume, cortical bone surface area, surface are to volume ratio of cortical bone, the calculated cortical bone volume, and the medullary surface area were similar between groups. The medullary volume, medullary calculated volume, and surface area to volume ratio of the medullary canal were all statistically significantly different between groups.

Discussion: These results supported the hypotheses that the medullary canal would be larger in the SP group and that there is less trabecular bone present in the SP group, but did not support the hypothesis that there was thinner cortical bone in SP femora. This is the first study to assess three dimensional proximal morphology in dogs with clinical hip dysplasia undergoing THR as well as the first that assesses medullary features that may affect initial press fit of THR implants

Keywords: total hip replacement, dog, femur

CR - 23

EQUINE INTRADERMAL TEST THRESHOLD CONCENTRATIONS FOR HOUSE DUST MITE AND STORAGE MITE ALLEGENS AND IDENTIFICATION OF STABLE FAUNA. H.A. Roberts[1], S.D.A. Hurcombe[1], A. Hillier[2], G. Lorch[1] [1]*Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA*. [2]*Companion Animal Division, Zoetis, Florham Park, NJ, USA*

The presence of house dust mite (HDM) and storage mite (SM) fauna in the stable of the U.S. Midwest region as well equine intradermal test (IDT) threshold concentrations (TCs) are unknown. The objectives of this study were to determine IDT TCs for two HDM and three SM species in clinically normal horses over two seasons as well as to characterize the mite fauna of a stable in this region across three seasons. Intradermal tests and TCs were determined using subjective scoring as well as statistically predicted dilution concentrations. Allergen testing concentrations ranged from 1:320,000-1:5,000 w/v. Subjective measurements of the IDT reactions were scored using a scale of 0 to 4+. Threshold concentrations were defined as the highest concentration of a mite allergen where ≤10% of horses had a positive subjective reaction (≥2+) at 15 min. A random-effects logistic regression model was generated to define the exact TCs for each of the five mite species in both seasons. Samples of bedding and feed were collected from nine locations on a horse farm over spring, late summer and winter. Mites were extracted via a modified flotation method and identified by a licensed acarologist. Subjectively determined TCs were: 1:80,000 w/v for *Dermatophagoides farinae* in both seasons, 1:80,000 w/v in spring and 1:160,000 w/v in late summer for *Dermatophagoides pteronyssinus*, 1:40,000 w/v in spring and 1:20,000 w/v in late summer for *Acarus siro*, 1:20,000 w/v for *Lepidoglyphus destructor* in both seasons, and 1:20,000 w/v in spring and 1:10,000 w/v in late summer for *Tyrophagus putrescentiae*. At least one mite from each of the four genera, *Dermatophagoides*, *Acarus*, *Lepidoglyphus* and *Tyrophagus*, were detected. The determined TCs from our study differ from published recommendations for equine HDM and SM IDT dilution concentrations, suggesting the need to consider seasonal and regional influences on IDT TCs and reactivity.

Keywords: equine, intradermal, threshold, mites

CR - 24

RETROSPECTIVE EVALUATION OF CONGENITAL HEART DISEASE IN CATS, 2003 to 2013. S. Savino and B.A. Scansen. Dept. of Veterinary Clinical Sciences

Congenital heart disease (CHD) is defined as a morphologic defect of the heart present at birth. Data on CHD prevalence in cats is lacking, though an understanding of the relative prevalence of CHD is critical to advising veterinarians who treat young cats. In this retrospective study, we sought to determine the prevalence of CHD in cats less than 1.5 years of age presented to The Ohio State University Veterinary Medical Center between January 2003 and May 2013. All cases of CHD during the same time frame were included in a secondary analysis regardless of age at presentation. All records were reviewed for completeness and pertinent clinical data recorded. The client or veterinarian for all cats with a final diagnosis of CHD was contacted to determine current clinical status and, for those cats that died, the age at and cause of death. There were 202 juvenile cats presented for cardiac evaluation; the reasons for evaluation included murmur (109), at-risk breed screening (41), tachypnea / labored breathing (30), arrhythmia (3), lethargy (3), cardiomegaly (3), cough (2), syncope (2), and other (9). Of the juvenile cats, 105 (52%) had a structurally normal heart. Ninety-nine cats with CHD were identified; the most common diagnoses were mitral valve dysplasia (31%) and ventricular septal defect (18%). Nineteen cats had more than one concurrent defect. Ninety-seven of 99 (98%) cats with CHD had a murmur on examination. These data may serve to inform veterinarians about the prevalence of CHD in young cats.

Keywords: Feline; Congenital heart defects

CR - 25

USE OF MULTI-MODAL DIGITAL IMAGING TO EVALUATE THE STRUCTURAL AND FUNCTIONAL EFFECTS OF A NOVEL CATHEPSIN K INHIBITOR ON BONE IN EXERCISING HORSES. L. Smanik, J. Dulin, W.T. Drost, H. Hussein, and A. Bertone. Department of Veterinary Clinical Sciences

Cathepsin K is the lysosomal protease expressed by osteoclasts responsible for degradation of type I collagen in bone. Cathepsin K inhibitors (CKI) may target osteo-inflammatory disorders such as equine dorsal metacarpal disease. This prospective, randomized, double-blinded study evaluated the effects of a novel CKI in normal exercising horses using orthopedic imaging. We hypothesized that cathepsin K inhibition would not result in detectable alteration of mid-dorsal cortical thickness or radiopharmaceutical uptake of the metacarpus (MCIII), but may increase parameters of bone density and volume. Twelve sound horses (2-6 yrs) were administered test (n=6; 4mg/kg) or placebo-control (n=6; vehicle) treatment orally once weekly for four weeks, and were exercised on a treadmill 3 days a week to mimic training and induce active bone remodeling. To detect differences in bone remodeling between groups, mid-dorsal cortical thickness of MCIII was measured (cm) using eFilm® digital radiographic software, and lateral scintigraphic images of each forelimb were analyzed pre/post-study. Using Mirage® software, three radioactive regions of interest (ROI) of standard size were drawn over the mid-dorsal cortex of MCIII, distal epiphysis of MCIII, and dorsoproximal first phalanx (P1). Radioactivity/ROI was compared between groups. To compare bone density, volume, and surface area:volume between groups, cortical and trabecular bone biopsies (≤ 4mm diameter) were trephined from P1 and tuber coxae (TC) post-study and analyzed using microcomputed tomography. Data showed no significant difference in mid-dorsal cortical thickness, ROI radioactivity, or P1 and TC cortical bone parameters between groups. Data for TC trabecular bone showed increased bone volume and decreased surface area:volume in the test treatment group compared to the control, reflecting thicker trabeculae and more trabecular bone overall. Results indicate this CKI may inhibit bone resorption in trabecular bone in horses treated for one month and validates the CTX-1 inhibition revealed through previous pharmacokinetic evaluation of this drug.

Keywords: cathepsin K, bone resorption, horses, dorsal metacarpal disease

CR - 26

MECHANICAL QUANTITATIVE SENSORY TESTING IN NORMAL DOGS AND DOGS WITH SPINAL CORD INJURY. RB Song, SA Moore, RC da Costa. College of Veterinary Medicine, Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH.

von Frey anesthesiometry (VFA) is a method of quantitative sensory testing that provides an objective measure of sensory threshold in rodent models of spinal cord injury (SCI) for evaluation of sensory abnormalities such as hypoalgesia or allodynia. The purpose of this study was to determine the utility of VFA to measure sensory thresholds (ST) in normal dogs and SCI-affected dogs, and to document change in ST with neurologic recovery.

ST of twenty normal small breed dogs were determined in all limbs using VFA at three separate time points. Twenty small breed dogs with spontaneous SCI caused by thoracolumbar intervertebral disc extrusion were then prospectively evaluated by a single investigator at 3, 10 and 30 days postoperatively. A mixed effect statistical model incorporating repeated measures was used for data analysis. In normal dogs, there was a progressive decrease in ST for all limbs across three testing sessions, and values from sessions two and three were significantly lower than those from session one ($p< 0.05$). There was no statistical difference between values from sessions two and three. In SCI-affected dogs, there was no significant change in thoracic limb sensory threshold values over time ($p >0.05$). However, there was a significant decline in ST between days 3 and 10 for both pelvic limbs ($p <0.01$) and between days 10 and 30 for the left pelvic limb ($p =0.04$).

These findings suggest a possible training effect for the investigator and/or an acclimation effect for the subject associated with VFA. With optimization of technique and an understanding of possible training or acclimation effects, we conclude that VFA may have utility for quantitative sensory testing in dogs with SCI for future clinical trials.

Keywords: spinal cord injury, trauma, intervertebral disc disease, sensory function

CR - 27

OUTDOOR CONTAINMENT SYSTEMS AND THEIR EFFECT ON CANINE BEHAVIOR AND PUBLIC SAFETY. N. Starinsky, M. Herron, and L. Lord. From the Depts of Veterinary Clinical Sciences and Preventive Medicine at The Ohio State University College of Veterinary Medicine.

Undesired behavior of dogs, particularly aggression, can have severe consequences for the human-animal bond as well as public safety. It is thus important that dogs with a history of fear or aggression issues be properly contained on their properties or separated from general public access. This study attempted to examine the effects of outdoor containment systems on dog behavior. Dog owners entering several local pet stores in Columbus, Ohio were surveyed about means of confining their dogs on their property and whether any adverse behaviors were noticed in their dogs. A total of 1,053 dogs had compete information returned from these pet owners. Four types of fencing were identified by dog owners, including solid fencing (n=163), see-through fencing (n=658), electronic fencing (n=150), and tethers/tie-outs (n=82). A total of 279 dogs (26.50%) were reported by owners to escape their containment systems. Dogs contained by invisible/electronic fence were significantly more likely to have escaped their property than dogs confined by other means ($p < 0.0001$). Importantly, 48 dogs (4.56%) have a history of biting a person entering their property while being contained. Dogs which had access to their yards while the owner was not home were significantly more likely to have bitten a person ($p=0.017$).Results of this study may have great impact on the decisions pet owners make in regards to confining animals on their property. If a public safety issue is discovered in this population of pets, perhaps dog and property owners will consider alternative forms of outdoor confinement.

Keywords: dog bites, public health, confinement, aggression, electronic fences, tethers

CR - 29

DEVELOPMENT AND VALIDATION OF A LOW-FIDELITY, LOW-COST SURGICAL SIMULATION MODEL TO TEACH CANINE CLEIOTOMY. E. Sweazy, T. Motta, B. Carter, L. Hill, and M. McLoughlin. Department of Veterinary Clinical Sciences

Financial and ethical constraints have created a need for model use in surgical training of veterinary students. The aim of this study was to develop a low-fidelity, low-cost canine celiotomy model, evaluate veterinary students' perception of the model, and determine the model's validity.

A low-fidelity, low-cost surgical model for celiotomy was created using silicone and different fabrics. Twenty-four first or second year veterinary students trained to perform a celiotomy using the following resources: live/podcasted lecture, handout, and videos. Half of these students received the celiotomy model and an hour of training with the model (simulation group) while the other half received no additional training (control group). Students were video recorded performing a celiotomy on a cadaver. A quiz was given before the surgery to access their knowledge retention. All students answered a questionnaire in order to determine students' perception of the model. The video was graded by an experienced surgeon according to a detailed rubric that included seven specific skill sets. Surgical time was documented at ten different steps.

Data from the questionnaire revealed that the most popular teaching resources were the simulator and the one-on-one training on how to use the simulator. Students voluntarily invested an average of 4.3h training with the simulator. The simulator was found to be extremely helpful (82% of students) and the model helped or somewhat helped improve their performance and confidence, and decreased their stress (100% of students) during their recorded procedure. The scores of recorded students' surgeries showed that the improvement on the performance score by students using the model was significant for the overall surgery ($p<0.001$) and six out of the seven skill sets sections. Currently only one of the graders has submitted their scores. This study improved students' surgical skills, ensuring new graduates will have less surgical complications improving animal well-being.

Keywords: surgery; training; simulator; low-fidelity; celiotomy

CR - 30

CHANGES OF THE EQUINE FECAL MICROBIOME IN RESPONSE TO ANTIMICROBIAL DRUGS. J. M. Swink, R. S. Liepman, S. D. Hurcombe, P. N. Boyaka, S. E. Dowd, and R. E. Toribio

Background: Antibiotic-induced enterocolitis is frequent and sometimes fatal condition of horses treated with antimicrobial drugs. It is likely it results from changes in composition of the intestinal microbiota. The development of high throughput bacterial DNA 16S rRNA gene sequencing has provided a tool to determine the baseline gastrointestinal microbiome in a number of species, however, microbiome information in horses is lacking. The **goals** of the study were to establish the baseline intestinal microbiome of horses in Ohio and investigate the effect of different antibiotics on this microbiota over time.
We **hypothesized** that 1) The equine microbiome will be unique with described and undescribed bacterial species, and 2) Antibiotic treatment will alter this balance.
Methods: Our investigation had two parts using 22 healthy horses: a pilot (n=6) and the main study (n=16). The pilot study consisted of three treatment groups: ceftiofur (n=2), enrofloxacin (n=2), and saline control (n=2). The main study included four groups: ceftiofur (n=4), enrofloxacin (n=4), oxytetracycline (n=4), and saline control (n=4). Horses were fed similar diets and were housed under the same conditions. Antibiotics were administered daily for 3 (pilot) and 5 (main) days. Fecal samples were collected daily for a week. Bacterial DNA was extracted and submitted to 454-pyrosequencing of the 16S rRNA gene. After processing, data analysis was performed using including R, MOTHUR, and Excel.
Results: Baseline data showed unique bacterial distribution with known and unknown bacteria that was influenced by antimicrobials. *Bacteroidetes, Firmicutes, Spirochaetes,* and *Tenericutes* were the most dominant phyla horses. Of interest was a decrease in the proportion of *Verrumicrobia* and *Proteobacteria* in response to enrofloxacin.
Conclusions: The equine gastrointestinal microbiome is a complex structure that can be affected by antibiotics used in equine practice. This is the first study to establish the baseline fecal microbiome of horses in central Ohio. This study has clinical relevance in understanding equine gastrointestinal biology as well as in the use of antimicrobials in horses.

Keywords: Microbiome, equine, antibiotics, pyrosequencing

CR - 31

SUTURE-FREE TECHNIQUE FOR CANINE URETERAL RESECTION-ANASTOMOSIS USING A MICROVASCULAR ANASTOMOTIC SYSTEM: A CADAVERIC STUDY. V. Wavreille, C. Adin, J. Arango, K. Ham, J. Byron and M. McLoughlin. Department of Veterinary Clinical Sciences.

Objective: To describe a suture-free technique of canine ureteral resection-anastomosis using a microvascular anastomotic system (MAS) and to compare surgical time and burst pressure of hand-sewn (HS) ureteral end-to-end anastomosis with the MAS technique.
Study Design: Experimental ex-vivo study.
Animals: Canine cadavers (n = 8).
Methods: For each cadaver, one ureter was randomly assigned to undergo HS anastomosis while the contralateral ureter underwent MAS anastomosis. The first 3 cadavers (6 ureters) were used to refine the MAS technique. In the remaining 5 dogs, surgical time and ureteral burst pressure were compared between groups (n = 5 ureters per group).
Results: Preliminary procedures showed that selective impaling of the mucosa and submucosa (without muscularis and adventitia) is necessary to allow complete mechanical interlock of the anastomotic rings for the MAS technique. Median anastomotic time was significantly shorter for the MAS technique (7.6 vs. 16.6 minutes, P = 0.0286) and burst pressure was significantly higher for the MAS technique (393 vs 180 cm H_2O, P = 0.0119).
Conclusion: This study demonstrated the feasibility of a suture-free technique of canine ureteral resection-anastomosis using a commercially available microvascular anastomotic system. The MAS anastomosis was faster and had higher burst strength compared to the HS anastomosis.

Keywords: ureter, canine, resection-anastomosis, suture free technique

CR - 32

DEVELOPMENT OF A VIRTUAL REALITY SIMULATOR FOR TEACHING CANINE ARTHROSCOPY. J. Yoo[1], T. Motta[1], B. Hittle[2], D. Stredney[2], M. Allen[1] [1]Dept of Veterinary Clinical Sciences and [2]Ohio Supercomputer Center. The Ohio State University Columbus OH 43210

Veterinary schools have been under pressure from both the public and their own students regarding the use of animals in teaching. Our overall objective was to limit the use of animals or cadavers and prepare surgeons in training by using simulation tools that provide for on-demand practice. Our specific goal was to develop a virtual simulator for performing a canine arthroscopy of the stifle joint. We present our current developments, and describe our future goals.

We developed a high-resolution 3-dimensional computer model of the canine stifle joint. To do so, images were acquired using computed tomography and magnetic resonance scans and the bony and soft-tissue structures were segmented. This model was then coupled to a force-feedback (haptic) device to create a virtual reality simulator. Currently our simulator provides visualization of the joint and allows for interactive manipulation. The simulator allows the surgeon in training to arbitrarily section through the hard and soft tissues providing an additional method for learning the spatial configurations of the regional anatomy.

Our ongoing development includes additional tools to physically investigate the canine stifle joint, during this stage, experts will assist in validating the simulator for its realism as well as help provide metrics for which trainees can be evaluated, thus providing an avenue to introduce automated assessment.

Once validated by experts, trainees can be evaluated and we will move towards our objective of employing simulation technologies to replace the use of cadaver materials in the formative development of surgical technique for canine arthroscopy.

Keywords: Surgery, Arthroscopy, Cadaver, Cruciate ligament, Canine stifle, Bony, Soft tissue, CT, MRI, 3D, Haptic feedback, Simulation, Validation

CR - 33

MOTION CAPTURE ANALYSIS OF THE MECHANICAL PERFORMANCE OF A NOVEL PEDICLE SCREW-ROD FIXATION SYSTEM FOR THE CANINE LUMBOSACRAL JOINT. C. Zindl[1], A.S. Litsky[2], N.R. Crawford[3], N. Fitzpatrick[4], M.J. Allen[1] ([1]Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio, USA, [2]Davis Medical Research Center, The Ohio State University, Columbus, Ohio, USA, [3]Barrow Neurological Institute, Phoenix, Arizona, USA, [4]Fitzpatrick Referrals, Eashing, UK)

Degenerative disorders of the canine lumbosacral (L-S) joint often result in pain and instability, necessitating surgical decompression and stabilization. The goal of the current study was to determine the effects of a combination of a new polyaxial pedicle screw-rod system and a tapered intervertebral traction screw on lumbosacral motion. We hypothesized that destabilization results in an increase in L-S motion and that the new spinal fixation system will effectively eliminate motion at the L-S joint.

Eight cadaveric lumbosacral spines (L4-Cd1) were harvested and prepared for mechanical testing. Specimens were mounted on a 4-point bending jig and tested in flexion, extension and lateral bending using axial loads of between 0 and 150 Newtons. Angular displacement was recorded from optical trackers rigidly secured to L6, L7 and S1. Data were collected from intact spines, after laminectomy/discectomy at the lumbosacral junction, and after surgical stabilization with the new implant system.

As compared with the intact spine, laminectomy resulted in a mild increase in angular displacement at L6-L7 and a modest increase at L7-S1. Instrumentation effectively eliminated motion at L7-S1 with significant decrease ($p<0.01$) of angular displacement irrespective of loading direction compared to intact and destabilized specimen. Instrumentation of L7-S1 was associated with an increase of motion at L6-L7.

Our data support the hypothesis that instrumentation with the new spinal fixation system results in measurable reductions in lumbosacral instability. Use of polyaxial screws allow for greater versatility when linking the screw to the connecting rod. Deployment of an interbody spacer permits distraction and prevents collapse of the interbody space. Although there was evidence of an increase in motion at the adjacent level (L6-L7), this effect was small.
In conclusion, the novel pedicle screw-rod fixation construct is a versatile surgical fixation system capable of restoring stability to the lumbosacral junction following surgical decompression in dogs.

Keywords: degenerative lumbosacral disease, surgical stabilization, pedicle screw-rod fixation, biomechanics

EPIDEMILOGY
AND
APPLIED RESEARCH

EAR - 1

PREVALENCE OF ANTIMICROBIAL RESISTANCE IN WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) IN NORTHEASTERN OHIO. G. Ballash, D. Mollenkopf, T. Wittum, P. Dennis

Environmental contamination with antimicrobial resistant (AMR) coliforms has been poorly characterized in population dense areas where intensive farming is not particularly abundant. In this study, we used white-tailed deer (*Odocoileus virginianus*) as an indicator species to characterize the presence and prevalence of AMR *E. coli* in suburban/urban reservations in Northeastern Ohio. *E. coli* was isolated from 119/122 deer fecal samples (97.5%) collected in the Cleveland Metroparks and screened for cephalosporin and fluoroquinolone resistance. Eighty-five cefoxitin resistant isolates (71.4%) and seven cefepime resistant isolates (8.2%) were phenotypically determined to be AmpC/ESBL producers. $bla_{CTXM-15}$ was the only ESBL gene identified to confer cefepime resistance to *E. coli*. A representative sample of the AmpC producing *E. coli* showed 72% (16/22) had the bla_{CMY-2} gene present. Twenty one isolates (17.2%) were resistant to ciprofloxacin, indicating mutations in quinolone resistance drug regions (QRDR) of genes *gyrA* and *parC*. Sequencing of these genes revealed that all isolates possessed both resistance-inducing mutations in the *gyrA* and *parC* gene sequences. These data demonstrate that white-tailed deer are commonly infected with AMR *E. coli* of animal and public health concern in suburban/urban habitats, suggesting environmental contamination with zoonotic AMR *E.coli*.

Keywords: Antimicrobial resistance, Cephalosporin, *E. coli*, Fluoroquinolone, Urban, White-tailed deer

EAR - 2

DETERMINING THE FREQUENCY OF TYPE A INFLUENZA VIRUS INFECTIONS IN EXHIBITION SWINE UPON ARRIVAL AT AGRICULTURE EXHIBITIONS AND ASSOCIATED RISK FACTORS. 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 viruses (IAV) infecting humans because pigs are considered to be a major “mixing vessel” in which reassortment of the IAV segmented genome can occur. Exhibition swine, due to the distinctive management practices under which they are reared, provide a critical human-swine interface allowing for the bidirectional zoonotic transmission of IAV. Uniquely, these exhibition pigs come into contact with not only their handlers/owners but also large numbers of other swine and the general public. While the presence of IAV at agriculture exhibitions has been documented post comingling of the pigs, little is known about the number of pigs that arrive at exhibitions already infected with IAV. In 2013, a pilot study was conducted at two agricultural exhibitions. Snout wipes were used to sample pigs as they entered the. Samples were screened using rRT-PCR and if positive inoculated onto MDCK cells for virus isolation. Based on this limited sampling suggestive of a prevalence level of 2.18% (48/2194). Expansions upon this project are needed to study a wider demographic of agriculture exhibitions in order to better estimate the true prevalence of IAV in the exhibition swine upon arrival. Thus in 2014, 300 exhibition swine will be sampled via snout wipes upon entry at 8 agricultural fairs for a total of 2400 samples. In addition, a survey will be administered to the swine farmers to determine the on farm management history of the exhibition swine. Snout wipes and surveys will be linked to asses if there is a correlation between IAV incidence and certain management practice such as vaccination and on farm biosecurity. This research will yield a better understanding of prevention and control of IAV in both the swine and human populations.

Key words: Influenza A virus, swine, risk factors, fairs, surveillance

EAR - 3

ANTIBIOTIC RESISTANCE IN AN ORGANIC DAIRY PRODUCTION SYSTEM

JK Cenera, BM DeWolf, DF Mollenkopf, CA King, TE Wittum; Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH

According to World Health Organization, the increasing development of bacterial resistance to critically important antimicrobial drugs is one of the most important food safety issues worldwide. The frequent use of antimicrobial drugs in agriculture and veterinary medicine has led to real concerns that the resulting selection pressure has driven the emergence of resistant bacteria of public health importance entering the food supply. This fear has resulted in an increase in consumer demand for products from organically raised animals due to the belief that they are healthier and free of antimicrobial-resistant bacteria. Additional scientific data are needed to better understand the public health risks and/or benefits associated with the consumption of products from organically-raised livestock compared to conventionally-raised animals. We investigated the prevalence of three extended-spectrum cephalosporin resistant *E. coli* phenotypes and *Salmonella* spp. from cows belonging to a large organic dairy production system. One hundred fecal samples were collected from six farms, 4 in Colorado and 2 in Texas for a total of 600 samples. Each sample was cultured for *E. coli* resistant to extended-spectrum cephalosporins and *Salmonella* spp. using selective media. *E. coli* with an AmpC resistance phenotype were recovered from 448 samples (74.7%) while *E. Coli* resistant to cefepime were recovered from 137 samples (22.8%). We were unable to recover meropenem-resistant *E. coli* from any samples. *Salmonella* spp. were recovered from 108 samples (18.0%). Characterization of samples (PCR to confirm genotype, plasmid profiles, conjugation experiments and sequencing) was performed to better evaluate and understand the presence of the antibiotic resistance pathogens recovered from the samples. We also reviewed the distribution of *Salmonella* and *E.coli* phenotypes between farms and found considerable variability in the recovery rates. The large differences will require further investigation of each facility's management practices in addition to the further characterization of our isolates.

Keywords: antimicrobial resistance; food-borne pathogens; antibiotic use; organic dairy; food supply; public health

EAR - 4

ASSESSMENT OF MANAGEMENT PRACTICES ASSOCIATED WITH *STAPHYLOCOCCUS AUREUS* PREVALENCE IN OHIO DAIRY FARMS. L. da Costa, PJ. Rajala-Schultz; G. Schuenemann Department of Veterinary Preventive Medicine

Mastitis remains a costly disease in dairy herds, with *Staphylococcus aureus* (SA) being the most important contagious pathogen causing mastitis. Management practices play a crucial role in controlling this disease and for a successful control plan, knowledge about the organism's prevalence and understanding of prevailing risk factors in a herd are fundamental.
The study objectives were 1- to estimate the prevalence of SA in Ohio dairy bulk tank milk (BTM) and 2- to determine management practices that are associated with isolation of SA.
Material/Methods Questionnaire about herd characteristics, mastitis control, biosecurity, and heifer raising practices was mailed to 780 Ohio dairy producers and permission to test BTM was requested. Up to three samples/herd were cultured and a herd was considered SA-positive if at least one BTM sample was positive. Logistic regression was used to assess the second objective.
Results Response rate was 49.2% and BTM samples from 308 herds were cultured. *SA* prevalence was 44% if one BTM sample/herd was considered, and 64% considering two BTM samples. Cumulative herd prevalence was 69% when using three samples/herd.
Individual milking procedures important for mastitis control were adopted by most herds; 59% of herds practiced pre-stripping, 89% pre-dipping and 97% post-dipping, but only 30% practiced all together. Of the herds reporting to be open, 82% did not quarantine new animals before introducing to the existing herd (71% of those SA-positive) and 18% practiced quarantine (54% SA-positive) (P=0.08). Suckling between heifers was noticed in 70% of the herds with 71% of those SA-positive, and 65% of herds where no suckling was observed were SA-positive (P=0.34). Chance of isolating SA was higher in herds having access to pasture or an exercise lot (*P*=0.04).
Conclusions Herd prevalence of SA in Ohio dairies is high with few producers applying all milking procedures simultaneously. Improvement needed in biosecurity.

Key words: prevalence, *Staphylococcus aureus*, management practices, mastitis.

EAR - 5

EXTENDED- SPECTRUM CEPHALOSPORIN RESISTANT SALMONELLA AND ESCHERICHIA COLI FROM BROILERS AT SLAUGHTER. B DeWolf, D Mollenkopf, J Cenera, C King, T Wittum. The Ohio State University, College of Veterinary Medicine, Columbus, OH.

Coliform bacteria harboring beta-lactamase genes conferring resistance to important extended-spectrum cephalosporin drugs are commonly present in US livestock populations. These bacterial resistance genes including *bla*$_{CMY-2}$ and *bla*$_{CTX-M}$ have been implicated in human food-borne infections resistant to antimicrobial therapy. The widespread use of ceftiofur in large populations of poults at the level of centralized hatcheries could support the emergence and dissemination of *E. coli* and *Salmonella* strains resistant to extended-spectrum cephalosporins that may ultimately contaminate fresh retail meat products. To quantify the frequency of recovery of coliform bacteria extended-spectrum cephalosporins, swabs of broiler transport crates containing fecal material at a single poultry processing facility in Ohio were screened using selective media in the summer of 2013. Samples were collected weekly from the floor of transport cages that had been recently unloaded at the processing facility. To date, 275 fecal samples and 12 ground chicken samples have been evaluated from a total project target of 500 fecal and 50 ground chicken samples. Coliform bacteria harboring *bla*$_{CMY}$ have been recovered from 98% of fecal and 88% of meat samples, while E. coli harboring *bla*$_{CTX-M}$ have been detected in 21% of fecal and 14% of meat samples. No samples have been found to carry carbapenemase-producing organisms to date. Salmonella have been recovered from 24% percent of fecal and 3% of meat samples. Pending work will fully characterize isolates expressing this phenotype using MICs, PFGE, plasmid profiling, plasmid incompatibility group typing, and gene sequencing. By quantifying the frequency and dissemination of extended-spectrum cephalosporin resistant enteric bacteria in broilers at slaughter, we expect to provide valuable information to assess the human health risk of exposure to food-borne zoonotic infections resistant to antimicrobial therapy.

Keywords: Antimicrobial resistance, salmonella, E. coli, cephalosporin resistance, coliform bacteria, poultry

EAR - 6

DISTRIBUTION AND DIVERSITY OF *SALMONELLA* FROM LESS COMMON SOURCES: HATCHLING CHICKS, DUCKLINGS AND PET HEDGEHOGS.

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Salmonella infections are commonly acquired from food but can also be acquired from live animal contact. Important animal sources of *Salmonella* infections in people, particularly children, are poultry hatchlings and pet hedgehogs. In 2013, eight multistate outbreaks and 500^{+} confirmed cases of Salmonellosis were traced to hatchling chick or duckling contact. In 2012-2013, one outbreak with 26 confirmed cases of Salmonellosis was associated with pet hedgehogs.

The objectives of this study are to characterize the diversity and antimicrobial resistance of *Salmonella* shed by hatchlings from mail-order hatcheries and hedgehogs from breeding farms.

During spring 2013, 36 farm stores in 13 states collected swabs from arriving hatchling shipments. Swabs were submitted to Ohio State University for *Salmonella* culture, with shipment tracking information for analysis. In summer 2013, swabs were collected from hedgehog cages at a breeding farm. Swabs were cultured for *Salmonella* and epidemiological characteristics were analyzed. 27% of swabs from hatchling shipment boxes and 53% of swabs from hedgehog cages cultured positive.

Serovar identification, pulsed field gel electrophoresis (PFGE), and antimicrobial susceptibility testing was performed on isolates at the National Veterinary Services Laboratory. Ten serovars and 23 distinct PFGE patterns were recovered from hatchlings, and these represented multiple source hatcheries in at least three different states. A single serovar and two PFGE patterns were recovered from the hedgehog herd, which represented one breeding farm in one state. The majority of recovered isolated were either pan-susceptible or resistant to only tetracycline or streptomycin.

From 2012-2013 *Salmonella* Typhimurium caused hatchling and hedgehog-associated outbreaks. *S.* Lille, *S*. Newport, and *S*. Infantis also caused hatchling-associated outbreaks. Study swabs were taken concurrent with human outbreak strain exposure and isolates recovered in this study reflect recovered human outbreak isolates. Shipment information form swabbed hatchling boxes was used to identify hatchery sources of outbreak associated *Salmonella* strains.

Keywords: *Salmonella*, hatchlings, hedgehogs, antimicrobial resistance

EAR - 7

THE ASSOCIATION BETWEEN METABOLIC STRESS LEVELS AND SHEDDING OF *Salmonella enterica* IN TRANSITION DAIRY CATTLE. R. Digianantonio**,** G. Schuenemann, S. Bas, L. Vargas-Munoz, and G. Habing. Dept. of Veterinary Preventive Medicine

As the most frequent cause of foodborne hospitalizations and deaths in the United States, *Salmonella enterica* has a significant impact on public health. The bacterium is a natural inhabitant of the gastrointestinal tract of most animals and is an opportunistic pathogen that is more prevalent in immunocompromised hosts. Food animals can be a significant source of the pathogen in consumer products, and it is important to understand what makes animals more likely to shed *Salmonella* . Increased fat mobilization due to negative energy balance in transition dairy cows is associated with immune suppression and increased susceptibility to pathogens. However, limited information is available on the association between levels of metabolic stress and the prevalence of important foodborne pathogens. Thus, the purpose of the present research was to perform a longitudinal observational study to test the hypothesis that cows with higher levels of metabolic stress are more likely to shed *Salmonella*. In total, 105 dairy cows were sampled every two weeks over a six week period, starting three weeks prior to their expected calving date. Fecal samples were collected on each sampling day and cultured for *Salmonella* using standard enrichment methods and plated onto selective media. Blood samples were collected and prepartum and postpartum non-esterified fatty acid concentrations and β hydroxybutyric acid concentrations were analyzed to determine metabolic stress. In total, 43% (162/378) of samples were positive for *Salmonella* across the sampling period. The data suggests that shedding was higher in samples collected closest to parturition, a known stressful period, with prevalence of 39%, 47%, 60%, and 39% at -3 weeks, -1 week, 1 week and 3 weeks relative to parturition, respectively. High levels of metabolic stress were frequent in the sampled cows; however, further analysis is necessary to determine the association between metabolic stress and *Salmonella* shedding in dairy herds.

Keywords: Salmonella enterica, Negative energy balance, Metabolic stress

EAR – 8

MULTILOCUS SEQUENCE TYPING OF *MYCOPLASMA SYNOVIAE.* M. El-Gazzar, K. McDonald, and R. Slemons. Department of Veterinary Preventive Medicine.

Mycoplasma synoviae (MS) is an important mycoplasma pathogen of poultry. The variable Lipoprotein Hemagglutinin A (*vlhA*) gene is currently the only target used for MS sequence typing. If two MS strains are identical on the partial *vlhA* sequence, clonality is inferred; however, if their sequence is different at this locus it is difficult to identify the degree of relatedness between them. In this study we propose the Multi-Locus Sequence typing (MLST) assay as an additional tool for MS sequence typing. Twenty-four house keeping genes were studied as potential targets, from which 7 genes were selected for the assay. An internal segment of ≈ 600 bp from each of the 7 genes was successfully amplified and sequenced for 58 different MS isolates or positive clinical samples. The collective sequence of all 7 gene segments was used for MS sequence typing. Using MLST; the 58 MS strains were organized into 30 different sequence types and also 30 sequence types when typed using the *vlhA* assay. Multiple positive clinical samples obtained from MS outbreaks in the same geographical vicinity differed in *vlhA* sequence. The same samples were identical on all 7 MLST gene sequences, showing that they belong to the same sequence type. Other samples were identical on *vlhA* but different on MLST. The phylogenetic tree generated by MLST was more congruent to the epidemiological information than the tree generated by *vlhA* assay. MLST identifies more coherent epidemiological relationships between outbreaks. We suggest that MLST and *vlhA* could be used in tandem. In our view MLST represents a valuable tool for MS sequence typing, providing better understanding of the source of infection and the epidemiology of the disease.

Keywords: Multilocus – sequence typing – *Mycoplasma synoviae* – MLST – Epidemiology – *vlhA* gene.

EAR - 9

THE GENETICS OF INFLUENZA A VIRUSES IN MISSISSIPPI FLYWAY MIGRATORY BIRDS. A.C. Fries, J.N. Nolting, R.D. Slemons Depts of Veterinary Preventive Medicine and Evolution, Ecology and Organismal Biology, Columbus, Ohio USA

Gene flow is often restricted by the geographic distance between populations but alternative ecological parameters may influence the successful migration of genes when distance does not affect population connectivity. It is widely assumed that wild bird migratory activity move the genetic diversity of influenza A viruses between geographic localities but this hypothesis is rarely tested using a comprehensive surveillance system that addresses both temporal and spatial distances. From 2008 to 2011, surveillance for avian-origin influenza A viruses (AIV) commonly infecting migratory waterfowl (Order Anseriformes) was conducted along the North American Mississippi migratory bird Flyway. This study sought to examine the temporal patterns of genetic structure and gene movement in AIV across multiple migratory seasons as birds left their northern staging areas and travelled to wintering locations in the southern United States. AIV isolates (n=297) were obtained from birds across three seasons and isolates were sequenced for all eight genomic segments. We observed high nucleotide sequence similarity (>99%) in pairwise comparisons of isolate segments across the entirety of the migratory flyway within a season. However, phylogenetic analyses identified genetic structuring of all eight AIV segments. In addition, similarity measurements identified patterns of regional persistence of viral segments over time as well as patterns of transient virus constellations that were negatively associated with time and distance. Results suggest that while AIV genetic segments do readily move across the Mississippi Flyway within a migratory season the pattern of genetic structuring and similarity over time indicates the persistence of regional genetic lineages. This study identifies potential factors influencing AIV natural history and indicates the importance of comprehensive surveillance efforts to understand virus infections in these key reservoir species for influenza A virus genetic diversity.

Keywords: Genetic structure, Influenza A virus, Isolation by distance, Migratory waterfowl, Phylogenetics

EAR - 10

COMPARISON OF REAL-TIME REVERSE TRANSCRIPTION-PCR AND VIRUS ISOLATION WITH EMBRYONATED CHICKEN EGGS FOR THE DETECTION OF INFLUENZA A VIRUS FROM WILD WATERFOWL E. Gerken, R.D. Slemons, J.M. Nolting, A.S. Bowman. Department of Veterinary Preventive Medicine

Surveillance of influenza A virus (IAV) in natural waterfowl reservoirs is crucial for early detection of virus strains that threaten the health of poultry and/or human populations. Virus isolation efforts are time consuming and expensive; advances in nucleic acid purification and PCR technology led us to re-compare rRT-PCR and virus isolation using embryonated chicken eggs (ECE) on cloacal samples from free-ranging, wild waterfowl. PCR screening methods were predicted to improve detection of IAV from wild waterfowl cloacal samples, when compared to current ECE methods. A total of 1373 cloacal swabs collected from wild waterfowl across six states during 2012 and 2013 were tested in parallel with rRT-PCR and ECE inoculation. Additionally, rRT-PCR positive samples that were negative for hemagglutinating activity after one passage in ECE were passaged in ECE a second time. Of the total samples, 351 (25.56%) were positive with rRT-PCR and 156 (11.36%) were positive through first passage in ECE. An additional 93 (6.77%) isolates were recovered with a second passage in ECE, increasing the total isolates by 59.62%, which resulted in 249 total isolates, an 18.14% overall frequency of virus isolation. While the sensitivity of rRT-PCR is high, 19 (1.38%) samples that yielded isolates through first pass ECE were rRT-PCR negative. The results of this study indicate screening original samples with rRT-PCR before isolation in ECE would be an effective way to conduct IAV surveillance in wild waterfowl. Other labs have used this approach successfully but did not utilize second passage in ECE to isolate IAV. Further studies are needed to investigate the impact of host and environmental variables on the accuracy of both rRT-PCR and virus isolation.

Keywords: Influenza A virus, Real-Time Reverse Transcription-PCR, Embryonated Chicken Eggs

IMPACT OF MILK CESSATION METHOD AND DAILY MILK YIELD AROUND DRY-OFF ON INTRAMAMMARY INFECTION STATUS AT CALVING. P.N. Gott, P.J. Rajala-Schultz, G.M. Schuenemann, and M.A. Masterson. Department of Veterinary Preventive Medicine.

Introduction

The dry period is crucial for maintenance of good udder health of dairy cows. Increasing milk yield at dry-off increases the risk of intramammary infections (IMI) at calving. Most cows are currently dried off via abrupt cessation of milking, even though earlier studies have shown that uninfected quarters dried off abruptly had significantly more IMI at calving than gradually dried quarters. The **objective** was to assess the impact of milk cessation method and daily milk yield near dry-off on IMI status at calving to identify the dry-off method most beneficial to udder health in the subsequent lactation.

Materials and Methods

Data from three Ohio dairy herds were used for these analyses. Cows were enrolled 7-12d prior to dry-off and randomly assigned to either abrupt or gradual cessation of milking. Gradual cessation cows were milked once per day for the final week of lactation while abrupt cessation cows kept the farm's regular milking schedule. Aseptic quarter foremilk samples were collected at enrollment (PRE), dry-off (DRY), and within 3d of calving (CALV) to determine quarter infection status at each event.

Results and Conclusion

Preliminary analyses were conducted on data from 582 quarters. Infection status between the study groups did not differ at any time point ($P>0.05$). The overall proportion of quarters infected at CALV was significantly lower than the proportion infected at PRE or DRY in both groups (McNemar's Chi^2 $P=0.013$, and $P=0.014$, respectively). On average, gradual cessation of milking decreased daily milk yield by 27% (11.6 lb). Additionally, for every 10-lb increase in daily milk yield at PRE, the odds of IMI at CALV were increased 32% (SAS-PROC GLIMMIX $P=0.1143$).

In conclusion, preliminary results suggest that gradual cessation of milking may help reduce IMI at calving by decreasing milk yield prior to dry-off compared with abrupt dry-off.

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

EAR - 12

THE ISOLATION AND CHARACTERIZATION OF SALMONELLA FROM SWINE FECES IN KENYA. A. Haftman (MPH-VPH), W. Gebreyes (VPM), G. Habing (VPM), M. Pennell (Public Health)

Non-typhoidal Salmonella (NTS) is an important pathogen that causes foodborne diseases in both humans and also gastrointestinal illness in animals. Since the 1990s, antimicrobial resistance in NTS has become a global concern. In Africa, non-typhoidal Salmonella is consistently a leading cause of bacteremia among immunocompromised people. Yet, the sources and transmission routes of Salmonella in developing countries are poorly understood.

In Kenya, swine production is one of the fastest growing food animal industry systems. We hypothesized that herd-level ecologic factors will have an impact on the prevalence and transmission of Salmonella in swine and these factors may contribute to the persistence of antimicrobial resistant strains. A total of 195 samples were collected from 30 farms located around Nairobi, Kenya. We found 99 isolates (17% prevalence) from 10 of the 30 (33% farm level prevalence) farms. Odds ratio analyses found that data collected on farm management practices was not a significant predictor of being Salmonella positive at the 0.05 confidence level. 65 isolates were found to belong to sergroup C. Using the Kirby-Bauer disk diffusion method most isolates (n=55) were pan susceptible. The second most frequent pattern was resistance to sulfisoxazole and ciprofloxacin (R-type SuCip) with a total of 19 isolates. Genotypically, pulsed-field gel electrophoresis (PFGE) was used to assess the persistence and transmission of the same strains within and across pig populations in this study. Dendrogram analysis of the PFGE profiling resulted in 18 genotypic clusters and nine sporadic clones. Several clusters had multiple farms within each cluster and/or multiple resistance patterns within each cluster. The outcome of this research might be useful as a baseline for a larger more longitudinal study to better understand any ecological management factors that are playing a role in the transmission of Salmonella.

Keywords: Salmonella, swine, antimicrobial resistance

EAR - 13

EXPLORING THE RELATIONSHIP BETWEEN MICRO-ECOSYSTEMS IN DRINKING WATER AND HUMAN HEALTH IN AN URBAN ENVIRONMENT IN CAMEROON. J. Healy Profitós, S. Lee, and J. Lee. Division of Environmental Health Sciences, The Ohio State University
A. Mouhaman. University of Maroua, Maroua, Cameroon
R. Garabed. Department of Veterinary Preventive Medicine, The Ohio State University
M. Mortiz and B. Piperata. Department of Anthropology, The Ohio State University
J. Tien. Department of Mathematics, The Ohio State University

This study was conducted in Maroua, a regional capital of Cameroon and home to over 300,000 people. The aims of this study were 1) to investigate the hottest spot of microbial contamination of drinking water along the water delivery chain within the city; 2) identify the potential sources of the microbial contamination using microbial source tracking; and 3) examine the relationship between water quality and gastrointestinal illnesses.

To achieve these aims, surveys covering demographic data and gastrointestinal health history 1 month prior to sampling were conducted in 120 households (785 people). Paired source and home drinking water storage (canary) samples were collected from 25 surveyed households, and microbial water quality tested by measuring *E. coli* levels (fecal contamination indicator). The degree of human fecal contamination was measured through microbial source tracking. Antibiotic resistance was also examined.

In the prior month, 12.5% of individuals experienced diarrhea. Sampling results from canaries had higher levels of contamination (average *E. coli* concentration of 383 CFU/100ml, GM= 112) compared to 71 CFU/100ml (GM=10) in source samples. Human genetic fecal marker (HF183), and the tetracycline resistant gene (*tetQ*) were detected in source and home samples. Statistical analysis found an inverse relationship between *tetQ* and *E. coli,* and *tetQ* was the parameter with the highest correlation coefficient with diarrhea (0.15, p=0.003). Combined survey and canary water quality analysis demonstrated that samples from the canaries of diarrhea cases were more likely to have *tetQ* and *E. coli* levels within the 1st quartile compared to non-cases (OR=5.05). When *E. coli* levels rose above the 1st quartile, no diarrhea cases were observed, despite presence of *tetQ*.

In summary, this study demonstrates that the greatest deterioration of drinking water quality occurs within the home. Therefore, further investigations of water handling practices and interactions between pathogens inside the canaries are warranted.

Keywords: drinking water quality, microbial source tracking, diarrhea, antibacterial resistance, home contamination

EAR - 14

CHARACTERIZATION OF THE ECTROMELIA VIRUS BALB/C MOUSE MODEL AS A SURROGATE FOR VARIOLA MAJOR. L. Huettner[1,2], E.M. Vela[2], B. Baur[2], D. Fischer[2], and B. Gillespie[2]. [1]The College of Public Health and Department of Veterinary Preventative Medicine , [2]Battelle Biomedical Research Center

Variola virus (VARV) is the causative agent of smallpox and has been globally eradicated with no naturally occurring infections reported since 1977. Because Variola virus (VARV) has been eradicated from the environment, alternative animal models are used to evaluate potential treatments for smallpox. One model of smallpox utilizes intranasal (IN) inoculation of BALB/c mice with ECTV, causative agent of mousepox disease in mice, due to the genetic and disease presentation similarities between ECTV and VARV. The ECTV-mouse model reproduces many features similar to that of human smallpox including severe, acute systemic disease caused by a low virus infectious dose; lack of pulmonary involvement early in the disease progression; and a presentation of a characteristic pustular rash in mice that survive the acute phase of disease. Thus, the BALB/c mouse model was developed to characterize and study ECTV infection and pathogenesis. First, the propagation of ECTV resulted in a certified working stock of virus and the LD_{90} and LD_{50} were determined to be 32.10 PFU and < 2.44 PFU, respectively. The natural history study resulted in the characterization of the disease progression resulting from intranasal infection in BALB/c mice. The clinical parameters that provided the earliest indication of disease onset following challenge were defined, in addition to the clinical parameters that correlated with mortality and time-to-death. The final study aimed to evaluate the combination of various clinical signs of disease as a reliable markers to define the onset of ECTV disease within the context clinical disease progression in the BALB/c mouse model using a combination of clinical and laboratory parameters. In all, the ECTV BALB/c mouse program resulted in a well-characterized small animal model that is suitable for use under the FDA “Animal Rule” in order to test the efficacy of therapeutics for smallpox.

Keywords: Mousepox, Ectromelia Virus, FDA Animal Rule, Surrogate Animal Modeling

EAR - 15

ELUTION OF TUMORICIDAL DOSES OF BORTEZOMIB FROM A RESORBABLE CEMENT CARRIER. B. Jones and M.J. Allen. Department of Veterinary Clinical Sciences

Chemotherapy, radiation therapy and surgery are used in the treatment of musculoskeletal tumors. Unfortunately, these treatments may be ineffective in eliminating tumors that are large, highly vascularized or locally invasive into soft tissues or neurovascular structures. An alternative approach would be to debulk the tumor and deliver local chemotherapy via a chemotherapy-loaded bone cement that acts as a structural support and drug reservoir. We hypothesized that bortezomib, a proteasome inhibitor with clinical approval for use against multiple myeloma, would show dose-dependent activity against primary and secondary bone tumors when administered locally or systemically. A canine osteosarcoma (OSA) cell line, Abrams, and a human breast cancer cell line, MDA-MB-231 were used in this work. Two sets of experiments were performed with each cell line, using either direct addition of bortezomib (1 ng/ml up to 10 µg/ml) to cell culture medium, or addition of drug-eluting beads made of a resorbable calcium phosphate cement mixed with bortezomib (1 ng/mg up to 200 µg/mg of cement). Viable cell numbers were quantified with MTT assay. Both cell lines were sensitive to the drug, with significant reductions in cell number at concentrations greater than or equal to 100 ng/ml. With the drug–eluting beads, the threshold for inhibition was 12.5 µg/g cement. When the drug was tested against normal primary rat calvarial osteoblasts, growth inhibition was also noted at a threshold of 100 ng/ml. Taken together, these data suggest that bortezomib delivered systemically or locally should be effective in decreasing the growth of OSA and breast cancer. However, more work needs to be done to better understand the safety of this approach with regards to the constituent cells of bone and bone marrow. Finally, in-vivo studies will be required to ensure that the results from this cell culture study are translatable into clinical practice in patients.

Keywords: bortezomib, cement carrier, breast cancer, osteosarcoma, osteoblasts

EAR - 16

THE IMPACT OF MOBILE PASTROALIST MOVEMENTS ON FOOT-AND-MOUTH DISEASE TRANSMISSION. H. Kim[1], N. Xiao[1], L. Pomeroy[2], R. Garabed[2] and M. Moritz[3]. [1]Department of Geography, [2]Department of Veterinary Preventative Medicine, [3]Department of Anthropology

Animal and human movements have a fundamental impact on disease transmission. Modeling such impact, however, presents a significant challenge to disease transmission models that often assume fully mixing populations where individuals have equal chance to contact each other. In this study, we model the impact of transhumance on foot-and-mouth disease (FMD), a highly contagious viral disease commonly found in cattle, transmission in the Far North Region of Cameroon. Our study area has a large number of mobile pastoralists who herd their livestock between rainy season and dry season pastures (transhumance). We develop an agent-based model coupled with a susceptible–infected–recovered (SIR) model. Each agent represents a mobile herd and each agent's movement in a year is modeled with a daily temporal resolution. We use a circle to represent the potential area that each herd can possibly reach for daily grazing to spread FMD directly from its location. We use a fixed radius for the circles for all herds for the entire year and we changed the radius to explore the impact of grazing behaviors on disease transmission. The simulation results show that grazing behaviour represented by the radius significantly affects the dynamics of FMD. When the grazing area is greater than 5 km, the 500 runs of the model for each parameter combination yield the almost same curves as the results using a fully mixing population under each transmission rate. Small grazing area (≤5 km radius), on the other hand, yields different results depending on where the first FMD infection occurs. Because of heterogeneity in herds' mobility, our simulation results produce, on average, multiple epidemic peaks a year. Even though this contrasts the standard SIR model, which produces only one peak, our results are in line with empirical evidence that we obtain by surveying herders from our study area over the last four years.

Keywords: foot-and-mouth disease, disease transmission, SIR model, agent-based model

EAR - 17

AMPHIBIAN MICOBIOMES AS INDICATORS OF INDIVIDUAL AND ENVIRONMENTAL HEALTH. S. Leyman, B. Wolfe, P. Mouser. Department of Veterinary Preventive Medicine

It is widely accepted that amphibians depend on the cutaneous microbial community (microbiome) for their innate immunity. However, very few studies have been performed to quantify or qualify the bacterial genera found on different amphibian species. The goal of this study was to classify the most abundant bacterial genera present on the skin of two *Lithobates* species living in lakes on the property of The Wilds in Cumberland, Ohio. Northern green frogs (*Lithobates clamitans melanota*) and American bullfrogs (*Lithobates catesbeiana*) were caught from 10 different lakes on the property and skin swabs, pharyngeal swabs and blood samples were taken. The skin swabs were taken after washing with a sterile saline solution for a bacterial sample and to test for chytridiomycosis, a disease of amphibians cause by the fungus *Batrachochytrium dendrobatiditis*. Pharygeal swabs were taken to test for ranavirus, another serious disease of amphibians. Blood smears were made immediately after swabbing to assess the neutrophil-lymphocyte ratio as an indicator of stress. Water quality parameters and samples were taken at the time of frog sampling for chemical analysis. The DNA was extracted from the bacterial swabs and sequenced using 454 pyro-sequencing. All sites from which frogs were sampled at the Wilds are positive for chytridiomycosis, but all of the frogs from the same sites are negative for ranavirus. The bacterial DNA from the frogs has been sequenced for one site. Sequences for the remaining sites and the analysis of the first site are pending. We hypothesize the composition of the amphibian cutaneous microbiomes will differ based on the water quality parameters. We also expect this background data on the formation of the microbiome communities will be useful for future studies to determine if changing environments or introduction of diseases changes the microbiomes of these species.

Key words: amphibian, microbiome, environmental health, chytridiomycosis, ranavirus, 454 pyro-sequencing

EAR - 18

EXTENEDED-SPECTRUM CEPHALOSPORIN RESISTENT ENTERIC BACTERIA FROM DOGS OBTAINING PARASITE SCREENING AT THE OSU VETERINARY MEDICAL CENTER. D. Mathys, D. Mollenkopf , T. Wittum,. Dept. of Veterinary Preventive Medicine. The Ohio State University, College of Veterinary Medicine.

Objective: *bla*$_{CMY-2}$ and *bla*$_{CTX-M}$ are genes that allow bacterial production of extended-spectrum beta lactamases that confer resistance to important antimicrobials. Their epidemiology has been described for food animals and clinical samples from companion animals. However, little is known about their frequency and distribution in the GI flora of healthy companion animals. The aim of this study is to estimate the prevalence of these resistance genes in the fecal flora of dogs providing samples for parasitology screening at the OSUVMC.

Methods: We obtained fecal samples from 223 dog submissions for parasite screening between January 22 and April 19, 2013. 4g of feces were enriched in a nutrient broth containing 2 ug/ml cefotaxime and inoculated onto MacConkey Agar with 8 ug/ml of cefoxitin, 4 ug/ml of cefepime, or 2 ug/ml of meropenem, to identify the *bla*$_{CMY,}$ *bla*$_{CTX-M,}$ and carbapenemase phenotypes. Genotypes were confirmed using PCR.

Results: Seventy-five (33.6%) canine fecal samples produced cefoxitin-resistant isolates, representing the expected phenotype of *bla*$_{CMY-2}$. Twelve (5.4%) samples produced cefepime resistant isolates, representing the expected phenotype of *bla*$_{CTX-M}$. Of these 87 isolates, 89% utilized both lactose and indole, indicating that they are *E. coli*. Of the 12 isolates expressing the *bla*$_{CTX-M}$ phenotype, 9 were confirmed to belong to CTX-M Group 1. Our estimated prevalence of *bla*$_{CTX-M}$ in canine fecal samples is thus 4.0% (95% CI 1.4% - 6.6%). We have also confirmed the AmpC geneotype for 23 of the 75 isolates expressing the *bla*$_{CMY-2}$ phenotype.

Discussion: Our results suggest that canine companion animals have similar fecal prevalence of *bla*$_{CTX-M}$ as livestock, while *bla*$_{CMY-2}$ prevalence appears to be lower. Additional investigation to identify subsets of dogs at greatest risk would be beneficial to determining risk factors such as chronic antibiotic use, surgical implant, ICU patient, exposure to livestock, or other high risk populations.

Keywords: antimicrobial resistance, extended-spectrum beta-lactamase, canines

EAR - 19

BEHAVIOR IN DAIRY COWS WITH LOW AND HIGH SOMATIC CELL COUNTS.

KE McCullough, PJ Rajala-Schultz, PN Gott, Department of Veterinary Preventative Medicine

Introduction: Milk losses associated with elevated somatic cell counts (SCC) have a substantial impact of the profitability of a dairy herd and this makes finding cows with increased SCC a priority. Animals display sickness behaviors around the time of most diseases. This behavior may be useful as a mastitis detection tool. **The objective of this study** was to compare behavior of cows with elevated SCC to cows with non-elevated SCC.

Materials and Methods: Activity monitors (IceQube™, IceRobotics, Edinburgh, Scotland) were placed on a hind leg of pregnant dairy cows one week before expected calving in one seasonal, grazing dairy herd in Ohio. Activity monitors were kept for two weeks after delivery. Cows were monitored for eight days and California Mastitis Test (CMT) was performed daily during this time. Somatic cell counts were estimated from CMT-positive quarters. A cow was classified as having an elevated SCC if she had at least one quarter with SCC over 200,000 cells/ml for two of the eight days. The activity monitors measured number of steps taken, lying time, number of lying bouts, and standing time and an average lying bout length was calculated. Descriptive statistics were computed and data were analyzed using MIXED procedure in SAS, v. 9.3 (SAS Institute Inc, Cary, NC), using daily summary data on different activity parameters as the outcomes. Cows with concurrent illnesses were excluded.

Results: Fourteen of the 30 cows were identified as having an elevated SCC. Number of lying bouts was found to be significantly different between cows with elevated and non-elevated SCC (P=0.0236). Our results suggest that cows with subclinical infections (high SCC) may exhibit altered behavior. The significance of this study is that new technology can be utilized to aid farmers in mastitis detection, which would improve cow welfare and provide consumers with higher quality milk.

Keywords: mastitis, somatic cell count, dairy cow, behavior

EAR - 20

EFFECTS OF SUPPLEMENTAL LACTOFERRIN ON CONCENTRATIONS OF TOTAL AND ANTIMICROBIAL RESISTANT FECAL COLIFORMS IN PREWEANED DAIRY CALVES.

M.M. Miller, L. Muñoz, S. DeWitt and G.G. Habing. Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, USA

Due to concerns about the human health impacts of agricultural antimicrobial use and antimicrobial resistance (AMR), alternatives to conventional antimicrobials are being sought. As a natural ingredient in milk, lactoferrin has antimicrobial and anti-LPS capabilities and may have a role for the treatment of calf diarrhea. Diaque (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, Missouri), is an oral electrolyte with lecithin-coated citrus fiber, purported to decrease the time to recovery in diarrheic calves. We hypothesized that supplementation of lactoferrin and/or Diaque would change the concentrations of total and AMR coliforms. Another objective of this research was to understand the dynamics of AMR coliforms early in the calf's life. Forty-four heifer calves were enrolled into one of four treatments: lactoferrin (2g), Diaque (500g), lactoferrin and Diaque, or pasteurized waste milk. The supplements were provided for the first two days of the trial, and fecal samples were collected for four consecutive days. Coliform concentrations were estimated using the Colilert (IDEXX, Westbrook, ME) growth indicator and Quanti-trays (IDEXX) to measure the most probable number (MPN) of total coliforms and coliforms growing in the presence of streptomycin, ampicillin, or cefotaxime. Other measurements included serum [IgG], calf weights and fecal scores. For all groups, over 95% of coliforms were resistant to ampicillin and streptomycin, whereas approximately 65% of coliforms were resistant to cefotaxime. Lactoferrin supplementation had no significant impact on concentrations of AMR coliforms, but significantly increased the concentration of total coliforms between the third and fourth day ($p < 0.023$). Passive transfer of IgG was mildly associated with higher concentrations of coliforms. Overall, age was the greatest predictor of both total and AMR coliforms with a peak between the ages of 3 to 4 days and a significant decrease in concentration by twelve days of age ($p < 0.05$).

Keywords: lactoferrin, antibiotic resistance, fecal coliforms, dairy

EAR - 21

EXPLORING THE ROLE OF COYOTES ON THE INFECTIOUS DISEASE DYNAMICS WITH AN EMPHASIS ON *N. CANINUM.* K. Moreno-Torres[1], B. Wolfe[1] and R. Garabed[1]
[1]Veterinary Preventive Medicine

The goal of our project is to assess the role of coyotes as a risk factor for disease transmission in non-domestic ruminant populations at the Wilds. Neosporosis—an infectious disease caused by a protozoan parasite and transmitted by dogs and coyotes— is an important cause of abortion and reproductive failure in ruminants worldly, leading to concerns for farmers, hunters and conservationists alike. Accordingly to the Ohio Department of Natural Resources coyotes' populations have increase dramatically in the last years, therefore it is expected to be an increment of infectious diseases related to this coyote-ruminant interaction. Thus improve knowledge of the environmental contamination of *Neospora caninum* due to coyotes may help to reduce ungulates reproductive failure by directing interventions on coyotes' populations. The Wilds, a safari zoo and conservation center with the main mission of breeding endangered species has reported a positive prevalence in American bison and Pere David deer. Therefore, management that leads to healthy predator populations is essential for the health and welfare of endanger populations. Coyote's populations may contribute with the drop of risk of diseases such as Leptospirosis and Hantavirus but also their increasing numbers may cause other infectious diseases to spread to ruminant populations. This project is in progress, thus we hope to increase sample size and expand our analysis. This work can identify the role of coyotes on the disease dynamics of *Neospora caninum* and help guide farmers, conservationist and researchers interventions on wildlife management from the point of view of the disease.

Keywords: Environmental contamination, conservation medicine, *Neospora caninum*, *Canis latrans*, parasites and infectious diseases

EAR - 22

2013 SURVEILLANCE OF INFLUENZA A VIRUSES IN EXHIBITION SWINE.

S. Nelson, J. Nolting, J. Edwards, J. Workman, N. Bliss, R. Slemons, A. Bowman. Department of Veterinary Preventive Medicine

The H3N2 variant influenza A virus (IAV) outbreak during 2012 resulted in 306 reported human cases, most of which were associated with exposure to swine at agricultural fairs in the Midwest. Continued IAV surveillance in exhibition swine at these swine-human interfaces is a key to understanding the epidemiology of IAV strains threatening human and animal health. In 2013, 2037 pigs were sampled at 100 exhibitions across Ohio, Indiana, Iowa, West Virginia, Colorado, Texas, and Kentucky. No IAV isolates were recovered from 388 pigs sampled in West Virginia, Colorado, and Kentucky (n=12 fairs). The frequency of virus isolation from the pigs sampled at the remaining 88 fairs in the other 4 states was: Ohio, 126/899 (14%) at 15 of 36 exhibitions; Indiana, 201/700 (29%) at 14 of 36 exhibitions; Iowa, 3/140 (2%) at 1 of 7 exhibitions; Texas, 16/200 (8%), at 2 of 9 exhibitions. Subtypes of the recovered isolates were H1N1 (55.5%), H3N2 (31.5%), H1N2 (7.8%), and mixed (5%). The high proportion of H1N1 isolates in 2013 is unlike the 2012 fair season when the vast majority of isolates recovered were H3N2 with the A(H1N1)pdm09 matrix gene. Continued reassortment with A(H1N1)pdm09 and previously established swine-origin IAV lineages was detected by sequencing of 147 isolates. Of the sequenced isolates, A(H1N1)pdm09 PA and NP genes were found in 72% and 49% of the sequenced isolates respectively. Only 19 human cases of variant H3N2 were reported in 2013. The divergence of swine and human H3N2 IAVs may have created an immunological gap which may allow for inter-species transmission. Increased H1N1 activity observed in exhibition pigs during 2013 is a possible explanation for the decreased number of variant IAV cases compared to 2012. Continued surveillance and characterization of swine-origin IAVs is required to develop and assess mitigation strategies aimed at protecting public health.

Keywords: pigs, influenza A virus, surveillance, fairs, H3N2v

EAR - 23

PREVALENCE OF ZOONOTIC ASCARID OVA (*TOXOCARA CANIS, TOXOCARA CATI, AND BAYLISASCARIS PROCYONIS*) IN COLUMBUS CITY PARKS. C.Shockling Dent, A. Dent, J. O'Quin, L. Capitini, C. Bremer, A. Hoet. Department of Veterinary Preventive Medicine

Zoonotic ascarids present an important Veterinary Public Health issue as it involves animal, human, and environment. This perfect storm is brought together in the playground, as it encourages humans and animals to interact in the same environment. A handful of prevalence studies on zoonotic ascarids in playgrounds have been published throughout the US, but none in Ohio. The objectives of the study were to determine the prevalence of zoonotic ascarid ova in soil in the Columbus City Parks and to identify potential risk factors associated with the presence of this parasite in playgrounds in these parks. There are a total of 220 parks in Columbus, of those only 127 parks had playgrounds and, therefore, qualified for the study. Of the 127 parks with playgrounds, a subset of 30 parks was then chosen. Ten soil/ground cover samples, as well as fecal samples (if present), were collected from each park (30 parks total) and analyzed for zoonotic ascarid ova. The prevalence of zoonotic ascarids ova in soil/ground cover in the Columbus City Parks was found to be 0.3% of the samples (1/289 samples) and 3.4% of the parks (1/29 parks), the thirtieth park was omitted. This low prevalence suggests that contamination of playgrounds in the central Ohio areas is not as large of a risk as in other locations in the country. This low prevalence may be due to multiple factors such as the type of ground cover used in playgrounds, time of year of sampling (late summer), or the behavior of dog owners in central Columbus (example, Columbus dog owners may see their veterinarian more regularly). This study will help to better inform the community of the potential risk of zoonotic ascarids ova in Columbus playgrounds and give the veterinary public health professionals a baseline for future studies and recommendations.

Keywords: *Toxocara canis, Toxocara cati, Baylisascaris procyonis,* Zoonotic ascarids Veterinary Public Health

EAR - 24

MICRO ISOLATOR FILTERED CAGE LIDS; VERIFICATION OF SANITATION STANDARDS.

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According to the *Guide for the Care and Use of Laboratory Animals*, enclosures and accessories, such as [cage] tops, should be sanitized every 2 weeks. Extended change out times have been proposed for cage lids on individually ventilated cages (IVC's) that do not directly contact the animal. IVC lids were tested for their ability to act as a barrier to compounds within cages and as a fomite for transmission to animals between cages. Sanitation levels of mouse IVC lids were tested every 2 weeks for 24 weeks using Replicate Organism Detection and Counting (RODAC) plates and ATP monitoring system. RODAC pass level was defined as 0-15 colonies and set the standard for ATP pass level of ≤ 17 RLU's. FVB mice were housed in cages treated with Krypton® glow powder. Black light was used to determine the spread of powder from the cage floor to the lid and IVC rack. Lids positive for fluorescence were placed on clean cages containing untreated FVB mice and black light was used to determine the spread of powder from the lid to the cage bottom. At 2 weeks, ~30% of cage lids passed according to RODAC testing and this level remained consistent until 18 weeks. In glow powder treated IVC's, fluorescence was not detectable within adjacent cages or within cages from treated lids. Currently, ~25% of cage lids are sanitized every 2 weeks. Sanitizing all lids every 2 weeks would increase labor cost >75%. The significance of the 30% RODAC pass rate remains unclear given the arbitrary nature of the guideline; however, extending sanitation intervals for non-animal contact accessories remains a significant source for cost savings. The use of glow powder indicates that filter tops are effective at containing material within IVC's.

Keywords: individually ventilated cages, RODAC plates, glow powder, sanitation levels, labor costs

EAR - 25

COMPARATIVE EVALUATION OF SNOUT WIPES FOR USE IN INFLUENZA A VIRUS SURVEILLANCE. C. Szablewski, S. Nelson, J. Nolting, R. Slemons, J. Edwards, J. Workman, and A. Bowman. Department of Veterinary Preventive Medicine

The current gold standard clinical sample material for influenza A virus (IAV) surveillance in swine is a synthetic fiber tipped nasal swab. However, the collection of nasal swabs requires restraining pigs which makes sampling labor intensive, stressful on the pigs, and aesthetically unpleasing to owners and observers. The hypothesis of this study was that non-invasive snout wipes could be an effective sampling alternative for the surveillance of IAV in exhibition swine populations. Three substrates were investigated for use as snout wipes: rayon polyester blend gauze, cotton gauze, and Swiffer® sweeping cloths. 2”x 2” pieces of each material, along with the gold standard polyester tipped swabs, were tested in triplicate. An H3N2 IAV was inoculated directly onto each material before being stored in viral transport medium. The samples were subsequently frozen and thawed with viral recovery from each substrate measured with qRT-PCR and $TCID_{50}$ assay. The rayon gauze had the worst viral recovery of the three in-vitro studied substrates. The cotton gauze and the Swiffer® had similar virus recovery results, both showed approximately one $\log_{10}$ less recoverable virus than the nasal swabs. Based on the in vitro results and the availability of pre-packaged, individual, sterile wipes, cotton gauze was selected for a subsequent field trial. In the field trial, paired polyester nasal swabs and cotton gauze snout wipes were collected from 295 pigs at 19 separate agricultural fairs. The samples were tested with rRT-PCR and virus isolation. The cotton gauze had good agreement with nasal swabs for both rRT-PCR (kappa= 0.44) and virus isolation (kappa=0.65). Veterinarians will benefit from the use of snout wipes because it will allow them to easily sample individual pigs with minimal labor and decreased animal stress. Additionally, the lack of restraint can lead to increased acceptance and owner participation, especially in public settings like agricultural fairs.

Keywords: Swine, Snout Wipe, Nasal Swab, Influenza A virus

EAR - 26

INTRODUCTION, CIRCULATION AND MAINTENANCE OF MRSA IN CONTACT SURFACES AT A LARGE EQUINE HOSPITAL: YEARLONG MOLECULAR EPIDEMIOLOGY OF ENVIRONMENTAL CONTAMINATION. J. Van Balen[1], J. Mowery[2], M. Piraino-Sandoval[2], R.C. Nava-Hoet[1], C. Kohn[3], A.E. Hoet[1,2]. [1]Department of Veterinary Preventive Medicine, College of Veterinary Medicine, [2]Division of Epidemiology, College of Public Health; [3]Department of Clinical Sciences, College of Veterinary Medicine; The Ohio State University, Columbus, OH.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important nosocomial pathogens affecting veterinary hospitals, and environmental contamination is considered a possible source for nosocomial infections. However, the presence of MRSA in equine hospital environments has been studied only during outbreaks or for very short periods. We hypothesized that if MRSA is present in humans working and/or visiting an equine hospital, as well as in horses admitted to such practice, then it will be found frequently contaminating surfaces across the hospital throughout the year. Therefore, the objectives of this study were to determine the monthly presence and distribution of MRSA in the environment of an equine hospital during one year, to characterize circulating strains, and to establish patterns of contamination overtime using molecular epidemiological tools. For this purpose, a yearlong active MRSA surveillance was performed. Antimicrobial susceptibility testing, SCC*mec* typing, PFGE typing, and dendrographic analysis were used to characterize/analyze these isolates. Overall, 8.6% of the surfaces and 5.8% of the horses sampled were positive for MRSA. The most common contaminated surfaces were: computers (16.7%), feed/water buckets (16.7%), and surgery tables/mats (15.6%). Ninety percent of the isolates carried SCC*mec* type IV, and 62.0% were classified as USA500, reflecting a low diversity among the strains circulating at the hospital. Moreover, 73.5% of the MRSA strains were classified as multidrug resistant. A constant introduction and reintroduction of new strains into the hospital was observed during the year. However, maintenance of strains in the environment was also observed when unique clones were detected for 2 consecutive months on the same surfaces. These findings highlight i) the importance of performing continuous surveillance and monitoring to identify surfaces that could act as hot spots and reservoir for this pathogen, ii) the need of steady and effective cleaning and disinfection to minimize environmental contamination.

Keywords: MRSA, Surveillance, Environment, Equine, Molecular Epidemiology, Veterinary Hospital

IMMUNOLOGY
AND
INFECTIOUS DISEASES

IMID - 1

THE ROLE OF TRANSDUCER LIKE PROTEINS (TLPS) OF *Campylobacter jejuni* IN NUTRIENT SENSING AND ITS PATHO-BIOLOGY. K. Chandrashekhar[1], S. Hwang[2], B. Jeon[3], S. Ryu[2] and G, Rajashekara[1]. [1]Food Animal Health Research Program, Department of Veterinary Preventive Medicine, The Ohio State University, Wooster, Ohio 44691. [2]Department of Food and Animal Biotechnology, Department of Agricultural Biotechnology, and Center for Agricultural Biomaterials, Seoul National University, Seoul 151-921, Republic of Korea. [3]Department of Public Health, University of Alberta, Canada

Campylobacter jejuni is a major cause of bacterial foodborne gastroenteritis worldwide. The chemotaxis system made up of Transducer Like proteins (Tlps) and other core signal transduction proteins allows *C. jejuni* to travel through environmental gradients and colonize different niches both in vivo and outside. The present work seeks to identify Tlp ligands and substrate specificity of Tlps (nutrient sensors) in *C. jejuni* and their role in colonization and pathobiology. Eight Tlp single deletion mutants were created and characterized for their function in substrate specific chemotaxis, motility, biofilm formation, in-vitro virulence and chicken colonization. Capillary chemotaxis assays revealed that certain Δ*tlp* mutants showed a decreased chemotaxis towards amino acids and organic acids. The *tlp3* and *tlp8* mutants showed increased motility and in-vitro virulence defects. Several *tlp* mutants showed a reduction in colonization of the chicken gut. Notably, the Δ*tlp2*mutant showed a decreased chemotaxis towards Pi and Fe^{2+} along with a growth defect under nutrient downshift and iron restriction conditions with an increased alkaline phosphatase (PhoX) activity. The gene organization along with the results of overlapping RT-PCR and Primer Extension Assay revealed that *phoX* gene is co-transcribed with *tlp2* from, the *tlp2* promoter (P_{tlp2}). Promoter fusion assays for *tlp2* demonstrated that P_{tlp2} was induced in the presence of 2mM of Pi and 40µM iron (Fe^{2+}). Real time PCR indicated that phosphate uptake genes were upregulated in the *tlp2* mutant. These findings reveal a novel ligand for Tlp2 in *C. jejuni* which can interact with underlying pathways of iron and phosphate regulation thus enhancing our understanding of *C. jejuni* biology.

Keywords: *Campylobacter jejuni*, chemotaxis, transducer like proteins, nutrient sensing

IMID - 2

Pseudomonas aeruginosa BIOFILM INDUCED NEUTROPHIL DYSFUNCTION

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Biofilms are linked to the development, severity, and persistence of numerous clinically significant chronic diseases including chronic wounds. Due to a historically single-cell, free-swimming or planktonic centered research paradigm, little is known about the role of the bacterial biofilm on innate host responses despite their unique sessile, surface associated mode of growth and pathogenic potential. The purpose of this ongoing study is to characterize the effects of Pseudomonas aeruginosa biofilm, a consistently isolated and important pathogen of various body systems, on neutrophils and investigate mechanisms of virulence expressed by biofilms. Human peripheral blood derived neutrophils are isolated and exposed to P. aeruginosa intact biofilms or extracellular products of biofilms. Readouts from these experiments included cytokine production, neutrophil cytotoxicity, bacterial viability and internalization. Extracellular products of P. aeruginosa biofilms, collected from conditioned media, produced reduced pro-inflammatory responses, as measured by IL-8 ELISA, in neutrophils when compared to similar products derived from planktonic cultures. These results indicate biofilms are able to augment their recognition by neutrophils. When neutrophils are directly applied to intact biofilms, bacterial viability is maintained while planktonic bacterial viability is significantly reduced. These findings are not due to loss of neutrophil viability or disparate neutrophil internalization, determined by lactate dehydrogenase assay and confocal microscopy quantification, respectively. Therefore, P. aeruginosa biofilms resist neutrophil killing mechanisms after phagocytosis. Future studies are aimed at elucidating biofilm mechanisms of resistance to neutrophil killing. Characterizing host-biofilm interactions may elucidate novel pathways to focus therapeutic development of chronic wounds and numerous other biofilm associated diseases.

Keywords: *Pseudomonas aeruginosa*, biofilm, neutrophil

IMID - 3

CADMIUM ENHANCES ALLERGIC RESPONSE IN ANTIBIOTIC-TREATED MICE.

K. Cornelius, S. DiBartola, A. Bonnegarde-Bernard, E. Kim, H. Steiner, J. Jee, E. Cormet-Boyaka, and P. N. Boyaka. Dept. of Veterinary Biosciences

The rate of allergy and asthma has increased in western countries since the 1960s. Several studies suggest a correlation between the alteration of mucosal microflora and this rising incidence. Moreover, changes in microflora have been linked with widespread antibiotic use and environmental pollutants. One such pollutant, cadmium, has contaminated drinking water through the improper disposal of industrial products such as batteries, fertilizers, and paints. Using a murine model, we examined how antibiotics and cadmium can affect sensitization to food allergens and alter subsequent allergic responses in the lung. We hypothesized that oral cadmium would enhance the allergic response in antibiotic-treated mice. We found that chronic ingestion of cadmium in drinking water decreased secretory IgA levels in fecal extracts over the 4 weeks of cadmium treatment. Similarly, oral administration of a cocktail of antibiotics also decreased secretory IgA levels. We next examined histology and allergen-specific antibody responses after oral sensitization with human serum albumin (HSA), as model antigen, in the presence of cholera toxin as an adjuvant. Small intestine histology showed an increase in goblet cells and mucus secretion in mice receiving either cadmium or antibiotics. Intestinal inflammation was notably less in mice that received both cadmium and antibiotics. The antigen-specific serum IgE levels were not significant in the mice that received only cadmium or only antibiotics. Antigen-specific serum IgE levels were increased oafter the combined cadmium and antibiotic treatment. However, unlike either treatment alone, the other antibody isotypes did not decrease. Finally, after nasal challenge with HSA, higher levels of lung inflammation were seen in orally sensitized mice that received either cadmium or antibiotics. Mice that received both cadmium and antibiotics exhibited more granulocytes and monocytes in the lung after nasal challenge and mucus secretion was also increased. We concluded that cadmium enhances airway allergic response in antibiotic treated mice, but the antibody response is different than in either treatment alone.

Keywords: cadmium, allergic-response, antibiotics, lungs, inflammation

IMID - 4

COMPARISON OF BIOCIDE TOLERANCE IN DIFFERENT STRAINS OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* N. Diana, O. Benear, V. Artuso Ponte, W. Gebreyes. Department of Veterinary Preventive Medicine

Methicillin-resistant Staphylococcus aureus (MRSA) is a pathogen that many people associate with humans, yet it is also isolated from domestic animals. Antibiotic resistance is a primary concern in the treatment of MRSA infections; however, another concern is the emergence of biocide-tolerant MRSA strains. Disinfectants play a major role in the prevention and spread of MRSA and increased biocide tolerance will lead to more MRSA infections. Misusing disinfectants such as not using enough or not allowing enough contact time with the area to be disinfected may accelerate biocide tolerance in MRSA. This study looked at 68 isolates of MRSA: 20 human, 18 porcine, 14 environmental, 10 canine, 3 equine, and 3 bovine isolates. These isolates were chosen based on methicillin-resistant characteristics and the sample's origin. The biocides were selected to have a range of common disinfectants. Biosentry is often used to disinfect animal barns, chlorohexidine is used in a hospital setting for disinfecting and triclosan is an antimicrobial agent found in many hand soaps. The goal of this study was to observe if there were differences in tolerance to biocides based on sample type. The MIC of these samples were determined for biosentry, chlorohexidine, and triclosan using Muellar Hinton agar plate dilutions that ranged from 1ug/ml to 160 ug/ml. None of the isolates showed tolerance to triclosan, but some isolates displayed tolerance to biosentry and chlorohexidine. Among the sample groups, environmental samples showed the most tolerance with 43% (6/14) of the environmental samples having an MIC over 20ug/ml for chlorohexidine, and 43% (6/14) having an MIC over 20ug/ml for biosentry. With this phenotypic trend, there is a possibility for MRSA to continue to evolve and become increasingly tolerant to these biocides, which poses a threat for increased survival and spread of MRSA to people and animals.

Keywords: S. aureus, antibiotic resistance, MRSA, biocide tolerance

IMID - 5

DIFFERENTIAL INDUCTION OF INTERFERON BY HTLV-1 AND HTLV-2 LEADS TO DISTINCT PATHOBIOLOGIES. N. Dissinger and PL Green. Department of Veterinary Biosciences.

Type I interferons (IFNs) are antiviral cytokines that play a key role in the innate immune system. Once IFNs are secreted, they can bind to their receptors inducing autocrine or paracrine signaling. This leads to an increase in antiviral IFN stimulated genes, such as OAS1 and viperin. These proteins are able to inhibit viral replication and transmission. The human T-leukemia viruses (HTLV-1 and HTLV-2) are retroviruses with differing pathobiologies. HTLV-1 can cause an array of diseases in infected individuals, including cancer and neurological disease, whereas HTLV-2 infection appears to be asymptomatic. Because the HTLVs and HIV share the same modes of transmission, it is common for there to be co-infected individuals. Data indicates that HTLV-2 has a protective effect on HTLV-2/HIV co-infected patients, leading to a significantly higher amount of long-term nonprogressors for AIDS as compared to HTLV-1/HIV co-infected or HIV monoinfected patients. Reports that type I IFN secreted by T-cells is able to inhibit HIV replication led us to hypothesize that HTLV-1 and HTLV-2 differentially regulate IFN expression. Using real-time PCR, we have demonstrated that HTLV-2 infected peripheral blood lymphocytes (PBLs) have an increased amount of IFN-beta expression over non-HTLV and HTLV-1 infected PBLs. We have further demonstrated using a paracrine activation assay that IFN-beta is secreted from HTLV-2 PBLs and able to induce virus restriction genes in bystander cells, resulting in an antiviral state. To determine the mechanism of IFN induction, we examined the ability of the HTLV-2 protein APH-2 to affect IFN-beta expression. We observed that over-expressed APH-2 had the ability to increase the activity of activated IRF-3 nearly 10-fold compared to activated IRF-3 without APH-2 present. Our data indicates that one mechanism of HTLV-2 repression of HIV infection is due to the increased amount of IFN-beta, which APH-2 plays a role in inducing.

Keywords: HTLV, HIV, interferon

IMID - 6

THE ROLE OF EOSINOPHILS IN THE PATHOGENESIS OF RESPIRATORY SYNCYTIAL VIRUS INFECTION. G. Green, N. Petrov, D. Huey , K. La Perle, and S. Niewiesk. Department of Veterinary Biosciences.

The role of eosinophils in the pathogenesis of respiratory syncytial virus (RSV) infection remains poorly understood. Eosinophils are of interest during RSV infection for three reasons. First, an anti-viral role has been suggested for esosinophils during RSV infection. Secondly, pulmonary eosinophilia is considered to be a hallmark of “vaccine-enhanced disease” in experimental animal models, and vaccine-enhanced disease has been a major obstacle in creating an RSV vaccine. Lastly, there is evidence to suggest that RSV infection may be linked to the development of asthma/allergy, 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, Staphyloccocus 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; Cotton Rat; Antiviral Immunity

IMID - 7

TREM2/DAP12 DIFFERENTIAL EXPRESSION IS ASSOCIATED WITH BAL CELLS THAT EXPRESS HIGH LEVELS OF ARG1 IN PIGS. J. B. Hiremath, B. Binjawadagi, K. Ouyang, S. Dhakal, C. Manickam, V. Dwivedi, and G. J. Renukaradhya. Food Animal Health Research Program (FAHRP), OARDC, Depts. of Veterinary Preventive Medicine, The Ohio State University, Wooster 44691.

Lung immunopathology is the major cause of influenza induced morbidity and mortality. DAP12 (DNAX-Activating Protein of 12kDa) is a membrane adaptor protein associated with varied surface receptors, and it is known to regulate influenza induced lung immunopathology. DAP12 associated receptor expression and their association with phenotype of bronchoalveolar lavage fluid (BAL) cells (containing >90% of macrophages) during swine influenza virus (SIV) infection in pigs is unknown. Since pig is a suitable large animal model for influenza research, our aim was to understand the effects of zoonotic SIV H1N1 infection on expression of DAP12 associated MDL-1, TREM-1 and TREM-2 receptors in pig BAL cells through qRT-PCR profiling. Results indicated that DAP12, MDL-1 and TREM-1 were constitutively expressed, but TREM-2 was upregulated. Stimulation of uninfected pig BAL cells *in vitro* using the cytokines IFN-γ and IL-4 revealed that upregulation of TREM-2 was associated with enhanced expression of ARG1, but not with high levels of expression of TNF-α and iNOS. ARG-1 is a phenotype of alternatively activated alveolar macrophages that are known to protect lung tissue; hence TREM2 upregulation in pig BAL cells with similar phenotype suggests the possible beneficial role of these molecules in preventing the lung immunopathology. In conclusion, DAP12 and associated receptors are differentially expressed in pig BAL cells and TREM2 appears to have a beneficial role in regulating the lung immunopathology in SIV infection.

Keywords: Influenza, Lungimmunopathology, TREM2/DAP12, M1& M2 Macrophages

IMID - 8

ALVEOLAR TYPE 2 CELL LOSS CONTRIBUTES TO THE PATHOGENESIS OF INFLUENZA-INDUCED ACUTE LUNG INJURY. C.C. Hofer, P.S. Woods, F. Aeffner, I.C. Davis. Department of Veterinary Biosciences

Seasonal influenza A virus (IAV) infections causes 300,000-500,000 deaths worldwide annually. Critically-ill patients exhibit severe pulmonary edema, resulting in respiratory failure consistent with development of acute lung injury (ALI). In the normal lung AT2 cells secrete surfactant lipids and proteins, and can also differentiate into type 1 (AT1) pneumocytes after lung injury. However, the role of AT2 cells in the pathogenesis of IAV-induced ALI is not well defined. Infection of wild-type (WT) C57BL/6 mice with IAV A/WSN/33 results in development of ALI within 2 days and 100% mortality by day 8. Infected mice develop severe pulmonary edema and hypoxemia. In contrast, C57BL/6-congenic mice that are heterozygous for the F508del mutation in the cystic fibrosis transmembrane conductance regulator anion channel (CF HETs) exhibit delayed mortality and do not develop significant respiratory failure or ALI. Protection from ALI in CF HETs is independent of viral replication, which does not differ from WT controls. In WT mice, development of ALI following IAV infection is temporally correlated with a progressive decline in AT2 cell numbers and expression of the Surfactant Protein-C (SP-C) gene and protein. This decline is accompanied by a progressive rise in T1-α gene and protein expression, indicative of increased differentiation to AT1 cells. Importantly, the effects of IAV on SP-C and T1-α expression are attenuated in CF HETs. Our data indicate that development of IAV-induced ALI is accompanied by increased differentiation of AT2 cells to AT1 cells in an attempt to repair the IAV-damaged alveolar epithelium. Importantly, this effect is independent of viral replication, suggesting that it is mediated by an indirect mechanism. Hence, AT2 cell loss may be an important component of IAV-induced ALI, and novel therapeutics that can either preserve AT2 cells, replenish them, or restore their function may be of value in treatment of patients with severe influenza.

Keywords: influenza, acute lung injury, pneumocyte

IMID - 9

CHRONIC EXPOSURE TO CADMIUM ALTERS GUT IMMUNE HOMEOSTASIS AND INNATE IMMUNITY. E. Kim, J. Jee, H. Steiner, E. Cormet-Boyaka and P.N. Boyaka
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Cadmium is a toxic heavy metal that can be ingested due to its presence in contaminated water and its accumulation in leafy vegetables, fish and grains. Since cadmium can compete with iron for intestinal absorption, long-term ingestion of cadmium can change composition of gut-commensal bacteria by inhibiting the growth of selected bacteria. Gut-commensal bacteria are critical to maintain intestinal homeostasis since they limit attachment of infectious agents, regulate pro-and anti-inflammatory milieu and secretory IgA levels. However, consequences of chronic exposure to cadmium for gut immune homeostasis, and mucosal innate immunity are poorly understood. To investigate the effect of cadmium on gut-homeostasis, C57BL/6 mice were treated with cadmium-contaminated water for 28 days. Mice exposed to cadmium exhibited a significant reduction of secretory IgA levels, which correlated with reduced expression of polymeric Ig receptor mRNA. These mice also showed increased intestinal permeability to macromolecules when compared to control untreated mice. The chronic exposure to cadmium also render these mice more responsive to oral administration of cholera toxin since the frequency of Paneth and goblet cells, as well as the levels of mucus production and anti-microbial mRNA responses seen 16 hours after ingestion of cholera toxin were higher than those of control mice. In summary, environmental pollutant cadmium is a major regulator of **gut immune** homeostasis and innate immunity. (1500/1500 limit)

Keywords: Cadmium, microbiota, gut, IgA

IMID – 10

IN VIVO TRANSCRIPTOME RESPONSES TO GRAM POSITIVE AND GRAM NEGATIVE PROBIOTICS IN NEONATAL GNOTOBIOTIC PIGLETS.

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Probiotics mediate gut homeostasis in a species and/ or strain specific manner however, mechanisms are largely undefined. Probiotics are often used in treatment or prevention of enteric disorders in adjunct to combinatorial therapies. Investigation of host global responses to administered probiotic in gnotobiotic (Gn) pigs (less complex but biologically relevant model) is an ideal approach to identify the specific molecules and pathways mediating beneficial effects. Since, Gram positive (G+) and Gram negative (G-) probiotics differ in the cell surface and cell wall constituents therefore, we hypothesized that they may differentially influence neonatal responses in host. We elucidated G+ probiotics (*Lactobacillus rhamnosus;* LGG and *L. acidophilus;* LA) specific gut transcriptome responses in duodenum and ileum in a neonatal Gn piglets. Our results indicated that both LA and LGG modulated common and unique responses related to host metabolism, gut integrity and immunity in Gn pigs.

Probiotic effects are mediated through crosstalk between enterocytes (IEC) and intestinal immune cells (eg. MNC). Hence, investigation of cell specific (eg. IEC and MNC) responses further provide comprehensible insight to host-probiotic interactions. Therefore, we also explored MNCs specific transcriptome responses to G+ (LA and LGG) and G- (*E. coli* Nissle1917; EcN) probiotics in duodenum and ileum. Overall, EcN regulated significantly higher number of gene transcripts than LA and LGG. Further, EcN establishment and beneficial effects in the host are guided by downregulation (eg.secretory leukocyte proteases inhibitor; SLPI and glutathione peroxidase 3; GPX3) or upregulation (eg. IgG and VDJ region) of immune response associated genes in the day1 and day7 post-colonization, respectively. Our overall goal is to understand differential effects of G+ and G- probiotics on neonatal immunity and gut homeostasis and its impact on human rotavirus diarrhea or vaccines.

Keywords: Probiotics, transcriptome, gnotobiotic pigs, gut, IECs, and MNCs

IMID - 11

EFFECTS OF PARENTERAL ANTIBIOTICS ON THE FECAL MICROBIOME OF HORSES. R. S. Liepman, J. M. Swink, S. D. A. Hurcombe, P. N. Boyaka, S. E. Dowd and R. E. Toribio. Departments of Veterinary Clinical Sciences and Veterinary Biosciences.

Antibiotic-associated diarrhea is the most common side effect of antibiotic therapy in horses and an etiologic diagnosis is rarely identified. However, our understanding of the complex equine fecal microbiota and how it temporally changes in response to antibiotic therapy remains unclear. The goal of this study was to describe the baseline intestinal microbiome of horses in central Ohio and investigate how it is altered by antibiotics. We hypothesized that the fecal microbiome of horses in Ohio will consist of a unique distribution of described and undescribed microbes, that it will change over time in response to cephalosporins, fluoroquinolone and tetracycline antibiotics, and that three days and one week of antibiotic treatment will allow demonstration of these changes in fecal samples.

The study was split into two parts: a preliminary and larger scale study. First, six healthy horses were treated intravenously with ceftiofur (n=2), enrofloxacin (n=2), or saline (n=2) for 3 days. Fecal samples were collected daily from the rectum. This procedure was repeated in the larger study with 16 healthy horses treated with ceftiofur (n=4), enrofloxacin (n=4), oxytetracycline (n=4) or saline (n=4) for 5 days and fecal samples collected for 1 week and 30 days later. Bacterial population diversity was assessed by 454-pyrosequencing of the 16S rRNA gene before and after antibiotic administration.

Baseline fecal microbiota was diverse both within and between subjects in each treatment group before and after antibiotic administration. The major phyla represented in baseline samples were *Firmicutes, Bacteroidetes, Spirochaetes,* and *Tenericutes*. No significant changes in diversity were noted over time, although trends were observed in subjects treated with enrofloxacin. Further results from the larger scale study are pending.

This is the first study to describe the fecal microbiome of horses in Ohio and to specifically demonstrate, based on 16S rRNA pyrosequencing, how the equine fecal microbiome is altered in response to intravenous antibiotics over time.

Keywords: fecal microbiome, antibiotic-associated diarrhea, pyrosequencing, horse

IMID - 12

INFECTIOUS ENTRY TRIGGERED BY *EHRLICHIA CHAFFEENSIS* ETPE VIA DNASE X IS MEDIATED BY CD147 AND HNRNP-K. D. Mohan Kumar and Y. Rikihisa. Department of Veterinary Biosciences

Ehrlichia chaffeensis is an obligatory intracellular bacterium that causes human monocytic ehrlichiosis, one of most prevalent emerging tick-borne zoonoses. *E. chaffeensis* uses its cell surface invasin EtpE C-terminus (EtpE-C) to engage mammalian glycosylphosphatidyl inositol-anchored protein DNase X to bind and enter host cells. How this interaction drives *E. chaffeensis* entry is unknown. In the present study, using affinity pull-down of host cell lysates with rEtpE-C followed by LC-MS/MS analysis we identified two additional interacting mammalian proteins: a transmembrane glycoprotein CD147 and a cytosolic protein hnRNP-K. Functional neutralization of surface-exposed CD147 with monoclonal antibodies or knock-down of CD147 using shRNA inhibited *E. chaffeensis* internalization and infection, but not binding. CD147 was recruited to the *E. chaffeensis* entry foci. Functional ablation of cytoplasmic hnRNP-K by intracellular antibody, while not affecting *E. chaffeensis* binding to host cells, significantly hampered bacterial entry. Analysis of the EtpE-C-DNase X-pulled down complex revealed CD147 and N-WASP. Actin and N-WASP localized to the entry foci of *E. chaffeensis* and rEtpE-C-coated beads; and chemicals that inhibit actin dynamics drastically inhibited *E. chaffeensis* entry and subsequent infection of cells. EtpE-C, but not EtpE-N terminus (EtpE-N) was able to stimulate actin polymerization in an N-WASP and DNase X-dependent manner in an *in vitro* pyrenyl actin polymerization assay. Compared with non-coated beads, internalized rEtpE-C-coated beads did not traffic to lysosomes or co-localized with NADPH oxidase components. rEtpE-C-coated beads did not induce reactive oxygen species generation by mouse bone marrow-derived macrophages, whereas non-coated or rEtpE-N-coated beads induced significant response. These results suggest that the engagement of DNase X by EtpE-C recruits CD147 to the sites of bacterial binding, which then recruit hnRNP-K and N-WASP to induce localized actin polymerization and consequent bacterial engulfment. Furthermore, EtpE-DNase X-mediated pathway inhibits or avoids anti-microbial mechanisms of phagocytes for *E. chaffeensis* survival.

Keywords: *Ehrlichia chaffeensis,* HME, Receptor, Infectious entry, DNase X, EtpE, CD147, hnRNP-K, N-WASP, actin, reactive oxygen species

IMID - 13

EVALUATING THE ABILITY OF RETINOIC ACID TO INDUCE REGULATORY MYELOID CELLS. M. Ormsby, Z.VanGundy, H. Strange, and TL Papenfuss. Department of Veterinary Biosciences

Myeloid cells (MCs) play an important role in antigen processing and bridge the gap between the innate and adaptive immune response. The contribution of MCs to inflammation has been well studied; however, their regulatory abilities are more recently being investigated for their potential to treat immune mediated disease. The vitamin A metabolite, retinoic acid (RA), has been shown to regulate immune responses at mucosal sites and promote MC differentiation. Our lab has previously found that retinoic acid (RA) induced the differentiation of a CD11c-CD11b+Ly6Clow regulatory MC population from bone marrow. The RA Reg MC population was CD11c-CD11b+Ly6Clow and had a mature activated phenotype (i.e. increased CD80, CD86, MHCII, PD-L1, PD-L2 and IL-10+) and could suppress the proliferation of responder immune cells. The ability of RA to induce Reg MC abilities in a mature myeloid cell is unknown. Although the mechanism is unknown, the protein Akt has been shown to influence macrophage polarization. A mouse macrophage cell line was used to determine whether RA affects a mature cell line in a similar way as primary bone marrow cells. RA was added to a mouse macrophage cell line for one to seven days and then the phenotype was assessed by flow cytometry. We assessed the cells' suppressive abilities by co-culturing them with responder splenocytes in a functional suppression assay. We found that RA did not provide consistent regulatory capabilities throughout the seven day course. An interesting effect of RA at days 3-5 of exposure was noted with an increased MHC class II, PD-L1 and Akt levels compared to other durations of RA exposure. Importantly, a 3-5 day exposure of macrophages to RA prevented their ability to suppress the proliferation of responder immune cells. Future studies will investigate the molecular signaling cascades that occur at day 3-5 of RA exposure in mature macrophages.

Keywords: Regulatory myeloid cells, macrophage, retinoic acid

IMID - 14

DEFINING THE ROLE OF EOSINOPHILS IN VIRAL INFECTIONS.

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According to current dogma, virus infections lead to recruitment of neutrophils whereas infection with parasites leads to recruitment of eosinophils. The presence of eosinophils during viral infections is considered to be a sign of a pathogenic process. In order to study this question, we used the cotton rat model (Sigmidon hispidus) because cotton rats are uniquely susceptible to a variety of human respiratory pathogens. We compared the influx of eosinophils into the lungs of cotton rats after infection with respiratory syncytial virus, measles virus, influenza virus, and Staphylococcus aureus. Inbred male cotton rats were intranasally inoculated with virus in a 100 µl volume. Four days after infection, a broncho-alveolar lavage (BAL) was performed and the lungs of the cotton rats were preserved in formalin for histological analysis. In order to identify eosinophils, BAL cells were stained with the (standard) Wright/Giemsa stain and the Hansel stain which is supposed to be specific for eosinophils. Electron microscopy was performed on BAL cells in order to determine the ultra structure of eosinophils and neutrophils. For histological analysis of the lungs, the Luna stain, specific for eosinophils, was compared with the standard H&E stain. The percentage of eosinophils in the naïve cotton rat lung is high. This correlates to the high percentage of eosinophils in their blood. Depending on the pathogen used, varying levels of eosinophils or neutrophils are seen to infiltrate the lungs. Comparing different pathogens there is no clear “virus pattern” versus “bacterial pattern”, but it is more dependent on the individual pathogen used.

Keywords: Eosinophils, viral infections, respiratory syncytial virus

IMID - 15

ACETALATED DEXTRAN MICROPARTICLES DELIVERY AS A MEANS TO GENERATE REGULATORY MYELOID CELLS. Z VanGundy, H Strange, T Papenfuss. Veterinary Bioscience department

Myeloid cells (MC) have potent regulatory abilities but factors influencing the development of such regulatory myeloid cells (MC_{regs}) remain poorly understood. Retinoic acid (RA) is a metabolite of vitamin A that we have previously shown to produce MC_{regs} that expressed increased IL-10, T_{reg} cells, maturation/regulatory phenotype and were able to suppress the proliferation/cytotoxicity of $CD4^+$/$CD8^+$ T cells respectively. We hypothesize that encapsulation of RA within acetalated dextran (Ac-DEX) microparticles (MPs) can be used for passive targeted generation of MC_{regs} *in vivo.* Acetalated dextran is a homopolysaccharide of glucose that can be manufactured by standard emulsion techniques and utilized as a passive delivery system. Ac-DEX MPs have the unique ability of being pH sensitive, tunable in size and degradation, and having non-toxic byproducts, making them ideal for passive targeting for therapeutic use. We used MPs containing encapsulated FITC-labeled BSA to determine the relative uptake of Ac-DEX MPs by myeloid cell populations *in vitro* and *in vivo.* We found that labeled MPs were readily taken up by numerous myeloid cell populations including macrophages and DCs *in vitro.* Following *in vivo* injection, increased percentage of FITC+ cells was seen in both $CD11c^+CD11b^-$ and $CD11c^-CD11b^+$ cells in draining lymph nodes (DLN) over controls of free FITC or empty MP administration. When RA was encapsulated into MPs, $CD11b^+$ cells within the DLNs had an increase expression of regulatory co-stimulatory molecular PD-L1. Also cells of the DLNs in mice receiving RA Ac-DEX MPs had reduced proliferation compared to control mice. These results suggest that *in vivo* targeted delivery of retinoic acid by Ac-Dex MPs can induce MC_{regs} which may have therapeutic application to treat inflammatory and immune-mediated diseases.

Keywords: Retinoic acid, Regulatory cells, Myeloid cells, Acetalated dextran Microparticles

IMID - 16

THE EFFECT OF MOUSE SAMHD1 PHOSPHORYLATION ON RETROVIRAL INFECTION

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Human SAM domain and HD domain-containing protein 1 (hSAMHD1) is a retroviral restriction factor, which blocks the infection of human immunodeficiency virus type-1 (HIV-1) and mouse leukemia virus (MLV) in human cells by hydrolyzing cellular deoxynucleoside triphosphates (dNTPs) and limiting viral reverse transcription. Recent studies have shown that phosphorylation of hSAMHD1 at threonine 592 (T592) impairs SAMHD1-mediated HIV-1 restriction. Mouse SAMHD1 (mSAMHD1) shares 72% identity with hSAMHD1 in protein sequences and also exhibits trisphosphohydrolase. Our mass spectrometry analysis has revealed a phosphorylation site at T603 in mSAMHD1, which is contained within a consensus cyclin-dependent kinase recognition sequence. Therefore, we hypothesized that mSAMHD1 inhibits MLV and HIV-1 infection by decreasing cellular dNTP concentrations, and that phosphorylation of mSAMHD1 at T603 impairs its restriction of MLV and HIV-1 infection. To this end mouse fibroblast cells (NIH3T3) were transduced with lentiviral vectors to stably overexpress wild-type (WT) hSAMHD1 or WT-mSAMHD1. Intracellular dNTP measurement indicated that overexpression of WT-hSAMHD1 or WT-mSAMHD1 reduced the intracellular dNTP pool in NIH3T3 cells. The infection of MLV, but not HIV-1, was reduced by two-fold in NIH3T3 cells expressing WT-hSAMHD1 and WT-mSAMHD1 compared to the empty vector-transduced cells. To test whether mSAMHD1 restricts HIV-1 infection in human non-dividing cells, we infected non-dividing human U937 monocytic cells that stably express human and mouse SAMHD1. The infection results showed that HIV-1 infection was significantly reduced by WT-hSAMHD1 and WT-mSAMHD1. We are currently testing the role of phosphorylation at position T603 in mSAMHD1-mediated restriction of HIV-1 using phopho-ablative and phosphor mimetic mutants in non-dividing U937 cells. Our results indicate that mSAMHD1 restricts MLV infection in NIH3T3 cells by reducing the cellular dNTP pool level, and will address the importance of T603 in mSAMHD1-mediated HIV-1 restriction. These results will extend the current knowledge about the mechanism of mSAMHD1-mediated retroviral restriction.

Keywords: mouse SAMHD1, MLV, HIV-1, dNTP

IMID - 17

ALTERNATIVELY ACTIVATED MACROPHAGES ATTENUATE INFLUENZA-INDUCED LUNG INJURY IN MICE HETEROZYGOUS FOR THE F508DEL MUTATION IN CFTR. P.S. Woods, C.C. Hofer, F.Aeffner, and I.C. Davis. Department of Veterinary Biosciences and The Ohio State College of Medicine.

Influenza viruses cause a highly contagious acute respiratory disease which is of major concern to public health. Severe primary influenza infection can rapidly progress to acute lung injury (ALI), which is defined by the onset of severe hypoxemia, pulmonary edema, and respiratory failure. Often ALI is not a direct result of viral replication, but rather a consequence of an exaggerated immune response to viral antigens. We have shown that development of ALI in influenza-infected C57BL/6 mice (WT mice) correlates with increased Cl- secretion via the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel expressed on alveolar epithelial cells. Influenza-induced ALI is greatly reduced in C57BL/6-congenic mice that are heterozygous for the F508del Mutation in CFTR, which results in a 50% reduction in CFTR-mediated Cl- transport (HET mice). This effect was associated with higher alveolar macrophage (AM) counts at 6 days post-infection (d.p.i.), and could be reversed by AM depletion using clodronate liposomes. We hypothesized that AMs from influenza-infected HET mice possess an “alternative” anti-inflammatory phenotype (M2), and thus are key mediators in the attenuation of influenza-induced ALI. AMs isolated from influenza-infected HET mice express more Arginase 1 protein and less inducible nitric oxide synthase (iNOS), which is characteristic of a M2 phenotype. In addition, brochoalveolar lavage fluid (BALF) from HET mice contained less nitrate/nitrite and more urea (indicators of iNOS and Arginase 1 activity, respectively) than BALF from WT mice. At 2 d.p.i., HET BALF also contained increased amounts of TGF-β, an anti-inflammatory cytokine that inhibits CFTR and promotes M2 polarization. These findings suggest that reduced CFTR expression/activity promotes an anti-inflammatory, M2-polarized response of HET AMs to influenza, resulting in attenuation of influenza-induced ALI. Hence, we propose that, by promoting M2 polarization of AMs, short-term inhibition of CFTR expression and/or CFTR-mediated Cl- transport may be a novel approach to preventing influenza-induced ALI.

Key Words: influenza, CFTR, macrophage, lung injury

MOLECULAR
AND
CELLULAR BIOLOGY

THE ROLE OF TAX-1 AND THE ALTERNATIVE NF-KB PATHWAY IN HTLV TRANSFORMATION. J. Al-Saleem and P. L. Green, Department of Veterinary Biosciences

Human T-cell Leukemia Virus Type-1 (HTLV-1) is a complex retrovirus infecting 15-25 million people worldwide. HTLV-1 is the etiological agent of an aggressive malignancy of CD4+ T cells termed Adult T-Cell Leukemia (ATL). ATL patients, on average, survive one year from disease onset. The HTLV-1 regulatory protein Tax is required for HTLV-1-mediated cellular transformation both *in vitro* and *in vivo*. Tax primarily functions to promote transcription of viral genes, but has also been shown to deregulate cellular genes leading to cell growth and genetic instability. Previous studies showed that Tax induces NF-κB by two distinct signaling/activation pathways: the classical pathway, which is rapid, and the alternative pathway, which is delayed. The exact role of the alternative pathway in HTLV-1-mediated transformation is unknown. We propose that Tax interaction with the alternative NF-κB pathway is important for HTLV-induced pathogenesis. To test this hypothesis we will identify and utilize Tax mutants that are deficient in their ability to activate the NF-κB pathways. Viruses containing these mutant forms of *tax* will be used to infect primary peripheral blood mononuclear cells (PBMCs) and transformation will be monitored via a cellular proliferation assay. We also plan to identify Tax-1 binding partners that are important for Tax-mediated activation of the NF-κB pathway. To identify these binding partners we generated S-protein epitope tagged Tax expression vectors for both wild type and NF-κB activation deficient *tax* genes. We expressed tagged Tax proteins in 293T cells and performed immunoprecipitation followed by mass spectrometry to identify binding partners. By comparing the differences between the wild type Tax and mutant Tax binding partners we will be able to identify candidate proteins required for Tax-mediated activation of the alternative NF-κB pathway. We hypothesis that these candidates could then be used in future studies as clinical targets to treat patients with ATL.

Keywords: HTLV, Tax, NF-κB

MCB - 2

BIOLOGIC ACTIVITY OF THE NOVEL ORALLY BIOAVAILABLE SELECTIVE INHIBITOR OF NUCLEAR EXPORT (SINE) KPT-335 AGAINST CANINE MELANOMA CELL LINES

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Background: Exportin 1 (XPO1), a protein responsible for the export of over 200 target proteins out of the nucleus, is upregulated in several human cancers and its expression is linked to chemotherapy resistance. Recent studies in human and murine tumor cell lines showed that XPO1 is a relevant target for therapeutic intervention. The purpose of this study was to characterize the biologic activity of a novel orally bioavailable selective inhibitor of nuclear export (SINE) compound, KPT-335, that irreversibly blocks the action of XPO1 in canine melanoma cell lines.
Methods: Four canine oral melanoma cell lines were evaluated. Cell proliferation was analyzed using the CyQuant assay to obtain the IC50 concentration for each line. Apoptosis was assessed using Annexin V/Propidium Iodide staining followed by flow cytometry. The ability of cells to form colonies was determined using a colony formation assay. Quantitative RT-PCR was used to assess alterations in gene expression. Changes in XPO1, p53 and p21 protein expression were assessed using Western blot analysis following SDS-PAGE of whole cell lysates or nuclear and cytoplasmic protein fractions. Lastly, confocal microscopy was used to evaluate the cellular localization of p53 and p21 after KPT-335 exposure.
Results: KPT-335 inhibited the proliferation of melanoma cell lines with IC50 values ranging from 0.071-0.330 μM. A significant increase in apoptosis and reduced ability of cells to form colonies *in vitro* were noted following XPO1 inhibition. KPT-335 promoted downregulation of XPO1 protein while inducing a concomitant increase in XPO1 mRNA. The tumor suppressor proteins p53 and p21 exhibited enhanced nuclear localization following KPT-335 exposure. Lastly, upregulation of mRNA for both MIC1 and p21 was observed after treatment with KPT-335.
Conclusion: KPT-335 demonstrates biologic activity against canine melanoma cell lines at physiologically relevant doses, suggesting that KPT-335 may represent a viable treatment option for dogs with malignant melanoma.

Keywords: XPO1, malignant melanoma, dog

MCB - 3

EVALUATION OF ANTITUMOR ACTIVITY OF SELECTED CHEMOTHERAPY AGENTS ON *IN VITRO* MODELS OF CANINE NON-SMALL CELL LUNG CANCER. F. Clemente-Vicario[1], S. Roy[1], W. Kisseberth[1], C. London[2], G. Lorch[1]. [1]Department of Veterinary Clinical Sciences and [2]Department of Veterinary Biosciences

Canine lung cancer has a metastatic incidence of approximately 71% at the time of diagnosis. Response rates to current chemotherapy drugs are poor. There is a criticial need to identify therapuetic vulnerabilities for these patients. Our objectives were to determine the biological activity, ICs_{50}, and downstream targets of platinum chemotherapies and targeted therapies on two canine lung adenocarcinoma cell lines (BACA and CLAC). Total RNA was isolated from both cell lines. RT-PCR was used to identify transcriptional targets that code for proteins which can be targeted with small molecule inhibitors. Changes in cell line viability were measured using the CyQUANT® cell proliferation assay after 72 hours of drug treatment. A logarithmic regression curve constructed from the cell viability data was used to calculate the ICs_{50} for the drugs. The inhibitory effects of treatment with the targeted therapies, toceranib and ganetespib, on receptors and downstream signaling activation were determined using immunoblotting. mRNA transcripts for predominately tyrosine kinase receptors and two downstream kinases were present in both cell lines and included NKX2-1, EGFR, cKit, HER2, VEGFR2, PDGFRα, PDGFRβ, Met, MAPK, Ret and Akt. Ganetespib, an HSP90 inhibitor, was the only agent that inhibited growth of both cell lines in a dose-dependent manner. The IC_{50} for ganetespib was 0.021 and 0.023 µM for BACA and CLAC, respectively. Ganetespib used at a biologically achievable dose significantly downregulated IGF-Irβ, EGFR, HER2, p-Akt, p-mTOR, and p-MAPK proteins in the CLAC line and HER2, p-Akt and p-MAPK protiens in the BACA line. Toceranib 's IC_{50} values were 0.15 µM for the BACA line and 0.27 µM for the CLAC line. Toceranib treatment did not decrease protein or phosphorylation levels of any receptors in either cell line. In conclusion, ganetespib exhibits potent *in vitro* activity against canine lung adenocarcinoma cell lines by destabilizing several HSP90 cellular clients.

Keywords: canine, lung cancer, ganetespib, toceranib, inhibitory concentration 50

MCB - 4

GENERATION AND EVALUATION OF A CANINE OSTEOSARCOMA CELL LINE RESISTANT TO THE SMALL MOLECULE STAT3 INHIBITOR LLL12. JI Couto[1], MD Bear[1], WC Kisseberth[2], and CA London[1]
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Osteosarcoma (OSA) is the most common primary bone neoplasm in both humans and canines. Despite multiple efforts, no improvements in survival times have been achieved over the past 15 years. STAT3 is a transcription factor important in transmitting signals from growth factor receptors and cytokines into the nucleus. We have previously shown that STAT3 is constitutively activated in canine OSA and that inhibition of STAT3 phosphorylation blocks canine OSA tumor cell growth. Specifically, we demonstrated that the novel small molecule STAT3 inhibitor LLL12 induces caspase 3,7 activation and apoptosis in canine OSA tumor cell lines, while having little effect on normal osteoblasts. To investigate potential mechanisms of resistance to LLL12, we generated a resistant canine OSA cell line through continual culture in drug (OSA 8R). Proliferation assays show clear resistance to LLL12, with 5- to 10-fold higher concentrations of drug needed to inhibit tumor cell growth. Utilizing a Proteome Profiler to evaluate changes in signal tranducers in the resistant line, we found pS727 STAT3 was significantly increased while phosphorylation of both β-catenin and WNK1 was decreased compared to the parent OSA8 cell line. Additionally, total protein levels of β-catenin were downregulated. Real-time PCR demonstrated that STAT3 and Bcl-2 transcript levels were increased in the resistant lines, while survivin levels remained unchanged. Treatment of OSA8 cells with LLL12 in combination with the exportin 1 (XPO1) inhibitor KPT-335 resulted in probable synergistic antiproliferative effects. As XPO1 is responsible for shuttling STAT3 out of the nucleus, it is possible that KPT-335 may be capable of mitigating resistance to LLL12 in the OSA8R cells. In summary, we have generated an OSA cell line resistant to the STAT3 inhibitor LLL12 and have shown that the mechanism of resistance is likely to be through altered STAT3 phosphorylation and expression.

Keywords: Cancer, STAT3, Osteosarcoma, Resistance

MCB - 5

EFFECT OF HEAT SHOCK PROTEIN 27 EXPRESSION ON PANCREATIC ISLET SURVIVAL AND FUNCTION. K. Fertal, F. Xu, G. Ilangovan, M. Velayutham, and C. Adin. Dept. of Veterinary Clinical Sciences

Recent advances in cellular therapy provide hope that transplantation of insulin-producing pancreatic islets may provide a cure for type 1 diabetes mellitus (T1DM). Unfortunately, pancreatic islets are extremely susceptible to injury and up to 70% of islets die within 72 hours after transplantation. Our laboratory is working to improve islet survival by manipulation of endogenous protective mechanisms. Heat shock proteins (HSPs) are endogenous molecules that provide non-specific protection against various stressors. While researchers have exploited the benefits of HSPs in averting reperfusion injury following by-pass surgery, manipulation of this system has not been investigated for use in islet cell transplantation. Collaborators at the Davis Heart and Lung Institute have provided us with access to two strains of mice, one of which exhibits over-expression of the key heat shock protein HSP27 (HSP 27tg) and another that encodes a non-functional form of the same protein (HSP 27tgm). Our overall hypothesis is that HSP over-expression will improve islet cell survival in models of transplant associated stress. In these studies, we will isolate islets from HSP 27tgm, HSP 27tg and from BALB/c (wildtype control) mice. The islets will then be subjected to hypoxic stress using an in vitro model that was developed in our lab. At 24 hours after exposure to hypoxia, cell viability will be determined using indirect fluorescent imaging and % cell death will be quantified using a custom islet macro for Image J. At the end of the experiment, conditioned media will be immediately frozen in liquid nitrogen and stored for measurement of cytokines (IL6, IL1b, TNF), DAMPs (HSP 70, HMGB1), and free radicals. Documentation of positive effects of HSP over-expression in transgenic mice would open the door to the use of viral vectors or targeted pharmacologic agents aimed at manipulation of the HSP system during islet transplantation.

Keywords: pancreatic islets, type 1 diabetes mellitus, HSP 27tg, HSP 27tgm

MCB - 6

A NOVEL MODEL TO ASSESS LAMINAR HYPOXIA IN SUPPORTING LIMB LAMINITIS

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The pathophysiology of equine supporting limb laminitis (SLL), a common and often fatal complication of equine orthopedic disease, is poorly understood. Often horses survive the initial catastrophic trauma, e.g. fracture, only to succumb to fulminant failure of the contralateral limb. Suggested causes of laminar failure include inflammatory injury, hypoxia and mechanical strain. We hypothesized that laminar hypoxia occurs in the supporting limb (SL) resulting in increase in laminar hypoxia-inducible factor-1 alpha (HIF-1). A novel model of SLL was used in this study in which a custom shoe insert causing instability to the sole surface upon weight bearing was placed on one forelimb of 8 Standardbred horses resulting in excessive weighting of the contralateral forelimb (SL). Laminae were harvested and immediately snap-frozen from all four limbs 48 h post-application of the shoe. Western hybridization (WH) and real time-quantitative PCR (qPCR) were used to assess markers of hypoxia (HIF-1A) inflammation (qPCR for IL-6, IL-1B, COX-2, NOS2), and stretch (phospho-ERK ½ and phospho-JNK). The only change noted was an increase ($P<0.05$) in laminar HIF-1A protein concentrations in the SL compared to both hindlimbs. Genes including those indicated in stretch, metabolism and inflammation were not upregulated. These results indicate that laminar hypoxia and HIF-1A may play a central role in SLL, and future research should focus on therapeutic options, including novel shoeing options on the SL which may allow increased vascular oxygen delivery to the distal limb. HIF-1A may have value as a biomarker of laminar hypoxia, and be used to assess the efficacy of SLL treatments.

Keywords: laminitis, equine, supporting limb, hypoxia, HIF-1A

MCB - 7

CYCLOSPORINE A INDUCES AUTPOPHAGIC CELL DEATH IN LENS EPITHELIAL CELLS TO PREVENT POSTERIOR CAPSULE OPACIFICATION EX VIVO. KJ Gervais, RB Matusow, EM Curto, HL Chandler. Department of Veterinary Clinical Sciences; College of Optometry

Purpose. To determine the appropriate Cyclosporine A (CsA) dose and minimum drug delivery time needed to prevent posterior capsule opacification (PCO) in an *ex vivo* model and evaluate the mechanism of CsA-induced cell death. **Methods.** Lens capsules were harvested from canine cadaver eyes using an established *ex vivo* model of PCO. Lens capsules were treated with 0, 5, or 10 µg/mL CsA for 0, 2, 3, 4, 5, 6, or 7 days, and then maintained in culture for a total of 28 days in the absence of drug. CsA treated lens epithelial cells (LEC) underwent routine transmission electron microscopy (TEM), western blotting, and fluorescent staining to evaluate the mechanism of cell death. **Results.** Lens capsules treated with 5, 6, or 7 days of 10 µg/mL CsA showed a significant decrease in *ex vivo* PCO formation; 7 days of drug delivery was sufficient to prevent PCO. Morphologically, CsA treated LEC were swollen, had intact nuclei, lacked peripheral chromatin condensation, and demonstrated prominent vacuolization; TEM revealed autophagosomes. LC3-II protein expression and acridine orange fluorescence increased in CsA treated cells. Dose dependent changes were observed in all experiments. **Conclusions.** Seven days of intracapsular CsA drug delivery prevented *ex vivo* PCO formation. Morphologic changes and TEM suggest that CsA is able to induce LEC death via autophagy; this is a novel finding in the lens. Acridine orange is a marker for acidic vesicles and LC3-II is a protein involved in mediating autophagic death. Expression of these markers in CsA treated LEC further supports this new mechanism of drug-induced LEC death. Funded in part by the American Kennel Club Canine Health Foundation.

Keywords: lens, Cyclosporine A, posterior capsule opacification, autophagy

MCB-8

CHARACTERIZING TRAMETINIB RESISTANCE IN BREAST CANCER.

Hicks, M., Macrae, E., DeWille, J. Department of Veterinary Biosciences

Our goal is to study the molecular susceptibilities of TNBC to targeted therapies and to drive the development of novel therapeutic strategies for future clinical trials. Triple negative breast cancer (TNBC) is a basal-like subtype characterized by tumors that lack expression of the estrogen receptor (ER), the progesterone receptor (PR) or the human epidermal growth factor receptor 2 (HER2). Recent targeted therapies for other subtypes of breast cancer have demonstrated success; for example, tamoxifen for ER+ breast cancer and trastuzumab for HER2+ breast cancer. TNBC patients are unresponsive to these therapies, resulting in a disproportionately high number of breast cancer related deaths. Whole transcriptome sequencing in TNBC patients reveal activation of the MAPK pathway. Treatment with trametinib (a MEK inhibitor) is currently FDA approved for use in melanoma, and currently being investigated in the first clinical trial for targeted therapy in TNBC (NCT01964924). **Because resistance is expected in 80% of patients, we hypothesize that by developing trametinib resistant cell lines we can uncover an altered molecular signature which can predict resistance to targeted therapeutics in TNBC**. To our knowledge, there are no TNBC pre-clinical models of resistance to trametinib. To address this unmet need we developed two trametinib resistant TNBC cell lines (PTEN WT MDA-MB-231 and PTEN knockout MDA-MB-468) using the dose escalation method. Protein reprogramming has been proposed as a mechanism to circumvent MEK inhibition. As an initial screen, Western Blotting was performed. Results demonstrated p-ERK was inhibited with short term and long term treatment with trametinib as expected. Interestingly p-AKT was induced in the PTEN knockout cell line with short term trametinib treatment and further enhanced in resistant PTEN knockout TNBC cells. Future studies will investigate global alterations in gene expression and proteomics in trametinib resistant TNBC cell lines using a transcriptome microarray and phopho-kinase antibody array.

Keywords: trametinib, breast cancer, chemotherapy resistance, MAPK

MCB - 9

CONDITIONAL DELETION OF PTEN WITHIN MURINE UTERINE EPITHELIUM RESULTS IN ENDOMETRIAL CARCINOMA: AN IMPORTANT MODEL FOR HUMAN ENDOMETRIOID CARCINOMAS. C. Koivisto[1], V. Bravo[2], A. Perez-Castro[2], A. Clements[3], K. La Perle[1,4], D. Cohn[3], C. Timmers[5] and G. Leone[2].
[1]Department of Veterinary Biosciences, [2]Department of Molecular Genetics, [3]Department of Obstretrics and Gynecology, [4]Comprehensive Cancer Center (CCC) Comparative Pathology & Mouse Phenotyping Shared Resource and [5]CCC Solid Tumor Translational Science Shared Resource, The Ohio State University, Columbus, OH.

Endometrial carcinoma is the most common malignancy in the female reproductive tract. Alterations in PTEN occur in up to 80% of type I (endometrioid) carcinomas in humans and appear to be an early event in the transformation process. To support these observations, we have analyzed a cohort of 320 human endometrioid carcinomas for *PTEN* mutations and find that 258 (80.6%) harbor *PTEN* mutations.

Previous mouse models designed to study the pathogenesis of endometrial cancer have had significant limitations primarily due to lack of endometrial-specific genes that could serve as promoters for driving transgene expression. The *Sprr2f* gene, identified through an organ-specific, gene-expression analysis as having uterine specificity had been used to generate a transgenic mouse model to conditionally express Cre-recombinase and was used to conditionally delete the tumor suppressor, *Lkb1* in endometrial epithelium, resulting in highly aggressive endometrial cancer. Using this same mouse model, we have conditionally deleted *Pten* and documented disease progression similar to that in human endometrioid cancers with foci of carcinoma *in-situ* progressing to invasive endometrial adenocarcinoma with myometrial and lymphatic invasion by 6 months of age. Immunohistochemical staining for Pten and phosphorylated Akt demonstrated loss of Pten expression and activation of Akt confined to the endometrial epithelium.

These studies emphasize the importance of PTEN in the pathogenesis of type I endometrial carcinoma. Importantly, this is the first mouse model to specifically delete Pten in the endometrial epithelium and parallels the morphology and molecular biology commonly observed in human endometrial cancer. There are conflicting reports in the literature regarding the status of PTEN and its association with disease prognosis. Further studies are underway to fully characterize the status of PTEN in our patient cohort at both the genetic and protein level and whether or not there is any correlation with clinical outcome.

Keywords: Endometrial Cancer, PTEN, Mouse Models

MCB - 10

TGF-α AND AMPHIREGULIN STIMULATION OF EGFR AND INDUCTION OF PTHRP IN FELINE SQUAMOUS CELL CARCINOMAS. K. Krammer, W. Dirksen, W. Supsavhad, and T.J. Rosol. Department of Veterinary Biosciences, The Ohio State University

Feline oral squamous cell carcinoma (FOSCC) is a destructive, malignant head and neck cancer associated with unsuccessful treatments and a poor prognosis. FOSCC is the most common oral tumor in cats, a viable treatment option is in demand, and the epidermal growth factor receptor (EGFR) is a possible target. EGFR is a tyrosine kinase receptor found in fibroblasts and epithelial cells, and its signaling pathways regulate cell growth, survival, adhesion, migration and differentiation. Human and feline EGFR have high gene sequence homology, which suggests the possibility of using human ligand and antagonist treatment strategies. Ligands that stimulate human EGFR include high affinity EGF, BTC, heparin binding-EGF, and low affinity EREG (epiregulin), TGF-α, AREG (amphiregulin), and EPGN (epigen). It is believed that low affinity signaling increases EGFR recycling, leading to increased signaling. In the clinically related human oral SCC, EGFR is overstimulated and deregulated, resulting in increased parathyroid hormone-related protein (PTHrP). Previous studies indicate that the FOSCC cell lines developed in our laboratory with the highest level of PTHrP expression, particularly SCCF2 cells, exhibit the highest degree of in vitro and in vivo bone resorption and osteoclastogenesis. Thus, it would be expected that agonist stimulation of feline EGFR by TGF-α and AREG will increase feline EGFR phosphorylation and feline PTHrP mRNA levels in SCCF1 (laryngeal), SCCF2 (maxillary), and SCCF3 (lingual) cell lines. Using human TGF-α and AREG, F1, F2, and F3 cell lines were treated for 0 (control), 3, and 24 hours. Real-Time RT-PCR was performed on RNA from these cells using primers for fEGFR and fPTHrP. It was found that F2 and F3 cells had increased expression of fEGFR and fPTHrP in response to both agonists. This indicates a need for more personalized FOSCC treatment and antagonist studies, using human monoclonal antibodies (mAb) or tyrosine kinase inhibitors (TKI) against fEGFR.

Keywords: feline, oral squamous cell carcinoma, EGFR, PTHrP

MCB - 11

CANINE PULMONARY ADENOCARCINOMA TYROSINE KINASE RECEPTOR EXPRESSION AND PHOSPHORYLATION. E. Mariotti, C. Premanandan, and G. Lorch. Depts. of Veterinary Biosciences and Veterinary Clinical Sciences.

This study evaluated tyrosine kinase receptor (TKR) expression and activation in canine pulmonary adenocarcinoma (cpAC) biospecimens. As histological similarities exist between human and cpAC, we hypothesized that cpACs will have increased TKR mRNA and protein expression as well as TKR phosphorylation. The molecular profile of cpAC has not been well characterized making the selection of therapeutic targets that would potentially have relevant biological activity impossible. The objectives of this study were to define TKR expression and their phosphorylation state in cpAC as well as to evaluate the tumors for the presence of potential epidermal growth factor receptor (EGFR) tyrosine kinase activating mutations in exons 18–21. Immunohistochemistry (IHC) for TKR expression was performed using a tissue microarray constructed from twelve canine tumors and companion normal lung samples. Staining intensities of the IHC were quantified by a veterinary pathologist as well as by two different digitalized algorithm image analyses software programs. An antibody array was used to evaluate TKR phosphorylation of the tumor relative to normal tissues with the resulting spot intensities quantified using array analysis software. Each EGFR exon PCR product from the tumors and non-affected lung tissues were sequenced. The pro-angiogenic growth factor receptor, PDGFRα, had increased cpAC tumor mRNA, protein expression and phosphorylation when compared to the normal lung tissue biospecimens. Similar to human pulmonary adenocarcinoma, significant increases in cpAC tumor mRNA expression and receptor phosphorylation of the anaplastic lymphoma kinase tyrosine receptor were present when compared to the corresponding normal lung tissue. The EGFR mRNA, protein expression and phosphorylation were not increased compared to the normal lung and no activating mutations were identified in exons 18–21.

Canine pulmonary adenocarcinoma TKRs are detected at both the mRNA and protein levels and are activated. Further investigation into the contribution of TKR activation in cpAC tumorigenesis is warranted.

Keywords: Canine; Epidermal growth factor receptor; Lung cancer; Platelet-derived growth factor receptor; Tyrosine kinase receptors

MCB - 12

CHARACTERIZATION OF THE PERFORMANCE OF A FILTRATION-BASED EQUINE BONE MARROW STEM CELL HARVEST SYSTEM FOR THE CONCENTRATION, VIABILITY, AND RECOVERY OF STEM CELLS. L. Mundy*, A. Ishihara*, M. Wellman+, A. Bertone*.
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The objective of this study was to assess the efficiency of gravity filtration to enrich equine bone marrow for blood component recovery, recovered cell type proportions, stem cell recovery, stem cell replication, and progenitor cell differentiation. Bone marrow aspirates were collected from twelve healthy, adult horses from the fifth sternebral body and filtered by gravitational flow to capture, and release, marrow elements. Raw, filtered, and harvested bone marrow were evaluated for white blood cell and platelet counts, automated and cytomorphologic cell differentials, bone marrow-derived mesenchymal stem cell colony forming units, cell viability by flow-cytometry, and differentiation capacity. Isolated marrow mesenchymal stromal cells were analyzed for CD90 and MHC class I and II antigens. Eleven of fourteen marrow aspirates (79%) successfully produced filtered marrow and 100% of filtered marrow produced a harvested marrow product. Mean cell viability of harvested marrow was 95.9%. Total white blood cells and platelets were efficiently captured by the filter (> 95%), but recovery in harvested marrow was a mean of 30%. Cytologic cell differentials indicated that neutrophils (%) were significantly less ($P<0.05$) and the progenitor cell population 1.56-fold greater in the harvested marrow compared to raw marrow. This represented a 37% recovery of progenitor cells from raw marrow. Flow cytometry and culture characterized harvested marrow cells as CD90+, MHCI-, and MHCII- indicating a stem cell phenotype that were multipotent and differentiated into chondrocytes, osteocytes, adipocytes, and tenocytes. Gravitational filtration of bone marrow efficiently captured platelets and cells and significantly enriched a viable progenitor/stem cell population while decreasing the neutrophil population. Filter modifications are anticipated to improve efficiency of cell harvest and result in greater concentration of cell components.

Keywords: bone marrow, stem cells, regenerative medicine, equine

MCB - 13

EXTRACELLULAR HEAT SHOCK PROTEIN 70 AS A POTENT STIMULATOR OF CANINE CORNEAL WOUND HEALING. CW Peterson and HL Chandler. The Ohio State University, Department of Veterinary Biosciences.

Purpose. The 70kDa heat shock protein (Hsp70) has been suggested to improve tissue repair and facilitate cellular migration in actively healing wounds. Expression has been localized to nuclei of corneal epithelial cells and stromal fibroblasts in normal and wounded canine corneas. Cytokine induction as a result of Hsp70 expression was investigated to elucidate the extracellular role for Hsp70 in normal canine corneal wound healing. **Methods.** Primary cultures of canine corneal stromal fibroblasts were treated with exogenous Hsp70 and an artificial wound was created *in vitro* to monitor restoration of the monolayer. Cell culture supernatants were analyzed for transforming growth factor-beta 1 (TGF-β1) with an ELISA to evaluate protein expression of pro-fibrotic cytokines downstream in the Hsp70 signaling pathway. Cellular lysates were subject to routine RT-PCR to evaluate for TGF-β1 and Toll-Like Receptor 4 (TLR4) mRNA expression. **Results.** Hsp70-treated fibroblasts demonstrated significantly ($p<0.001$) increased migration and proliferation at 12 hours, with 95.8% of the original wound area healed compared to 37.6% in the vehicle-treated group. Fibroblast culture supernatants subject to ELISA did not demonstrate any appreciable expression of TGF-β1. Additionally, RT-PCR of lysates from Hsp70 treated cells found no significant differences in TLR4 or TGF-β1 mRNA when compared to controls. **Conclusions.** Extracellular Hsp70 may have an instrumental role in facilitating appropriate corneal wound healing as a potent inducer of cellular migration and proliferation. This is independent of TLR4 or TGF-β1 expression, a signaling pathway that has been documented in other models of epithelial healing and fibrosis. Supported by The Ohio State University Internal Research Funds.

Keywords: Hsp70, canine corneal wound healing, TLR4, TGF-β1

MCB - 14

SUBCELLULAR LOCALIZATION OF RNA HELICASE A BY CONFOCAL MICROSCOPY. S. Peteya and K. Boris-Lawrie. Dept. of Veterinary Biosciences and OSU Center for Retrovirus Research.

RNA helicase A (RHA) is important in many cellular processes, including development, immunity, and viral infections. It is required for translation of certain RNAs and enhances synthesis of some viral proteins, including those of Human T-lymphotropic Virus Type 1 (HTLV-1). RHA is mainly a nuclear protein with some cytoplasmic accumulation and the C-terminal end is thought to drive most of its subcellular localization, but few have investigated the role of specific domains in localization of RHA. We attempted to characterize the domains of RHA, hypothesizing that deletion of various domains would change its subcellular accumulation. Hela cells were transfected with deletion mutants of RHA tagged with fluorescent proteins and stains for different organelles and cellular membranes applied. These cells were imaged via confocal microscopy to determine which domains of RHA are necessary to accumulate in different subcellular compartments, including the nucleus, cytoplasm and cytoplasmic aggregates, lysosomes, and cell, plasma, and intracellular membranes. Our images suggest that different domains of RHA are involved in localizing to different cellular compartments, with the most c-terminal domain appearing necessary for membrane accumulation and the RG rich domain for fine cytoplasmic aggregation. We demonstrated RHA mutant localization in all cellular compartments examined, and membrane and lysosomal accumulation are consistent with our ongoing hypothesis that RHA is involved in intercellular communication in retroviral infections. The ability to accumulate in cellular compartments beyond the cytoplasm and nucleus, such as cellular membranes, could be important in RHA functions, particularly in immune responses in which packaged cellular components like RNA and proteins can communicate viral infections to nearby cells.

Keywords: RNA helicase A (RHA), microvesicles, confocal microscopy, localization

MCB - 15

TRANSIENT GENE DELIVERY INTO EQUINE FETAL BONE MARROW-DERIVED MESENCHYMAL STEM CELLS: THE EFFECTIVENESS OF ADENOVIRAL-MEDIATED TRANSDUCTION. JE Santiago-Torres[1,2], JO Vera[3], AL Bertone, DVM, PhD[1]. [1]Comparative Orthopedics Research Laboratory, Department of Veterinary Clinical Sciences and Biosciences, College of Veterinary Medicine, [2]College of Medicine, The Ohio State University, and [3]Department of Natural Science, University of Puerto Rico at Aguadilla

Umbilical and placental stroma could be regarded as an alternative source of mesenchymal stem cells (MSC) in conditions where adult MSCs are depleted. Gene delivery to young MSCs has not been well studied. In the current work, equine fetal MSC (efMSC) were used as a model. It was hypothesized that efMSC prematurity would alter efficiency of adenoviral vector (Ad) transduction due to several reasons, including possible lack of receptors and mature shuttle proteins. Transient gene delivery via chemical transfection may present an alternative to overcome this critical barrier. Efficiency, self-renewal, viability, and differentiation capacity were assessed in efMSCs and eaMSCs after green fluorescent protein (GFP) gene delivery. During screening for a transient gene delivery reagent, the water-soluble polymer polyethylenimine (PEI) provided better transfection and viability of efMSCs than two proprietary chemical reagents. Ad-GFP transduction at a MOI of 15 resulted in efficiency of 80.9±0.8% and 78.1±1.1% at 48 hr post-transduction in efMSCs and eaMSCs, respectively and was greater than PEI's 35.0±1.6% as assessed by flow cytometry. Mean fluorescence intensity (MFI) was unexpectedly lower for Ad-GFP-transduced efMSCs than eaMSCs ($4.40\pm0.14x10^6$ vs. $7.43\pm0.05x10^6$ channel numbers, respectively). GFP transgene expression was maintained at >70% during the first week post-transduction in eaMSCs, while only at >40% in efMSCs. Cell viability, doubling times, and differentiation capacity were not significantly different in either efMSCs or eaMSCs. These data show that adenoviral transduction is efficient in both, eaMSC and efMSC, providing similar level of transfection efficiency, but suggests that expression is significantly less intense ($p<0.05$) and of shorter duration in efMSC (1.2±0.05% vs 11.4±1.2% at 2-week post-tranduction, $p<0.05$). Also, adenoviral transduction provided a statistically significant superior efficiency than chemical transfection. Lower GFP expression in efMSCs than eaMSCs may suggest transduction of fewer viral particles per cell or less receptive machinery for expression of exogenous proteins in efMSCs.

Keywords: gene delivery, adenovirus, mesenchymal stem cells

MCB - 16

WNT SIGNALING AND PROSTATE CANCER BONE METASTASIS. JK Simmons, WP Dirksen, and TJ Rosol. Department of Veterinary Biosciences

Introduction: The molecular mechanisms by which prostate cancer cells metastasize and grow in bone are not fully understood, however we hypothesized that the Wnt signaling pathways play an important role in the pathogenesis. We over-expressed the Wnt/JNK pathway agonist, Dkk-1, in the mixed osteoblastic and osteolytic Ace-1 prostate cancer cells to investigate the contribution of the Wnt signaling pathways in prostate cancer bone metastases,. Previous work had shown that Dkk-1 expression increased the number and lytic nature of bone metastases in vivo. This study focused on elucidating how enhanced Wnt/JNK signaling could be altering the bone microenvironment.

Methods and Experimental Design: Ace-1 cells stably expressing human DKK-1 or empty vector were cultured in vitro. Wnt/JNK signaling was investigated by AP-1 reporter activity, Affymetrix mRNA microarray, and qRT-PCR. Treatment with a non-canonical Wnt agonist and antagonist were performed and the resultant changes in reporter activity, gene expression, proliferation and migration were investigated.

Results: DKK-1 significantly increased non-canonical Wnt/JNK signaling. Subsequent gene expression alterations include a dramatic decrease in mRNA expression of genes important in osteoblast maturation. Treatment with non-canonical Wnt/JNK agonist enhanced tumor cell proliferation and migration, while an effect reduction seen with the antagonist treatment.

Conclusion The present study showed that DKK-1 is a potent activator of non-canonical Wnt/JNK signaling and provides possible mechanisms whereby DKK-1 expression inhibits bone growth in prostate cancer metastases.

Impact Statement: This research highlights a potential pathway to target to reduce the morbidity and mortality of prostate cancer bone metastases.

Keywords: Non-canonical Wnt/JNK signaling, prostate cancer, bone metastasis, Ace-1

MCB - 17

COMPARATIVE TOXICITY OF CRYOPROTECTANTS ON *STAPHYLOCOCCUS AUREUS* AT ROOM TEMPERATURE. R. C. Soltys, J. Van Balen, and A. E. Hoet, Dept. of Veterinary Preventive Medicine

Staphylococcus aureus and Methicillin-Resistant *S. aureus* (MRSA) are important human and veterinary pathogens. Each year, thousands of researchers study them; thus, it is common practice for *S. aureus* and MRSA isolates to be cryopreserved. Cryopreservation is a method by which bacterial samples are prepared and frozen for long periods of time, enabling future analysis and research. However, a comparison of different cryoprotectant methods for *S. aureus* preservation has not yet been done. The objective of this study was to determine which cryoprotectant is the most efficient in preserving *S. aureus*. If the cryoprotectant dimethyl sulfoxide (DMSO) is considered highly toxic to bacteria at room temperature, then an alternative cryoprotectant such as 60% glycerol, a 50:50 solution of 7% milk and 60% glycerol, or a commercially available kit could be less toxic and thus better suited for cryoprotection. We froze eight strains of MRSA and two strains of *S. aureus* using these four cryoprotectants. The samples were then thawed and allowed to sit at room temperature for up to 72 hours, with sampling occurring at predetermined intervals. The samples were serially diluted and plated on Mueller-Hinton agar, and colony-forming units were counted after 24 hours of incubation at 37°C to determine concentration and loss in bacterial population over time. After 72 hours at room temperature, the concentration of bacteria preserved with DMSO showed an average decrease of 98.78% from the concentration determined at 0 hours. Samples preserved with glycerol and milk/glycerol solution respectively decreased by 91.89% and 68.69%. Samples preserved with the commercial kit increased in concentration. This suggests that using DMSO as a cryoprotectant is risky because of its toxicity. Glycerol, milk and glycerol solution, or a commercial kit is recommended instead based on their lower toxicities. Careful selection of a cryoprotectant is key to preserving valuable strains of *S. aureus*.

Keywords: MRSA, *Staphylococcus aureus*, cryopreservation, microbiology, laboratory methods

MCB - 18

TELOMERASE ACTIVITY AND FELINE TERT MRNA EXPRESSION IN FELINE ORAL SQUAMOUS CELL CARCINOMA. 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 causes poor quality of life and short survival rate. Few treatment options are available with limited success. Telomerase has recently been reported as an attractive novel target for various human cancer therapies. The biological characteristics of telomerase appear to be similar between human and cat. However, telomerase has not been investigated in FOSCC. The telomerase repeat amplification real-time PCR assay has also never been applied in cat studies. Increased telomerase activity and feline telomerase catalytic subunit (fTERT) mRNA expression are expected to be found in FOSCC. Both human and feline TERT and telomerase RNA component (TERC) sequences were aligned revealing a high degree of similarity between the two species. Telomerase activity in 3 FOSCC cell lines and frozen normal cat tissues was quantified using the quantitative telomerase detection kit. Real-time RT-PCR was used to quantify fTERT mRNA expression in 3 FOSCC cell lines and frozen cat biopsy samples. Telomerase activity and fTERT mRNA expression in 3 FOSCC cell lines were significantly higher than normal cat oral tissues. Telomerase activity and fTERT mRNA expression will be quantified in frozen FOSCC samples. In conclusion, telomerase activity and fTERT mRNA expression in FOSCC cell lines are comparable with human cancer cells. The feasibility of using FOSCC as a model for telomerase therapeutic target in feline cancers will be further investigated.

Keywords: Feline, oral squamous cell carcinoma, telomerase

STRUCTURE/FUNCTION

CONCENTRATIONS OF PROGESTERONE DURING EARLY FOLLICULAR DEVELOPMENT AND PREGNANCY RATE TO AI IN BEEF COWS. F. M. Abreu, M. L. Day, M. A. Coutinho da Silva, C. A. Madsen, T. Martins, L. H. Cruppe, B. R. Harstine, G. A. Bridges, T. W. Geary; Depts. of Animal Sciences and Veterinary Clinical Sciences

The objective was to investigate if decreased progesterone (P4) concentrations during follicular growth would impact fertility in beef cows. Crossbred (Angus x Hereford) cows (n = 261) received estradiol benzoate (EB; 1 mg) and previously used CIDR on d-7, to induce emergence of new follicular wave approximately 3 days later (d-4). On d0, all cows received 100 µg GnRH and were randomly assigned to one of the two treatments. In the high-P4 (H; n = 131) treatment, the previously inserted CIDR was replaced with new CIDR on d0. In the low-P4 (L; n = 130) treatment, 25 mg PGF was administered on d0, and the CIDR previously inserted on d-7 remained. On d5, blood samples to determine P4 concentrations were collected, all cows received two 25 mg PGF doses, and CIDRs were removed. Estrous detection coupled with artificial insemination (AI) 12h later (Estrus-AI) was performed for 60h after PGF. Cows not detected in estrus within this period were bred by timed-AI (TAI) and received 100µg GnRH at 72h. Pregnancy diagnosis was performed approximately 40d after AI. P4 concentrations at CIDR withdrawal (d5) were greater ($P < 0.01$) in the H (2.81 ± 0.10 ng/ml) than in the L (1.73 ± 0.05 ng/ml) treatment. Within the first 60h after PGF, estrus response (82% vs. 85%) and estrus distribution (56.1 ± 0.7 h vs. 54.0 ± 0.7 h) did not differ between H and L treatments, respectively. Synchronized pregnancy rate was similar between H (77.1%) and L (82.3%) treatments. Across treatments, pregnancy rates were greater ($P < 0.01$) with Estrus-AI (82.9%) than TAI (63.6%). Concentrations of P4 on d5 were negatively related ($P < 0.01$) with estrus response and time to estrus; across treatments. In conclusion, P4 concentrations during early follicular development did not influence synchronized pregnancy rate in beef cows.

Keywords: Beef cattle, Progesterone, Pregnancy rate

SF - 2

EX-VIVO EQUINE MEDIAL TIBIAL PLATEAU CONTACT PRESSURE WITH AN INTACT MEDIAL FEMORAL CONDYLE, WITH A MEDIAL FEMORAL CONDYLAR DEFECT, AND AFTER PLACEMENT OF A TRANSCONDYLAR SCREW THROUGH THE CONDYLAR DEFECT.

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The stifle (knee) is a common source of equine lameness, but its anatomical complexity has limited biomechanical research. One of the goals of the study was to create a stifle model to determine contact pressures on the medial tibial plateau. Additionally, we determined contact pressures after creation of an osteochondral defect in the medial femoral condyle (MFC). This defect mimics the location and size of a subchondral cystic lesion (SCL) of the MFC, a common pathology seen in horses. Lastly, a novel technique to treat SCL based on a transcondylar screw placed through the MFC was also tested.

Stifle joints were axially loaded to 1800N at 155º, 145º and 130º, under 3 conditions: Intact, MFC with a 15 mm circular osteochondral defect, and with a transcondylar lag screw through the defect. An electronic pressure sensor (Tekscan®) on the medial tibial plateau recorded contact area (cm^2), force (N), peak pressure (MPa) and contact maps. Stress load (Newton/cm^2) was calculated for the entire medial plateau and in 3 sub-regions; cranial, caudal and central. Significance was set at $p \leq 0.05$.

Flexion increased force, contact area, and stress load for all conditions. A MFC defect significantly reduced force at both flexion angles and contact area at 145°. The transcondylar screw returned force to intact values at 130° and reduced contact area in extension. Qualitative analysis of intact MFC contact maps revealed pressure peaks on the central cartilage at all angles and increase contact pressure and area expansion and caudal movement with flexion. Contact maps with a MFC defect amplified the caudal and abaxial pressure movement during flexion, and the screw did not further change them.

These findings suggest a biomechanical explanation for caudal meniscal tears and meniscal injuries associated with SCL. A transcondylar screw may reverse some of the biomechanical alterations found after defect creation.

Keywords: Equine, Stifle, Biomechanics, Femoro-tibial joint, Orthopaedics, Pressure

SF - 3

THROMBOELASTOGRAPHIC PARAMETERS OF CLOT FORMATION OF AUTOLOGOUS BLOOD PRODUCTS ACTIVATED BY VARIOUS CLOTTING AGENTS. S Ghassab[1], M Srinivasan[1], AL Bertone[2]. [1]Department of Mechanical and Aerospace Engineering, [2]Department of Veterinary Clinical Sciences

Objective: Compare clotting efficiency of platelet-rich plasma (PRP) and concentrated platelet-poor plasma (cPPP) to citrated whole blood when activated by autologous thrombin, bovine thrombin or calcium chloride ($CaCl_2$) to assess the dynamic properties of fibrin and platelet bonding for clinical use.

Study Design: Experimental

Animals: 6 horses (2 Thoroughbred, 3 Quarter Horse, 1 Standardbred)

Methods: PRP and cPPP were prepared by commercial devices and tested against autologous whole blood. Autologous blood, PRP and cPPP were activated by each of three clotting agents (autologous thrombin, bovine thrombin and $CaCl_2$) and compared for clotting parameters using a thromboelastograph. PT and aPTT testing were performed to establish normality of test subjects. Platelet counts of whole blood and PRP were measured.

Results: Whole blood, PRP, and cPPP clotted with all agents. Among blood products, PRP demonstrated the greatest clot strength, quickest clot rate, and shortest clot initiation time; cPPP had the longest clot initiation time. Among clotting agents, bovine thrombin had the shortest clot initiation time and greatest clot strength; Calcium chloride had the longest clot initiation time and time to reach maximum clot strength (TMA); Autologous thrombin had the lowest clot strength, but had a comparable TMA to bovine thrombin.

Conclusions: When combined with either bovine thrombin (rapidly) or $CaCl_2$ (slowly), PRP provided the best combinations for clinical use. Autologous thrombin, as prepared here, was suboptimal, but would be an autologous alternative for clinical application. As used here, cPPP was inefficient at clotting, but may be sufficient for a plasma spray indication.

Keywords: PRP, PPP, autologous, thrombin, horse

SF - 4

PHARMACOLOGY OF THE GLP-1 ANALOG LIRAGLUTIDE IN HEALTHY CATS. MJ Hall, CA Adin, S Borin-Crivellenti, AJ Rudinsky, and C Gilor. Dept. of Veterinary Clinical Sciences

GLP-1 is an intestinal hormone that induces glucose-dependent stimulation of insulin secretion while suppressing glucagon secretion and increasing beta cell mass, satiety and gastric-emptying time. Liraglutide is a fatty-acid derivative of GLP-1 with a protracted pharmacokinetic profile that is used in people for treatment of type II diabetes mellitus and obesity. The aim of this study was to determine the pharmacodynamics of liraglutide in healthy cats.

A hyperglycemic clamp was performed on day -1 (Clamp-I) and 13 (Clamp-II) in seven healthy cats. Liraglutide was administered subcutaneously (0.6 mg/cat) once daily on days 7 through 13. During the clamp blood glucose concentrations were measured every 5 minutes and 20% dextrose infusion was adjusted to achieve hyperglycemia (225 mg/dl) at 30 min and to maintain that level of glycemia for 60 min. Plasma insulin and glucagon concentrations were measured at -15, 0, 30, 45, 60, 75, and 90 min.

Weight loss was recorded in all cats at day 13 (9% ± 3; P=0.006). Appetite was subjectively decreased in all cats and one cat was withdrawn on day 10 because of 48 hrs of anorexia.

Compared to Clamp-I, there was a trend during Clamp-II towards increased 60 min total glucose infused (median [range] 29% [1 – 178%], P=0.087) and insulin concentrations (47% [-11 – 234%], P=0.084). Glucagon concentrations (P=0.67) and baseline glucose concentrations (P=0.66) did not differ significantly between clamps.

Liraglutide may aid in weight loss in overweight cats but further evaluation is needed to determine its efficacy on improving glycemic control in diabetic cats.

Keywords: liraglutide, pharmacology, GLP-1 analog, diabetes mellitus, cat

SF - 5

PARATHYROID HORMONE-RELATED PROTEIN (PTHRP) REGULATES STEM CELL PLURIPOTENCY THROUGH THE ACTIONS OF ITS NLS AND C-TERMINUS.

B.E. Hildreth III, K.M. Hernon, J. Leong, P.N. Boyaka, T.J. Rosol, R.E. Toribio
Departments of Veterinary Biosciences and Veterinary Clinical Sciences

The N-terminus of PTHrP stimulates proliferation and promotes bone and cartilage formation while inhibiting fat and muscle formation from mesenchymal stem cells (MSCs). Since the roles of the NLS and C-terminus are unknown, we investigated the effects of deleting these regions on MSC pluripotency, proliferation, and differentiation.

MSCs were isolated from neonatal mice lacking the NLS and C-terminus (*Pthrp*$^{\Delta/\Delta}$) and wild-type littermates. Genes regulating pluripotency and lineage commitment were then measured. Proliferation was assessed by a MTT assay and direct counting. MSCs were differentiated into osteoblasts, chondrocytes, adipocytes, and myocytes for 24 days and lineage-specific protein secretion, gene expression, and flow cytometric/histochemical/morphological indices were compared.

Pthrp$^{\Delta/\Delta}$ MSCs expressed greater levels of pluripotency genes (*Sox2*, *c-Myc,* and *Klf4*; $P < 0.016$) and *Gsk-3*β ($P = 0.005$). *Pthrp*$^{\Delta/\Delta}$ MSCs proliferated faster with greater ALP activity ($P < 0.0001$). However, *Pthrp*$^{\Delta/\Delta}$ MSCs had reduced osteoblast maturation (decreased mineralization and osteocalcin secretion; $P = 0.044$ and 0.029). *Pthrp*$^{\Delta/\Delta}$ MSCs produced less cartilage ($P < 0.0001$) and expressed less *Sox9* ($P = 0.042$), but greater *Ihh* ($P = 0.035$). *Pthrp*$^{\Delta/\Delta}$ MSCs produced a greater number of adipocytes ($P = 0.023$) with concomitant increases in *PPAR*γ and *C/EBP*α expression ($P < 0.049$). Interestingly, *Pthrp*$^{\Delta/\Delta}$ MSCs produced more myocytes ($P < 0.0001$), expressing more *desmin* and *myogenin* ($P < 0.031$).

In conclusion, regions distinct from the N-terminus of PTHrP influence MSC pluripotency, proliferation, and differentiation. Increased proliferation, ALP, *c-Myc,* and *Ihh* by *Pthrp*$^{\Delta/\Delta}$ MSC, but less bone and cartilage formation, indicate that the NLS and C-terminus inhibit proliferation while promoting osteoblast and chondrocyte maturation. Mechanistically, increases in *Sox2*, *Klf4, and Gsk-3*β favor adipogenesis at the expense of osteogenesis. This is the first study demonstrating that additional regions of PTHrP are involved in regulating pluripotency, adipogenesis, and myogenesis, of which they complement the inhibitory functions of the N-terminus.

Keywords: PTHrP, MSC, pluripotency, proliferation, differentiation

SF - 6

MINOCYCLINE, A PUTATIVE NEUROPROTECTANT, CO-ADMINISTERED WITH DOXORUBICIN-CYCLOPHOSPHAMIDE CHEMOTHERAPY IN A XENOGRAFT MODEL OF TRIPLE-NEGATIVE BREAST CANCER. L.E. Himmel[1], M.B. Lustberg[2], A.C. DeVries[3], C.L. Shapiro[2], C.-S. Chen[4], and S.K. Kulp[4]
Departments of [1]Veterinary Biosciences, [2]Internal Medicine, [3]Neuroscience, [4]Medicinal Chemistry and Pharmacognosy, The Ohio State University, Columbus, OH

Introduction: Minocycline is purported to have neuroprotective properties in experimental models of some human neurologic diseases, and has thus been identified as a putative neuroprotectant for chemotherapy-induced cognitive impairment in breast cancer patients. However, because its mechanism of action is believed to be mediated through anti-inflammatory, anti-apoptotic, and anti-oxidant pathways, co-administration of minocycline with chemotherapeutic agents could reduce the efficacy of anticancer drugs. The objective of this study is to evaluate the effect of minocycline on the activity of the AC chemotherapeutic regimen (Adriamycin [doxorubicin], Cytoxan [cyclophosphamide]) in *in vitro* and *in vivo* models of triple-negative breast cancer (TNBC). **Methods**: Clonogenic and methylthiazol tetrazolium assays were used to assess cell survival and viability in two TNBC cell lines treated with increasing concentrations of AC in the presence or absence of minocycline. Biomarkers of apoptosis, cell stress, and DNA damage were characterized by western blot. The *in vivo* effects of AC and minocycline, each alone and in combination, on tumor growth were evaluated in a xenograft model of TNBC in female athymic nude mice by tumor volume measurement, body and organ weight measurement, and histopathology. Immunohistochemistry was used to characterize apoptosis and proliferation in the xenografts. **Results**: Data from these *in vitro* and *in vivo* studies demonstrate that minocycline does not diminish the cytotoxic and tumor-suppressive effects of this chemotherapeutic drug combination. **Conclusion**: We posit that minocycline may be useful clinically for its reported neuroprotective activity in breast cancer patients receiving AC without loss of efficacy of these cytotoxic drugs.

Keywords: Triple-negative breast cancer, minocycline, chemotherapy, experimental pathology, chemo fog

EFFECT OF EXPOSURE TO DIM LIGHT AT NIGHT AND PARTICULATE MATTER ON C3H/HENHSD MICE. M. K. Hogan, Q. Sun, R. J. Nelson. Department of Veterinary Preventive Medicine and University of Laboratory Animal Resources

Increased industrialization and populations have led to harmful contaminants in the atmosphere as well as exposure to light at night. 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 anxiety, depression, mood disorders, and cognitive malfunction. C3H/HeNHsd mice, with intact melatonin production, were used to model humans exposed to circadian disruptions and contaminated environmental air (e.g., night shift employees). We hypothesized exposure to 2.5μm 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 argued exposure to $PM_{2.5}$ and dLAN provoke anxiety-like and depressive-like behaviors.

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 (open field assay, elevated plus maze, forced swim test, sucrose anhedonia). At termination of the study, hippocampi were collected for qPCR and morphological analyses.

Here we report that mice exposed to $PM_{2.5}$+dLAN and FA+dLAN increase depressive-like and anxiety-like responses in the sucrose anhedonia and open field assays, respectively. These results are comparable to previous $PM_{2.5}$ and dLAN studies conducted on mice. Initial results along with previous work implicate $PM_{2.5}$ and dLAN as potential factors contributing to depression and anxiety. Ongoing molecular and morphological analyses will help to further characterize the deleterious effects of air pollutants and exposure to light at night on rodent inflammatory responses and hippocampal function and structure.

Keywords: Dim light at night, Particulate matter, Melatonin, Circadian disruptions, Central Nervous System Disorders, Anxiety, Depression, C3H/HeNHsd

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PHARMACOKINETICS AND BONE RESORPTION EVALUATION OF A NOVEL CATHEPSIN K INHIBITOR (VEL-0230) IN HEALTHY ADULT HORSES. H. Hussein [1], A. Ishihara [2], M. Menendez [1], A. Bertone [1]. Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA [1]. School of Veterinary Medicine, Azabu University, Kanagawa, Japan [2].

VEL-0230 is an irreversible inhibitor of bone Cathepsin K shown to suppress osteoclast-mediated bone resorption. Unlike bisphosphonate anti-resorptive drugs which lead to over-suppression of bone resorption, VEL-0230 was found to induce a rapid short-acting inhibitory effect. The drug also inhibits pro-inflammatory cytokines mediated by Toll-like receptor 9. VEL-0230 could target osseous-inflammatory disorders common in horses. This study determined an oral dose, dose interval, and inhibition of a bone biomarker of VEL-0230 in horses. Plasma pharmacokinetic and bone resorption biomarker [carboxy-terminal cross-linking telopeptide of type I collagen (CTX-1)] analyses were performed following administration of single or multiple oral dosing protocols of VEL-0230. In the oral dose study, three doses [2, 4, and 8 mg/kg body weight (b.w.)] were administered in a latin-square design to three mares and plasma and urine sampled for one week. Based on a sustained and greatest inhibition of plasma CTX-1 at day 5 (mean 29.9 ± 22.7 SEM), which was not different from baseline by day 7, a dose of 4mg/kg b.w. was selected for the multiple dose study. In the multiple dose study, 3.25 day (d) and 7d interdosing intervals were studied for 3 doses using 4 exercising young adult horses in a latin-square design. The 3.25d and 7d interdosing intervals at 4 mg/kg b.w. provided a rapid inhibition in plasma CTX-1. Although CTX-1 inhibition prior to next dose administration was not statistically different between the 3.25 d or 7 d protocols for the first 3 days, the sustained plasma CTX-1 inhibition in the 7d protocol along with the cost and logistic benefits for weekly administration, made the 7d protocol preferable. In conclusion, weekly administration of VEL-0230 at a dose of 4 mg/kg b.w. may inhibit bone resorption in young exercising horses that returns to baseline within 7 days after drug withdrawal even after multiple doses.

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

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TUMOR ANTIGEN ROR1 TARGETED DELIVERY OF FTY720 DERIVATIVE OSU-2S PROLONGS SURVIVAL IN ROR1 ENGINEERED MOUSE MODEL OF CHRONIC LYMPHOCYTIC LEUKEMIA. R. Mani[1-3], Y. Mao[2,4,5], F. Frissora[2,3], C. Chiang[2,3], J. Wang[2], Y. Zhao[4], Y. Wu[5], B. Yu[2,5], R. Yan[6], X. Mo[7], L. Yu[7], J. Flynn[3], J. Jones[3], L. Andritsos[3], S. Baskar[8], C. Rader[9], M. Phelps[2,4], C. Chen[1,2,6], R. J. Lee[2,4,5], J. Byrd[1-3,6], L. J Lee[5,10] and N. Muthusamy[1-3]

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Selective cytotoxicity to cancer cells without compromising their normal counterparts pose a huge challenge for traditional drug design. We have recently identified cytotoxic activity of OSU-2S, a novel non-immunosuppressive FTY720 derivative and protein phosphatase 2A (PP2A) activator against human B-cell chronic lymphocytic leukemia (CLL). Here, we demonstrate the molecular mechanisms and a rational approach for developing this novel agent for preclinical and clinical studies. OSU-2S induces activation of PP2A, phosphorylation and nuclear translocation of $SHP1^{S591}$ and deregulation of multiple cellular processes in CLL resulting in potent cytotoxicity. Gene expression studies by microarray analysis of RNA isolated from OSU-2S treated CLL cells revealed 260 genes that have changed by at least two fold ($p<0.0005$). Moreover, with relevant to CLL disease, Tcl1A oncogene expression that was identified to be down regulated in response to OSU-2S in the gene expression profile was independently confirmed to be down regulated both at the mRNA and protein levels. To preclude OSU-2S mediated effects on the ubiquitous phosphatases in unintended cells and avoid potential adverse effects, we developed OSU-2S targeted delivery immunonanoparticles (2A2-OSU-2S-ILP), that mediated selective cytotoxicity of CLL but not normal B cells through targeting receptor tyrosine kinase ROR1. ROR1 is an orphan receptor tyrosine kinase that is expressed exclusively in malignant B but not normal B cell surface of CLL patients. Developing a novel spontaneous CLL mouse model expressing human ROR1 (hROR1) in all leukemic B cells, we demonstrate the therapeutic benefit of enhanced survival with 2A2-OSU-2S-ILP in Eμ-hROR1-Tcl1 mouse model of CLL ($p<0.0002$). The newly developed non-immunosuppressive OSU-2S, its delivery using human CLL directed immunonanoparticles and the novel transgenic mouse model of CLL that expresses hROR1 exclusively in leukemic B cell surface are highly innovative and can be applied to CLL and other ROR1+ malignancies including mantle cell lymphoma and acute lymphoblastic leukemia.

Keywords: Chronic lymphocytic leukemia(CLL), OSU-2S, ROR1, ROR1 Transgenic Mice, Targeted drug delivery, Immunonanoparticles

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HIGH-FIELD MAGNETIC RESONANCE IMAGING OF THE CANINE KNEE.

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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 progression. 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 evaluate the usefulness of MRI to qualitatively assess the canine knee.

Materials: Six canine cadaver knees were used in the study. The ex-vivo knees were examined with a 3T whole body system using an 8-channel knee coil. Protocol sequences included: PD-TSE-SPIR was calculated using TE= 15 ms and TR= 2.6 s. Flip angle= 90°and 2 mm slice thickness. T1Weighted-TSE was calculated using TE= 20 ms and TR= 433 ms. Flip angle 90° and 3 mm slice thickness. PD-TSE was calculated using TE= 12 ms and TR: 1.1 s. Flip angle 90° and 3 mm slice thickness. PD-TSE FS PHASE SWAP was calculated using TE=45 ms and TR= 2.8 s. Flip angle 90° and 3 mm slice thickness. And 3D-WATsf was calculated using TE= 5 ms and TR= 10 ms. Flip angle 35° and 3 mm slice thickness.

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

Discussion: This study showed that 3T MRI in combination with the 8 channels knee coil is suitable and provided new sequences to assess qualitatively the canine knee.
However, quantitative as well as *in vivo* MRI assessment would added valuable additional information.

Conclusion: This study demonstrated that MRI is a useful technology to provide a non-invasive and reliable dog knee assessment. Providing a promising tool to evaluate joint changes during the development of osteoarthritis.

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

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THE PHARMACOLOGY OF EXENATIDE EXTENDED-RELEASE IN HEALTHY CATS.

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GLP-1 is an intestinal hormone that induces glucose-dependent stimulation of insulin secretion while suppressing glucagon secretion and increasing beta cell mass, satiety and gastric-emptying time. Exenatide extended-release (ER) is a microencapsulated formulation of the GLP-1-receptor agonist exenatide. It has a protracted pharmacokinetic profile that allows a once-weekly injection to replace insulin therapy safely and effectively in type-II diabetic people.

Here we studied the pharmacology of Exenatide-ER in six healthy cats. A single subcutaneous injection of Exenatide-ER (0.13 mg/kg) was administered on day 0. A hyperglycemic clamp was performed on days -7 (Clamp-I) and 21 (Clamp-II). During the clamp, blood glucose concentrations (BG) were measured every 5 minutes and 20% dextrose infusion was adjusted to achieve hyperglycemia (225 mg/dl) at 30 min and to maintain that level of glycemia for the subsequent 60 min. Plasma insulin and glucagon concentrations were measured at -15, 0, 30, 45, 60, 75, and 90 min. Glucose tolerance was defined as the amount of glucose required to maintain hyperglycemia during the 60 minutes of the clamp.

Comparing Clamp-1 to Clamp-2 using paired t-tests, fasting BG decreased (mean [±SD] = -11± 8 mg/dl, P=0.02), glucose tolerance improved (median [range] +33% [4-138%], P=0.04) and median glucagon concentrations decreased (-4.7% [0-12.1%], P=0.04). Insulin concentrations did not differ significantly. No side effects were observed throughout the study.

Exenatide-ER was safe and effective in improving glucose tolerance 3 weeks after a single injection. Further evaluation is needed to determine its efficacy and duration of action in diabetic cats.

Keywords: diabetes mellitus, incretin, GLP-1, exenatide

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INTRAVENOUS MAGNESIUM SULFATE INDUCES CALMING IN HORSES AND IS DETECTABLE IN BLOOD AND URINE: A PILOT STUDY. S. Schumacher DVM, R. Toribio, DVM, PHD, J. Hubbell DVM, MSc, A. Bertone, DVM, PhD. Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio 43210

Magnesium is potentially the single most abuse substance in equine competition. From racing to the show ring, magnesium sulfate ($MgSO_4$) has become a substitute for training. No published work to date confirms it is effective at calming, but it is anecdotally used as to not permit nervous energy to sap the athlete's racing potential, or to calm horses going into the hunter ring. The regulation of magnesium carries significant challenges. Magnesium is an essential cofactor in more than 300 enzymatic reactions in the body and is the second most common intracellular cation. The goal of this pilot study was to document the changes in serum electrolytes and in the fractional excretion of magnesium (FMg) and calcium (FCa) subsequent to IV magnesium sulfate administration. Three healthy horses were instrumented with bilateral jugular intravenous catheters, a urinary catheter, and continuous ECG recording. Baseline physiological parameters and blood and urine electrolytes (Na, K, Cl, Mg, Ca, and ionized Mg and Ca) were obtained. Hypermagnesemia was induced by administering 50% $MgSO_4$ solution (30 grams/horse) intravenously over 5 minutes. Within minutes of injection, measures of calming were noted including a drop in head carriage and reduction in the startle response. Urine production immediately reduced and blood values revealed an increase in serum Mg levels above the reference ranges for the laboratory. Serum total Mg (tMg) and ionized Mg (Mg^{2+}) concentrations increased by 2-3 fold while serum total calcium (tCa) and ionized calcium (Ca^{2+}) concentrations decreased by 15-20%. Serum Mg^{2+} and Ca^{2+} did not return to baseline values for at least 2 hrs. It was also documented that hypermagnesemia increased FCa by 3 folds, while FMg only doubled despite this dose of parenteral $MgSO_4$. No cardiac abnormalities were detected in heart rate or rhythm. Additional research is necessary to address the clinical and regulatory relevance of these findings.

Keywords: Hypermagnesemia, Magnesium sulfate, Fractional excretion

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DIFFERENCES IN BLOOD PRESSURE, MICROALBUMINURIA, AND VASO-PROTECTIVE EICOSANOIDS BETWEEN GREYHOUND AND NON-GREYHOUND DOGS. J. Tucker, L. K. Rogers, C. Iazbik, T. Hoepf, C. G. Couto, C. Kellogg, M. J. Radin. Depts. of Veterinary Biosciences and Veterinary Clinical Sciences

Greyhound dogs have been shown to have marked differences in blood pressure and microalbuminura relative to other dog breeds. These apparent abnormalities in vascular function have been well documented, but the mechanism underlying the alterations remains unclear. In humans and rodent models, arachidonic acid (AA) metabolites play a role in vascular reactivity and cardiovascular and renal health. Epoxyeicosatrienoic acids (EET) are produced when AA is metabolized by cytochrome P450 (CYP). In turn, dihydroxyeicosatrienoic acids (DHET) are produced when EETs are degraded by the enzyme soluble epoxide hydrolase (sEH). EETs have been shown to have vaso-protective effects, including vasodilatory, anti-hypertensive, and renoprotective properties while DHETs are less protective. AA metabolic products of lipoxygenase pathways, the hydroxyeicosatetraenoic acids (HETE), are vasoconstrictive and pro-inflammatory and have been implicated in renal and cerebrovascular disease. We hypothesized that there would be significant differences in levels of various eicosanoids between Greyhounds and non-greyhound dogs. In this study, we evaluated plasma eicosanoids in Greyhounds and non-greyhounds using high pressure liquid chromatography (HPLC) followed by electrospray mass spectrometry. We also measured arterial blood pressure and urine microalbumin levels across groups. We found that Greyhounds have significantly higher systolic blood pressure and microalbuminuria than non-greyhounds. Most interestingly, we also found that Greyhounds have significantly higher plasma levels of 14,15-EET and some DHET metabolites, specificially 8,9-, 11,2- and 14,15-DHET isomers. Higher levels of DHETs in Greyhounds may indicate increased activity of sEH or potentially increased activity of the entire CYP pathway. Greyhounds also had significantly increased plasma HETE metabolites (specifically 5(s), 8(s), 9(s), and 12(s)-HETE) compared to non-greyhounds, suggesting increased lipoxygenase activity. Higher levels of DHETs and HETEs in Greyhounds may help to explain some of the cardiovascular and renal anomalies observed in this breed.

Keywords: Greyhound, microalbuminuria, epoxyeicosatrienoic acid (EET), dihydroxyeicosatrienoic acid (DHET), soluble epoxide hydrolase (sEH), hydroxyeicosatetraenoic acid (HETE)