PROGRAM

April 12, 2012

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

KEYNOTE SPEAKER
Veterinary Medical Center Auditorium
immediately following the awards presentation

Dr. Steven Arnoczky
Wade O. Brinker Endowed Professor in Surgery
Director, Laboratory for Comparative Orthopaedic Research
College of Veterinary Medicine
Michigan State University

“Rebuilding the Injured Knee: The Science Behind the Surgery”

POSTER SESSION
Anatomy Lab Hallway – Sisson Hall
and
Main Hallway – Vet Med Academic Building
11:00 am – 5:00 pm

PROGRAM CHAIR
Dr. Matthew Allen

ORGANIZED BY
Michele Morscher

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Special thanks to the College’s Technology Services for creating the posters
ADVANCES IN VETERINARY MEDICINE RESEARCH WEEK

VETERINARY MEDICAL CENTER AUDITORIUM
Corner of Coffey Road and Tharp Street
Enter from Coffey Road and go up the stairs

THURSDAY, APRIL 12th
12:15 – 2:00 pm

AWARDS PRESENTATION AND KEYNOTE ADDRESS

“Rebuilding the Injured Knee: The Science Behind the Surgery”

Dr. Steven Arnoczky
Wade O. Brinker Endowed Professor in Surgery
Director, Laboratory for Comparative Orthopaedic Research
College of Veterinary Medicine
Michigan State University

Posters will be on display in the Veterinary Medical Academic Building and The Anatomy Lab Hallway in Sisson Hall

Poster Judging Schedule:
April 11th: 2:00-5:00 pm for Professional Students
April 12th: 8:00-10:30 am for Graduate Students

2012 SPONSORS:
POSTER JUDGING SESSIONS

Wednesday, April 11, 2012
2:00 – 5:00 pm
Veterinary Student Poster Judging

Thursday, April 12, 2012
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 89 posters.

Raj Anupam   Jim Belknap   Alicia Bertone
Brad Bolon   Andrew Bowman   Prosper Boyaka
Mary Carsillo   Andrew Dahlem   Adrienne Dardenne
Leighann Daristotle   Rami Doueiri   Kat Ham
Kate Hayes-Ozello   Kazu Ishihara   Eason Hildreth
John Hubbell   Bill Kisseberth   Krista La Perle
Xin Li   Larry Mathes   Sharell Mikesell
Stefan Niewiesk   Mike Oglesbee   Tracey Papenfuss
Barrak Pressler   Judith Radin   Paivi Rajala-Schultz
John Sagartz   Liz Santschi   Bill Saville
Jean Schelhorn   Judi Stella   Karen Tefft
Li Wu
IMID – 1 THE ROLE OF ADENOSINE IN THE DEVELOPMENT OF PULMONARY FUNCTIONAL IMPAIRMENT IN CD73- AND A1-ADENOSINE RECEPTOR-KNOCKOUT MICE INFECTED WITH INFLUENZA A (H1N1) F. Aeffner, A.A. Gaughan, L.M. Joseph, I.C. Davis. Department of Veterinary Biosciences

IMID – 2 INTESTINAL EPITHELIAL CELLS REGULATE AIRWAY ALLERGIC INFLAMMATION THROUGH INDUCTION OF IgA AND IL-17A A. Bonnegarde-Bernard, J. Jee, I. Davis, P. Boyaka. Department of Veterinary Biosciences

IMID – 3 POLYMORPHISMS OF THE SAMHD1 GENE ARE NOT ASSOCIATED WITH THE INFECTION AND NATURAL CONTROL OF HIV-1 IN AFRICAN AMERICANS AND EUROPEANS S. Coon, D. Wang, L. Wu. Departments of Veterinary Biosciences and Pharmacology

IMID – 4 DISTINCT TRANSFORMATION TROPISM EXHIBITED BY HUMAN T LYMPHOTROPIC VIRUS TYPE (HTLV-1) AND TYPE 2 (HTLV-2) IS THE RESULT OF POST-INFECTION T CELL CLONAL EXPANSION P. Kannian, H. Yin, R. Doueiri, M. D. Lairmore, S. Fernandez, P. L. Green. Department of Veterinary Biosciences

IMID – 5 HTLV-1 ORAL EXPOSURE IN THE RABBIT MODEL: ESTABLISHMENT OF A MODEL FOR MOTHER TO CHILD TRANSMISSION R. Haines, R. Urbiztondo, J. Stanley, R. Haynes, M. Lairmore. Department of Veterinary Biosciences

IMID – 6 HSP70-MEDIATED PROTECTION AGAINST VIRAL NEUROVIRULENCE IS TYPE 1 DEPENDENT M. Y. Kim, Y. Shu, T. Carsillo, J. Zhang, L. Yu, C. Peterson, S. Girod, S. Niewiesk, M. Oglesbee. Department of Veterinary Biosciences and Center for Biostatistics

IMID - 7 EHRLICHTIA TYPE IV SECRETION EFFECTOR ECH0825 IS TRANSLOCATED TO MITOCHONDRIA AND CURBS ROS AND APOPTOSIS BY UPREGULATING HOST MNSOD H. Liu, W. Bao, M. Lin, H. Niu, Y. Rikihisa. Dept. of Veterinary Biosciences

IMID – 8 TOLERGENIC DCS DIFFERENTIATION AND DEVELOPMENT OF IMMUNOSUPPRESSIVE DC PRECURSORS Z. VanGundy, C. Taylor, A. White, T. Papenfuss. Department of Veterinary Biosciences
IMID – 9  LOSS OF MAPK PHOSPHATASE 1 (MKP-1) ENHANCES BACTERIAL CLEARANCE AND IMPROVES SURVIVAL DURING ACUTE PRIMARY STREPTOCOCCUS PNEUMONIAE PULMONARY INFECTION  L. M. Wancket, Y. Liu, Department of Veterinary Biosciences and the Center for Perinatal Research, The Research Institute at Nationwide Children’s Hospital

Molecular and Cellular Biology

MCB – 1  COMPARATIVE HOST PROTEIN INTERACTIONS WITH HTLV-1 P30 AND HTLV-2 P28: INSIGHTS INTO DIFFERENCE IN PATHO BIOLOGY OF HUMAN RETROVIRUSES  R. Anupam‡1,2, R. Doueihi‡1,2, M. Kvaratskhelia1,5, K. Green-Church5, M. D. Lairmore1,2,4,7, P. L. Green*1,2,3,4 (‡ Equal authors), 5Center for Retrovirus Research, 2Department of Veterinary Biosciences, 3Department of Molecular Virology, Immunology, and Medical Genetics, 4Comprehensive Cancer Center and Solove Research Institute, 5College of Pharmacy, 6Mass Spectrometry and Proteomics Facility, The Ohio State University, Columbus, OH 7Department of Pathology, Microbiology, and Immunology, University of California, Davis, CA

MCB – 2  BIOLOGIC ACTIVITY OF THE NOVEL SMALL MOLECULE STAT3 INHIBITOR AGAINST CANINE OSTEOSARCOMA CELL LINES  J. Couto1, M. Bear1, J. Lin2, C.-L. Li3, P. Houghton2, W. Kisseberth1, C. London1.  1Department of Veterinary Clinical Sciences, 2Nationwide Children’s Hospital, 3College of Pharmacy

MCB – 3  COMPARISON OF HTLV-1 AND HTLV-2 ANTI SENSE PROTEINS’ EFFECTS ON CELLULAR SIGNALING PATHWAYS  N. Dissinger, H. Yin, P. Green. Department of Veterinary Biosciences

MCB – 4  CHARACTERIZATION OF microRNA DYSREGULATION IN CANINE MAST CELL TUMORS  J. M. Fenger1, B. Harrington1, T. Lin2, S. Volinia3,4, W. C. Kisseberth1,4, C. A. London1,2,4 1Department of Veterinary Clinical Sciences, 2Department of Veterinary Biosciences, 3Department of Molecular Virology, Immunology, and Medical Genetics, 4Ohio State University Comprehensive Cancer Center

MCB – 5  RNA HELICASE A INTERACTS WITH TRANSLATIONAL REGULATORY PROTEINS TO CONTROL THE EXPRESSION OF VIRAL AND CELLULAR mRNAs.  S. Fritz1-3, A. Ranji2,3, K. Boris-Lawrie1,5.  1Integrated Biomedical Sciences Graduate Program1, Department of Veterinary Biosciences2, Center for Retrovirus Research3, Center for RNA Biology4, Comprehensive Cancer Center5
MCB – 6 JUNB EXHIBITS LOSS OF “PAUSED” RNA POLYMERASE II REGULATION AND PROMOTES SURVIVAL IN METASTATIC MCF10CA1A CELLS. Q. Hu, M. Hicks, J. DeWille. Department of Veterinary Biosciences

MCB – 7 CELL DELIVERY ROUTE, CELL SOURCE, AND BMP2 GENE TRANSDUCTION ON ENGRAFTMENT AND BONE DENSITY IN RABBIT MODEL A. Ishihara, K. Ohmine, S. Jump, D. Russell, S. Weisbrode, and A. Bertone. Department of Veterinary Clinical Sciences, The Ohio State University; Department of Medicine, University of Washington

MCB – 8 REGULATING THE REGULATOR: TRANSLATIONAL REGULATION BY RNA HELICASE A (RHA) OF THE RNA STABILITY REGULATOR HUMAN ANTIGEN R (HuR) A.R. Martinez1-4, D. Singh1-2, M. Singh6, B. S. Lee6, K. Boris-Lawrie1-5 Department of Veterinary Biosciences1, Center for Retrovirus Research2, Integrated Biomedical Sciences Graduate Program3, Center for RNA Biology4, Comprehensive Cancer Center5, Department of Physiology and Cell Biology6

MCB – 9 MECHANISTIC ROLE OF PROTEOLYSIS IN THE FUNCTIONAL CONVERSION OF RNA HELICASE A FROM AGONIST TO ANTAGONIST OF VIRAL AND CELLULAR TRANSLATION J. Picking, A. Sharma, K. Boris-Lawrie. Department of Veterinary Biosciences, Center for Retrovirus Research

MCB – 10 WNT SIGNALING AND PROSTATE CANCER BONE METASTASIS J. K. Simmons, W. P. Dirksen, L. G. Lanigan, T. J. Rosol. Department of Veterinary Biosciences

MCB – 11 THE ROLE OF P16 IN FELINE ORAL SQUAMOUS CELL CARCINOMA. W. Supsavhad, W. Dirksen, C. Martin, S. Pillai, and T. Rosol. Department of Veterinary Biosciences

MCB – 12 THE NLS AND C-TERMINUS OF PTHRP MODULATE THE WNT/ß-CATENIN PATHWAY IN OSTEOBLASTS. F. Wang, B.E. Hildreth, K.M. Hernon, R.E. Toribio, Departments of Veterinary Clinical Sciences and Veterinary Biosciences

Structure/Function

SF – 1 EFFECTS OF MECLOFENAMIC ACID ON LUTEAL FUNCTION OF BEEF CATTLE. C. A. Messerschmidt, F. M. Abreu, L. H. Cruppe, M. V. Biehl, M. L. Day, C. R. F. Pinto, M. A. Coutinho da Silva; Departments of Veterinary Clinical Sciences and Animal Sciences

SF – 2 EFFECTS OF HEMATOCRIT AND RED BLOOD CELL-INDEPENDENT VISCOSITY MANIPULATION ON THROMBOELASTOGRAPHIC VARIABLES IN DOGS. A. C. Brooks, J. Guillaumin, E. Cooper, G. Couto. Department of Veterinary Clinical Sciences

SF – 3 THE USE OF RADIOGRAPHS DUAL-ENERGY X-RAY ABSORPTIOMETRY, QUANTITATIVE COMPUTED TOMOGRAPHY AND MICRO COMPUTED TOMOGRAPHY TO DETERMINE LOCAL CANCELLOUS BONE QUALITY IN THE PROXIMAL FEMUR. K. L. Townsend1, R. Hart2, V. Samii1, T. Motta1, G. Noble2, J. Dyce1, M. J. Allen1. Department of Veterinary Clinical Sciences1, Department of Biomedical Engineering2

SF – 4 BIOMECHANICAL EVALUATION OF MEDIAL FEMORAL CONDYLAR SUBCHONDRAL CYSTIC LESIONS AND THE EFFECTS OF TREATMENT WITH INTERNAL FIXATION J. M. Williams1, E. M. Santschi1, M. J. Allen1, A. S. Litsky2 Department of Clinical Sciences1, The Orthopaedic BioMaterials Laboratory2

Clinical Research

CR – 1 MUSCULOSKELETAL RESEARCH PROGRAMS
College of Veterinary Medicine, The Ohio State University

CR – 2 PLATELET ENHANCEMENT THERAPY FOR CANINE OSTEOARTHRITIS A. Bertone, M. Fahie, V. Guercio, J. Schaffer, G. Johnston, J. Au, B. Hettlich, T. Phillips, M. Allen, and G. Ortolano. Department of Veterinary Clinical Sciences; Western University of Health Sciences
CR – 3  NON-INVASIVE MEASURE OF BONE DENSITY TO PREDICT MECHANICAL PROPERTIES OF THE VERTEBRAL ENDPLATE IN THE CANINE CERVICAL SPINE  J. Bertran, N. Fitzpatrick, M. J. Allen. Department of Veterinary Clinical Sciences

CR – 4  MEDIAL TIBIAL PLATEAU CONTACT PRESSURE IN HORSES  A. Bonilla¹, J. Williams¹, A. Litsky², E. Santschi¹. Department of Clinical Sciences¹, The Orthopaedic BioMaterials Laboratory²

CR – 5  A COMPARISON OF A TRADITIONAL FLUID WARMING METHOD FOR PERITONEAL LAVAGE TO AN ON-DEMAND FLUID WARMING SYSTEM  A. N. Brooks, C. Adin, and K. Ham. Department of Veterinary Clinical Sciences

CR – 6  RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM (RAAS) RESPONSE AND RATIOS IN HOSPITALIZED FOALS  K.A. Dembek¹, K. Onasch¹, S.D. Hurcombe¹, N.M. Slovis², B. Barr³, R.E. Toribio¹. Department of Veterinary Clinical Sciences¹, Hagyard Equine Medical Institute, Lexington, KY², Rood and Riddle Equine Hospital, Lexington, KY³

CR – 7  KINEMATIC GAIT ANALYSIS USING 3-D MOTION CAPTURE IN DOBERMAN PINCHERS WITH AND WITHOUT CERVICAL SPONDYLOMYELOPATHY  K. Foss, R.C. da Costa, S. Moore. Department of Veterinary Clinical Sciences

CR – 8  THE EFFECT OF KETOCONAZOLE ON BLOOD AND SKIN CONCENTRATIONS IN CANINES  L. L. Gray, A. Hillier, L. K. Cole, P. J. Rajala-Schultz. Departments of Veterinary Clinical Sciences and Veterinary Biosciences


CR – 10  EFFECT OF MODIFIED CYCLOSPORINE A ON LENS EPITHELIAL CELL AND CORNEAL ENDOTHELIAL CELL VIABILITY  E. A. Lutz, D.A. Wilkie, A.J. Gemensky-Metzler, H.L. Chandler. Department of Veterinary Clinical Sciences
FACTORS AFFECTING IN VITRO MATURATION OF ALPACA OOCYTES
C. A. Messerschmidt, M. A. Coutinho da Silva, B. S. Forshey, E. A. Coffman, C. R. F. Pinto. Department of Veterinary Clinical Sciences

EFFECT OF BODY POSITION ON ABDOMINAL PRESSURES IN ADULT HORSES.
V. H. L. Scott, J. M. Williams, M. C. Mudge, and S. D. A. Hurcombe. Department of Veterinary Clinical Sciences

NOVEL PUBLIC HEALTH RISK FROM SUBCLINICAL INFLUENZA A VIRUS INFECTIONS AT SWINE SHOWS
A. Bowman, J. Nolting, S. Nelson, R. Slemons. Department of Veterinary Preventive Medicine

CHARACTERIZATION OF AN ORTHOTOPIC MOUSE MODEL OF OSTEOSARCOMA
B. K. Chaffee, F. Xu, M. J. Allen, Department of Veterinary Clinical Sciences

GENOTYPIC VARIATION OF STAPHYLOCOCCUS AUREUS IN DAIRY CATTLE.
L. da Costa, P. Rajala-Schultz. Department of Preventive Medicine

BIOMECHANICAL COMPARISON OF 3 FIXATION CONSTRUCTS IN THE CANINE CADAVERIC CERVICAL VERTEBRAL COLUMN
B. Hettlich, M. Allen¹, D. Pascetta¹, G. Fosgate³, and A. Litsky². Departments of Veterinary Clinical Sciences¹, Orthopaedics and Biomedical Engineering²; Production Animal Studies³, University of Pretoria, South Africa.

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN DOGS, CATS, AND HORSES AT A VETERINARY TEACHING HOSPITAL FROM 2007 TO 2010
J. Mathews¹, N. Tiao¹, P. Patchanee², W. Gebreyes¹ Department of Veterinary Preventive Medicine¹; Veterinary Public Health Centre for Asia Pacific, Faculty of Veterinary Medicine, Chiang Mai University, Thailand²

VALIDATING METHODS FOR ISOLATING CONTEMPORARY INFLUENZA A VIRUSES FROM SWINE

FIRST RECOVERY OF RARE H14 INFLUENZA A VIRUSES IN THE WESTERN HEMISPHERE.
J. Nolting, A. Fries, J. Pedersen, N. Hines, M.L. Killian, C. Courtney, and R Slemons. Department of Veterinary
Preventive Medicine and USDA, APHIS, National Veterinary Services Laboratories

EAR – 8  ENVIRONMENTAL FACTORS THAT AFFECT THE BEHAVIOR AND WELFARE OF DOMESTIC CATS (FELIS SYLVESTRIS CATUS) HOUSED IN CAGES  J. Stella¹, C. Croney², T. Buffington  Department of Veterinary Preventative Medicine, Veterinary Clinical Sciences³, Animal Sciences, Purdue University, West Lafayette, Indiana²

Core and Shared Resources

COR – 1  RESEARCH PATHOLOGY SUPPORT FOR EXPERIMENTAL ANIMAL MODELS PROVIDED BY THE COMPARATIVE PATHOLOGY & MOUSE PHENOTYPING SHARED RESOURCE  K. La Perle and B. Bolon. Department of Veterinary Biosciences.

COR – 2  CLINICAL TRIALS OFFICE, ADVANCING THE HEALTH OF ANIMALS AND HUMANS  N. Stingle, T. Mathie, N. Ruffin, H. Borghese, C. A. London, and WC Kisseberth, Departments of Veterinary Biosciences and Veterinary Clinical Sciences

COR – 3  VETERINARY BIOSPECIMEN REPOSITORY (TISSUE BANK)  H. L. Borghese, N. Ruffin, W. C. Kisseberth, C. A. London, M. L. Wellman. Departments of Veterinary Biosciences and Veterinary Clinical Sciences
Veterinary Students – Clinical Research

VME – 1  ECHOCARDIOGRAPHIC ASSESSMENT OF RIGHT VENTRICULAR FUNCTION IN 150 DOGS WITH PULMONIC STENOSIS A.T. Amburgy, and B.A. Scansen. Department of Veterinary Clinical Sciences

VME – 2  EFFECT OF CARRIER TRAINING AND SIMULATED CAR RIDES ON OWNED DOMESTIC CATS M Boretsky, C A T Buffington, Department of Veterinary Clinical Sciences

VME – 3  A NOVEL METHOD TO REMOVE SEMINAL PLASMA FROM CANINE SEMEN PRIOR TO CRYOPRESERVATION E. Clark, C. Messerschmidt, D. Howell, M. Coutinho da Silva. Department of Veterinary Clinical Sciences

VME – 4  CAN SERUM AMYLOID A BE USED AS A MARKER FOR PLACENTITIS IN MARES? B.S. Forsheya, D.G. Howella, M.L. Macphersonb, C.R.F. Pintoa, and M.A. Coutinho da Silvaa a Department of Clinical Sciences b University of Florida, College of Veterinary Medicine, Department of LACS

VME – 5  ANATOMICAL CHARACTERIZATION AND EFFICACY OF THREE TECHNIQUES FOR MEDIAL MENISCAL RELEASE IN THE CANINE STIFLE D. Gale, J. Au, B. Hettlich, T. Motta. Dept. of Veterinary Clinical Sciences

VME – 6  EFFECTS OF PREADOPTION COUNSELING FOR OWNERS ON SEPARATION ANXIETY IN SHELTER DOGS. M. Herron, L. Lord, S. Husseini. Depts. of Veterinary Clinical Sciences (Herron) and Veterinary Preventive Medicine (Lord).


VME – 8  HEMATOLOGY AND CHEMISTRY REFERENCE RANGES IN RACING GREYHOUNDS. K. Kontur1, OSU CVM Class of 2014; G. Couto1, DVM, DACVIM; L. Bohenko2, DVM; S. J. Horvath1, K. Yant1; J. Chase3, BS; M. Frye3, MS, DVM; D. DeNicola3, DVM, PhD, DACVP.  
1The Ohio State University College of Veterinary Medicine; 2The West Virginia Racing Commission; 3IDEXXX Laboratories.

VME – 9  THE EFFECTS OF AUTOLOGOUS PROTEIN SOLUTION ON LAMENESS SCORES AND FORCE PLATE GAIT ANALYSES IN
EQUINE OSTEOARTHRITIS  K.Lewis, A. Ishihara, L. Zekas, A.L. Bertone. Department of Veterinary Clinical Sciences

VME – 10  SERUM VITAMIN D, CALCIUM, AND PHOSPHORUS CONCENTRATIONS IN HORSES FROM THE UNITED STATES AND TWO LOCATIONS IN THAILAND  M. Pozza, T. Kaewsakhorn, C. Treenarone, N. Inpanbutr, and R.E. Toribio. The Ohio State University Dept. of Veterinary Clinical Sciences, Chiang Mai University Faculty of Veterinary Medicine, Thailand

VME – 11  EVALUATION OF THE ARTICULAR ANTI-INFLAMMATORY EFFECTS OF INTRA-ARTICULAR AUTOLOGOUS PROTEIN SOLUTION IN HORSES WITH OSTEOARTHRITIS  R. Schwarze, A. Ishihara, A. Barnaba, L. Zekas, M. Wellman, A. Bertone. Department of Veterinary Clinical Sciences, The Ohio State University, College of Veterinary Medicine, 1900 Coffey Road, Columbus, Ohio 43210

VME – 12  USE OF A BLOOD BIOMARKER FOR PAINFUL BLADDER SYNDROME/INTESTINAL CYSTITIS  K.L. Sesemann, R.E. Rodriguez-Saona, C. A. T. Buffington. Departments of Veterinary Clinical Sciences and Food Science and Technology

VME – 13  EFFECT OF ASSISTED AND UNASSISTED BIRTHS ON COW BEHAVIOR AT CALVING IN HOLSTEIN DAIRY COWS  M. Titler, S. Bas and G.M. Schuenemann. Department of Veterinary Preventive Medicine

VME – 14  ACID-BASE AND ELECTROLYTE CONCENTRATIONS IN GREYHOUNDS AFTER RACING  K.Yant, C. G. Couto, L. Bohenko, S. Horvath, K. Kontur, L. Marin, J. Chase, M. Frye, D. DeNicola; 1The Ohio State University College of Veterinary Medicine, Department of Veterinary Clinical Sciences; 2The West Virginia Racing Commission; 3IDEXX Laboratories.

VME – 15  EFFECTS OF ATENOLOL ON FIVE-YEAR SURVIVAL IN CATS WITH PRECLINICAL HYPERTROPHIC CARDIOMYOPATHY  K.E. Schober, J.F. Zientek, X. Li, V. Luis Fuentes, J.D. Bonagura; Department of Veterinary Clinical Sciences

Veterinary Students – Epidemiology and Applied Research

VME – 16  EFFECT OF DRYING-OFF PRACTICES ON DAIRY COW BEHAVIOR  K. Brunson, P. Rajala-Schultz, L. da Costa, L. Heider, G. Schuenemann. Department of Veterinary Preventive Medicine
EMERGENCE OF THE LYME DISEASE VECTOR AND AGENT IN OHIO

P. Wang1, M. Glowacki1, D. Acosta1, M. Wellman1, C. G. Couto1, A. Hoet1, R. Gary3, K. Smith3, G. Needham4,5,6, and X. Li1

1Veterinary Medicine, 2Public Health, 4Natural and Mathematical Sciences, 5Food, Agricultural, and Environmental Sciences, 3The Ohio Department of Health, 6OSU Extension

BOVINE ABORTIONS DUE TO ZOONOTIC ETIOLOGY IN OHIO, 2001-2008

S. Greenbaum, P.J. Rajala-Schultz1, J. Hayes2

Department of Veterinary Preventive Medicine1, Ohio Department of Agriculture Animal Disease and Diagnostic Laboratory2

DETECTION AND SURVEILLANCE OF TUBERCULOSIS IN RAW MILK SAMPLES FROM THE SEMI-ARID REGION OF BRAZIL

W. Gebreyes, A. Guimarães, Department of Veterinary Preventive Medicine and M. Matiuzzi, Universidade Federal do Vale do São Francisco

NUTRITIONAL PROFILE COMPARISONS OF VARIOUS POPULAR PET FOODS

A. Hanthorn, T. Buffington. Department of Veterinary Clinical Sciences

CONSEQUENCES OF CUTANEOUS INJURIES FROM RADIO TRANSMITTER ANTENNAS ON REINTRODUCED EASTERN PLAINS GARTER SNAKES (Thamnophis radix radix).

R. Lauer, D. Wynn, J. Mckinley, and N. Reichenbach. College of Veterinary Medicine, Department of Evolution, Ecology, and Organismal Biology, and Liberty University, Dept. of Biology and Chemistry

INVESTIGATION OF EPIDEMIOLOGICAL AND NUTRITIONAL FACTORS ASSOCIATED WITH THE HIGH PREVALENCE OF TRANSITIONAL CELL CARCINOMA IN FISHING CATS (PRIONAILURUS VIVERRINUS).

E. Marshall1, W. Swanson2, R. Kelley3, T. Vennard3 and T. Buffington1

1College of Veterinary Medicine, Ohio State University, Columbus, OH 43210; 2Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden, Cincinnati, OH 45220; 3Procter & Gamble Pet Care, Mason, OH 45040

IMPACT OF CEFTIOFUR USE ON THE DISSEMINATION OF EXTENDED-SPECTRUM CEPHALOSPORING RESISTANT ENTERIC BACTERIA IN FINISHING SWINE POPULATIONS.

J. McGintee, D. Mollenkopf, T. Wittum, PhD. Veterinary Preventive Medicine. The Ohio State University
VME – 24  **INTESTINAL PARASITE PREVALENCE IN HUMANS AND DOMESTIC ANIMAL SPECIES: AN EXPLORATORY STUDY IN THE FAR NORTH REGION OF CAMEROON**  V. Nesser and Dr. R. Garabed. Department of Veterinary Preventive Medicine

VME – 25  **BIOMECHANICAL COMPARISON BETWEEN 2 CONSTRUCTS IN THE CANINE CERVICAL SPINE.**  D. Pascetta, A. Litsky, M. Allen, and B. Hettlich. Departments of Veterinary Clinical Sciences, Orthopaedics and Biomedical Engineering, College of Medicine, The Ohio State University


VME – 27  **IN VITRO CULTURE OF FRESHWATER MUSSELS**  M Shoemaker, B Wolfe. The Ohio State University College of Veterinary Medicine, The Wilds, Columbus Zoo Freshwater Mussel Conservation and Research Facility

VME – 28  **HIGH SEROPREVALENCE OF TOXOPLASMA GONDII IN FERAL CATS IN ADDIS ABABA, ETHIOPIA.**  N. Tiao¹, C. Darrington¹, B. Molla¹, W.J.A. Saville¹, G. Tilahun², O.C.H. Kwok³, W.A. Gebreyes¹, M.R. Lappin⁴, J.L. Jones⁵, and J.P. Dubey³ ¹Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210-1092, USA. ²Akililu Lema Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia ³United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Parasite Biology, Epidemiology and Systematics Laboratory, Beltsville, Maryland 20705-2350, USA ⁴Department of Clinical Sciences, College of Veterinary Medicine, Colorado State University, Fort Collins, Colorado 80523, USA. ⁵Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, 4770 Buford Highway, MS: F22, Chamblee, GA 30341, USA

VME – 29  **HEALTH ASSESSMENTS OF FOUR TURTLE SPECIES WITHIN THE OHIO ERIE CANAL**  D. Vajda, S. Koeth, O. Lockhart, P. Dennis. Ohio State University College of Veterinary Medicine, Cleveland Metroparks Zoo and Cleveland State University

VME – 30  **COMPARING ENVIRONMENTAL FACTORS OF TSETSE (GLOSSINA SPP) FLY POPULATIONS TO MODEL TRYPANOSOMIASIS RISK IN**
THE FAR NORTH REGION OF CAMEROON  S. Valerius and R. Garabed. Department of Veterinary Preventive Medicine

VME – 31  THE EFFECT OF HABITAT BURNING ON TSETSE FLY POPULATIONS IN CAMEROON L. Wagner and R. Garabed, Department of Veterinary Preventive Medicine

VME – 32  CHANGES IN ANTIMICROBIAL RESISTANCE ON OHIO DAIRY HERDS  M. Weeman, D. Mollenkopf, T. Wittum. Veterinary Preventive Medicine

VME – 33  EPIDEMIOLOGY AND GENOTYPIC DIVERSITY OF CAMPYLOBACTER SPP. FROM BROILER FLOCKS IN CHIANG MAI, THAILAND IN COMPARISON TO ISOLATES FOUND IN THE MIDWEST, UNITED STATES  C. Chokboonmongkol1, W. Gebreyes2, K-H Zessin3, T. Alter4, C. Widmann2, P. Patchanee1 Veterinary Public Health Center for Asia Pacific (VPHCAP) Faculty of Veterinary Medicine, Chiang Mai University, Thailand1; Ohio State University, College of Veterinary Medicine2; Department Panel, Veterinary Public Health, Freie Universitat Berlin, Germany3; Institute of Food Hygiene, Freie Universitat Berlin, Germany4

Veterinary Students – Immunology and Infectious Diseases

VME – 34  BRAIN MICROGLIAL ACTIVATION AS A BASIS FOR HSP70-MEDIATED PROTECTION IN THE VIRUS INFECTED BRAIN  S. Girod, M. Kim, C. Peterson, Y. Shu, and M.J. Oglesbee. Department of Veterinary Biosciences

VME – 35  IN VITRO REPLICATION OF PORCINE TORQUE TENO VIRUS  E. Ihms, S. Ringler, R. Jackwood, S. Krakowka. Department of Veterinary Biosciences


VME – 37  RETINOIC ACID FOR REGULATION OF HOST RESPONSE TO SUBLINGUAL VACCINE AGAINST RESPIRATORY PATHOGENS  J. Morrison, J. Jee, M. Fial, H. Steiner, A. Bonnegarde and P. Boyaka. Department of Veterinary Biosciences

VME – 38  A COMPARISON OF THREE ANTIBODIES FOR THE IMMUNOPURIFICATION OF EQUINE MONOCYTES  M. O’Brien1, W.M. Yeo2, and T. Stokol2 1The Ohio State University College of Veterinary
VME – 39  BROAD RELEVANCE OF HSP70 MEDIATED INNATE IMMUNITY IN THE VIRUS INFECTED BRAIN  
C. Peterson, M. Y. Kim, P. R. Dell 
Armelina Rocha, Y. Shu, M. Oglesbee. Department of Veterinary Biosciences

VME – 40  EFFECT OF AUTOANTIBODY-MEDIATED INFLAMMATION ON BORRELIA BURGDORFERI SURVIVAL AND GENE EXPRESSION IN VIVO  
L. Ramos, D. Acosta, and X. Li. Department of Veterinary Biosciences

VME – 41  CHARACTERIZATION OF A MODEL OF HTLV-1 ORAL TRANSMISSION IN THE RABBIT MODEL  
E. M. Simpson,¹ R. A. Haines,¹ M. D. Lairmore¹ ² ¹Center for Retrovirus Research and Department of Veterinary Biosciences, ²Comprehensive Cancer Center, Arthur G. James Cancer Hospital and Solove Research Institute

VME – 42  SUPPRESSION OF CANINE MYELOID CELLS BY SOLUBLE FACTORS FROM CULTURED CANINE TUMOR CELLS  
J. Wasserman, T. Papenfuss. Department of Veterinary Biosciences

VME – 43  CROSS-REACTIVITY OF HUMAN FOXP3 ANTIBODIES: IDENTIFICATION OF REGULATORY T CELLS IN RABBIT GALT  
T. Wyszynski¹, R. Haines¹, M. Lairmore¹ ², K. Landes¹, and E. Simpson¹. ¹Center for Retrovirus Research and Department of Veterinary Biosciences, College of Veterinary Medicine¹, Comprehensive Cancer Center, Arthur G. James Cancer Hospital and Solove Research Institute²

Veterinary Students – Molecular and Cellular Biology

VME – 44  EXPRESSION OF MIR-21 AND MIR-720 IN CANINE MELANOMA AND TRANSITIONAL CELL CARCINOMA  
D.L.H. Smith, S. Murahari, J.M. Fenger, and W.C. Kisseberth. Department of Veterinary Clinical Science, College of Veterinary Medicine

Veterinary Students – Structure/Function

VME – 45  CALCITRIOL AND CURCUMIN POTENTIATE ANTIPROLIFERATIVE EFFECTS OF GEMCITABINE AND CARBOPLATIN ON CANINE TRANSITIONAL CELL CARCINOMA  
K. Bakewell, N. Inpanbutr. Department of Veterinary Biosciences
VME – 46  LENGTH CHANGES OF THE COLLATERAL LIGAMENTS OF THE CANINE STIFLE JOINT: A COMPARISON OF IMAGING VERSUS SURGICAL NAVIGATION C. Clark, M. Allen. Department of Veterinary Clinical Sciences

VME – 47  THE EFFECT OF OSMOLALITY ON SNAKE SEMEN STORAGE AT TWO DIFFERENT TEMPERATURES E. Ferris, M. Cox, A. Santas, B. Wolfe, The Ohio State University College of Veterinary Medicine, Muskingum University Department of Biology, The Wilds Department of Conservation Medicine

VME – 48  EFFECTS OF RACING ON RETICULOCYTE CONCENTRATIONS IN GREYHOUNDS SJ Horvath¹, CG Couto¹, K Yant¹, K Kontur¹, L Bohenko², MC Iazbik³, LM Marín¹, D Hudson¹, J Chase³, M Frye³, DB DeNicola³. ¹ The Ohio State University College of Veterinary Medicine, Department of Veterinary Clinical Sciences; ² The West Virginia Racing Commission; ³ IDEXX Laboratories, Inc.

VME – 49  EFFECTS OF LACTOFERRIN ON STALLION SPERM SURVIVAL AND FUNCTION IN VITRO D.G. Howell, E.E. Clark, C.R.F. Pinto, and M.A. Coutinho da Silva. Department of Veterinary Clinical Sciences

VME – 50  SKIN LEVELS OF CYCLOSPORINE IN VARIOUS TOPOGRAPHIC LOCATIONS IN NORMAL DOGS K. Moning, A. Hillier DVM Dept. of Veterinary Dermatology

VME – 51  RELATIONSHIP OF SALIVARY HORMONE CONCENTRATIONS TO URINARY HORMONE EXCRETION PROFILES IN THE INDIAN RHINOCEROS (Rhinoceros unicornis). M. Nau¹, C. Pinto¹, R. Pairan², R. Sims³ and M. Stoops¹ College of Veterinary Medicine, Ohio State University, Columbus, OH 43210; ²Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden, Cincinnati, OH 45220; ³Fort Worth Zoo, Fort Worth, TX 76110.

VME – 52  COMPARISON OF BLOOD SUPPLY TO THE CANINE AND FELINE RECTUM: QUANTITATIVE MEASUREMENT OF THE CRANIAL RECTAL ARTERY, MEDIAN SACRAL ARTERY AND THE INTERNAL PUDEBDAL ARTERY V. Taylor-Lehman, T. Motta, C. Frasure, J. Masty, C. Premanandand, J. Richards, and N. Inpanbutr. Departments of Veterinary Biosciences and Veterinary Clinical Sciences
THE ROLE OF ADENOSINE IN THE DEVELOPMENT OF PULMONARY FUNCTIONAL IMPAIRMENT IN CD73- AND A1-ADENOSINE RECEPTOR-KNOCKOUT MICE INFECTED WITH INFLUENZA A (H1N1)

F. Aeffner, A.A. Gaughan, L.M. Joseph, I.C. Davis. Department of Veterinary Biosciences; The Ohio State University; Columbus OH, USA

RATIONALE: Influenza virus causes highly-contagious acute respiratory disease with significant morbidity and mortality. We have shown that H1N1 influenza A virus infection results in CD73-mediated generation of adenosine and activation of A1-subtype adenosine receptors (ADORA1) in mice at 2-6 days post-infection (d.p.i.). We hypothesized that ADORA1 activation by adenosine plays a key role in influenza-induced respiratory failure.

METHODS: 8 week-old C57BL/6 and congenic CD73-/- or ADORA1-/- mice were infected intranasally with influenza A/WSN/33 (10,000 PFU/mouse). Body weight, survival, peripheral O2 saturation (S\textsubscript{O}2), heart rate and pulse distention were monitored. Parameters of lung mechanics were measured by the forced oscillation technique (Scireq, flexiVent). Bronchoalveolar lavage fluid (BALF) cytokines were detected by ELISA. Viral replication was assessed by plaque assay.

RESULTS: No differences in outcome were found between influenza-infected CD73-/- mice and C57BL/6 controls. However, survival rate, respiratory rate, heart rate, S\textsubscript{O}2, and lung mechanics were improved in ADORA1-/- mice relative to wild-type controls, despite comparable levels of viral replication in both strains. BALF neutrophils, protein, IFN-\gamma and IL-10 content were also significantly reduced in influenza-infected ADORA1-/- mice, while BALF IL-6 and KC levels were higher.

CONCLUSIONS: Although CD73 is the rate-limiting enzyme for adenosine generation in the lung, absence of CD73 did not modulate influenza outcomes relative to wild-type C57BL/6 mice. In contrast, ADORA1 knockout significantly ameliorated cardiopulmonary dysfunction in H1N1-infected mice. ADORA1 may therefore represent a novel therapeutic target for prevention of respiratory failure in influenza infection.

Keywords: H1N1, influenza, ARDS, adenosine, mouse, pulmonary pathophysiology
IKKβ-NF-κB signaling controls a large number of biological processes via tissue-specific regulation of inflammatory and anti-inflammatory responses. Using a model of oral antigen sensitization and mice with cell-specific deletion of IKKβ, we addressed the contribution of intestinal epithelial cells (IECs) to regulatory crosstalk between distant mucosal sites of the gastrointestinal tract and the airways. Mice with localized alteration of IKKβ-NF-κB signaling in IECs (IKKβΔIEC) exhibited unaltered IgE, but enhanced Th1-associated IgG2a Abs and IgA responses after oral antigen sensitization in the presence of cholera toxin. Interestingly, these mice showed reduced lung inflammation and mucus production after nasal allergen challenge. This protection required oral sensitization and was associated with the presence of IgA in bronchoalveolar fluids and increased IL-17A in the lungs. When orally sensitized with the same regimen, mice with a generalized IKKβ deficiency in cells of the myeloid lineage (IKKβΔMye) also were protected against nasal allergen challenge. Distinct mechanisms contributed to protection in IKKβΔIEC and IKKβΔMye mice since airway hyper-responsiveness and lung inflammation were completely abolished in IKKβΔMye mice, which also showed no IgA in bronchoalveolar fluid and lower IL-17A in the lung than IKKβΔIEC mice. In summary, IKKβ in IECs is sufficient for shaping immune responses to ingested antigens, and subsequent responses to antigen exposure via the airways.

Keywords: allergy, gut, lungs, airway inflammation, NF-κB
POLYMORPHISMS OF THE SAMHD1 GENE ARE NOT ASSOCIATED WITH THE INFECTION AND NATURAL CONTROL OF HIV-1 IN AFRICAN AMERICANS AND EUROPEANS. S. Coon, D. Wang, and L. Wu. Departments of Veterinary Biosciences and Pharmacology

Background: The HIV-1 restriction factor SAM domain and HD domain-containing protein 1 (SAMHD1) blocks HIV-1 infection in human myeloid cells. Mutations in the SAMHD1 gene are associated with rare genetic diseases including Aicardi-Goutieres syndrome. However, it is unknown whether polymorphisms of SAMHD1 are associated with infection and natural control of HIV-1 in humans. Our objective was to determine whether the expression of SAMHD1 mRNA is affected by common single nucleotide polymorphisms (SNPs) in SAMHD1 and whether the SNPs are associated with HIV-1 infection status.

Results: Using a tagging SNP approach, we determined the association between eight tagging SNPs in SAMHD1 and the mRNA expression in B-lymphocyte cell lines from 70 healthy Caucasian donors. We identified one SNP (rs1291142) that was significantly associated with SAMHD1 mRNA expression, with minor allele carriers having 30% less mRNA levels ($P=0.015$). However, after analyzing the published genome-wide association study data of 857 HIV-1 controllers and 2,088 HIV-1 progressors from the African American and European cohorts, we did not find a significant association between SNPs in SAMHD1 and HIV-1 infection status, including SNP rs1291142 ($P>0.05$).

Conclusions: Our results suggest that common regulatory polymorphism(s) exist in the SAMHD1 gene that affects its mRNA expression in B-lymphocyte cell lines from healthy Caucasians. However, polymorphisms of SAMHD1 are unlikely to contribute to the infection and natural control of HIV-1 in African American and European individuals.

Keywords: HIV-1, SAMHD1, Single nucleotide polymorphisms, HIV-1 controllers, HIV-1 progressors.
DISTINCT TRANSFORMATION TROPISM EXHIBITED BY HUMAN T LYMPHOTROPIC VIRUS TYPE (HTLV-1) AND TYPE 2 (HTLV-2) IS THE RESULT OF POST-INFECTION T CELL CLONAL EXPANSION. P. Kannian, H. Yin, R. Doueiri, M. D. Lairmore, S. Fernandez and P. L. Green. Veterinary Biosciences

Objective: HTLV-1 and HTLV-2 are related but pathogenically distinct viruses. HTLV-1 causes adult T cell leukemia and a neurodegenerative disease termed HAM/TSP. HTLV-2 is not associated with leukemia, but a few infected individuals develop HAM/TSP like disease. In vitro HTLV-1 predominantly transforms CD4+ cells while HTLV-2 predominantly transform CD8+ T cells. The genetic determinant maps to the viral envelope, which contains the surface unit (SU) and the fusigenic transmembrane (TM). Herein, we investigate whether this transformation tropism occurs during initial infection, or during the cellular transformation process.

Methodology: Since most individuals are chronically infected at the time of detection, we utilized an established rabbit model to longitudinally measure the early HTLV-1 and HTLV-2 infection and replication kinetics in purified CD4+ and CD8+ T cells by measuring the proviral load and viral gene expression.

Results and conclusion: HTLV-1 and HTLV-2 were detected in both CD4+ and CD8+ T cells within one-week post-inoculation. In HTLV-1-infected rabbit CD4+ T cells, proviral burden and tax/rex mRNA expression peaked early and expression levels were directly proportional to each other. The late expression of the antisense transcript Hbz correlated directly with a late proviral burden peak in HTLV-1, similarly to Aph-2 in HTLV-2-infected rabbit CD8+ T cells. We evaluated the transformation tropism of HTLV-1 and HTLV-2 over a nine-week period using in vitro cell growth/immortalization assays. At the early weeks, both HTLV-1 and HTLV-2 showed proportionate growth of CD4+ and CD8+ T cells. However, beyond week 5, the predominance of one particular T cell type emerged supporting the conclusion that transformation tropism is a post-infection event due to selective clonal expansion over time.

Significance: This study is the first to provide in vivo evidence that HTLV-1 and HTLV-2 do not exhibit cellular preference during initial infection, however subsequent signaling cascades culminate to the observed tropism.

Keywords: HTLV-1, HTLV-2, transformation tropism, CD4+ T cells, CD8+ T cells
IMID - 5


Objective of the Study: Human T-cell leukemia/lymphoma virus 1 (HTLV-1), a deltaretrovirus, is the causative agent of a highly aggressive T-cell malignancy, adult T-cell leukemia/lymphoma (ATL). This virus infects more than 20 million people world wide, and vertical transmission of the virus from mother to child via breast milk is thought to be the primary route of exposure in endemic areas. There are currently fundamental gaps in the knowledge of the early immunologic events involved in the mucosal transmission of orally acquired HTLV-1 infection. The objectives of this study were to develop an oral model of HTLV-1 infection in the rabbit model, and to examine this model through hematology, serology (for humoral response), proviral load, and ex vivo cultures for detection of pro-viral antigen. We next examined the early spatial and temporal distribution of the virus within the GALT during the first four weeks of infection. Our overall goal is to determine the location of early virus populations and reservoirs so we are able to focus future studies to elucidate the mechanisms HTLV-1 uses to cross the mucosal barrier and establish persistent infections.

Methodology: In phase one, 12 week old female New Zealand White rabbits were orally inoculated with HTLV-1 positive lymphocytes. The rabbits were monitored with regular complete blood counts and differential white blood cell counts, serum antibody response to HTLV-1 antigens via western blot, detection of p19 viral antigen, and detection of pro-viral load by PCR. In phase two, we applied the same methods in a serial necropsy study where rabbits were sacrificed at 1, 2, 3 and 4 week PI, and key lymphoid organs, including the GALT, were examined for evidence of virus. Results and Conclusions: Phase one results confirm that we have established a method for orally inoculating rabbits with HTLV-1 that results in the establishment of a persistent infection, and this infection mimics infant infection following repeated exposure through breast feeding. These orally exposed rabbits had a delayed and often less intense humoral response, a delayed and variable leukocytosis, and a delayed and less robust p19 matrix antigen production as compared to IV exposed rabbits. In phase two, we determined that evidence of infection within the tissues examined was not detectable in any of the rabbits until 4 weeks PI. At 4 weeks PI, virus was detected within several GALT compartments as well as within systemic lymphoid tissues. Phase two results show that immediately following oral transmission, HTLV-1 infected lymphocytes exist in low numbers, undetectable by our current methods until the establishment of systemic infection at 4 weeks PI. This may represent a viral strategy to evade immune response during early infection.

Significance: This established model of oral infectivity will provide fundamental information about the mucosal microenvironment during the early stages of orally-acquired HTLV-1 in gut-associated lymphoid tissue. Determination of the early immunological events necessary for the establishment of persistent infection will provide the information needed to develop effective strategies to prevent mother to child transmission.

Keywords: HTLV-1, mucosal immunology, oral transmission
HSP70-MEDIATED PROTECTION AGAINST VIRAL NEUROVIRULENCE IS TYPE 1 DEPENDENT. M.Y.Kim¹, Y.Shu¹, T.Carsillo¹, J.Zhang², L.Yu², C.Peterson¹, S.Girod¹, S.Niewiesk¹, M. Oglesbee¹
¹Department of Veterinary Biosciences and ²Center for Biostatistics, The Ohio State University, Columbus, Ohio 43210

The major inducible 70 kDa heat shock protein (hsp70) both stimulates virus gene expression and innate immunity in vitro. However, the in vivo significance of virus-hsp70 interaction is only partially understood. Infection of the mouse brain with measles virus (MeV) demonstrates that hsp70 can be host protective. Transgenic constitutive expression of hsp70 in neurons protects neonatal H-2d congenic C57BL/6 mice from MeV neurovirulence, and a significant level of protection is retained after depletion of T lymphocytes which suggests innate immune mechanisms. The focus of the present work was to elucidate the basis for hsp70-dependent innate immunity in this model. Transcriptome analysis of brains from transgenic (TG) and non-transgenic (NT) mice 5 days after infection identified macrophage activation/antigen presentation and type 1 interferon (IFN) signaling as the main differences linked to survival. Evidence of enhanced macrophage activation in infected TG mice was further supported by MHC II immunohistochemistry. The activation of a type 1 IFN response was confirmed by RT-PCR analysis early after infection (1 d p.i.), where significant induction of IFN-β transcripts were observed in infected TG but not NT mice. Use of mice with a genetically deleted type 1 IFN receptor (IFNAR−/−) established the pivotal role for type 1 IFN in hsp70-mediated protection; virus-induced mortality was identical between infected hsp70 TG IFNAR−/− and NT IFNAR−/− mice. Results indicate that hsp70, in context of virus infection, is associated with accelerated induction of host protective innate immunity that is type 1 IFN dependent. This study has potentially broad virological relevance and support a novel axis of innate antiviral immunity driven by hsp70.

Keywords: interferon, hsp70 and measles virus neurovirulence
Ehrlichia chaffeensis infects monocytes/macrophages and causes human monocytic ehrlichiosis. To determine the role of type IV secretion (T4S) system in infection, candidates for T4S effectors were identified by bacterial two-hybrid screening of E. chaffeensis hypothetical proteins with positively charged C-terminus using E. chaffeensis VirD4 as bait. Of three potential T4S effectors, ECH0825 was highly upregulated early during exponential growth in a human monocytic cell line. ECH0825 was translocated from the bacterium into the host-cell cytoplasm and localized to mitochondria. Delivery of anti-ECH0825 into infected host cells significantly reduced bacterial infection. Ectopically expressed ECH0825 also localized to mitochondria and inhibited apoptosis of transfected cells in response to etoposide treatment. In double transformed yeast, ECH0825 localized to mitochondria and inhibited human Bax-induced apoptosis. Mitochondrial manganese superoxide dismutase (MnSOD) was increased over 9-fold in E. chaffeensis-infected cells, and the amount of reactive oxygen species (ROS) in infected cells was significantly lower than that in uninfected cells. Similarly, MnSOD was upregulated and the ROS level was reduced in ECH0825-transfected cells. These data suggest that, by upregulating MnSOD, ECH0825 prevents ROS-induced cellular damage and apoptosis to allow intracellular infection. This is the first example of host ROS levels linked to a bacterial T4S effector.

Keywords: Ehrlichia chaffeensis, type IV secretion effector, mitochondria, apoptosis, BAX, ROS, MnSOD
TOLEROGENIC DCS DIFFERENTIATION AND DEVELOPMENT OF IMMUNOSUPPRESSIVE DC PRECURSORS. **VanGundy, Z.** Taylor, C. White, A. Papenfuss, T. Dept. of Veterinary Biosciences

Tolerogenic dendritic cells (tDCs) are important myeloid cells which potently regulate innate and adaptive immune responses. We have previously found that the steroid hormone estriol (E3) generates tDCs *in vivo* which protect mice from developing autoimmune disease. To better understand tDC generation, we developed an *in vitro* tDC differentiation model. Myeloid progenitors were differentiated with GM-CSF in the presence of steroid hormones E3 and retinoic acid (RA) and both differentiated tDCs and their precursors (pre-tDCs) were phenotypically and functionally evaluated. We found that both E3 and RA tDCs had increased expression of stimulatory and inhibitory costimulatory markers and suppressed proliferation of responder immune cells in a dose-dependent manner, similar to *in vivo* tDCs. Importantly, we also found that tDC precursors had a marked immunosuppressive ability without marked changes in phenotypic cell composition. Although CD11b+Gr1+ populations were unchanged, increases in FoxP3+ expression were seen in responder splenocyte populations when co-cultured with pre-tDCs. These results suggest that pre-tDCs may be acting as myeloid derived suppressor cells (MDSCs) and are a potently immunosuppressive immature myeloid population that gives rise to tDCs. A better understanding of how tDCs are generated has potential therapeutic relevance for the treatment of numerous inflammatory diseases.

Keywords: Tolerogenic dendritic cells, myeloid derived suppressor cells, estriol, retinoic acid.
LOSS OF MAPK PHOSPHATASE 1 (MKP-1) ENHANCES BACTERIAL CLEARANCE AND IMPROVES SURVIVAL DURING ACUTE PRIMARY STREPTOCOCCUS PNEUMONIAE PULMONARY INFECTION L. M. Wancket and Y. Liu, Department of Veterinary Biosciences and the Center for Perinatal Research, The Research Institute at Nationwide Children's Hospital

MAPK phosphatase (MKP)-1 is a critical negative regulator of p38 and JNK MAP kinases. Mkp-1/- mice exposed to bacteria and bacterial ligands have prolonged p38 and JNK activation, enhanced TNF-alpha and IL-6 cytokine production, multi-organ failure. Additionally, in response to bacterial ligands, Mkp-1/- mice consistently produce more IL-10 (an inhibitor of IFN-gamma production) and less IFN-gamma. IFN-gamma has been shown to inhibit pulmonary clearance of Streptococcus pneumoniae through inhibition of alveolar macrophages. In this study, we evaluated the role of Mkp-1 in the host response to primary pulmonary S. pneumoniae infection. Male wildtype and Mkp-1/- mice received a single intratracheal instillation of either vehicle or the TIGR4 (subtype 4) strain of S. pneumoniae. TIGR4 LD50 levels were determined for both strains. Additionally, mice were euthanized at 24 or 48 hours post inoculation with 5E4 CFU (colony forming units) of TIGR4 to assess cytokine production and bacterial burdens. Separately, wildtype mice received an IP injection of 2mg of either isotype control or an IFN-gamma neutralizing antibody 3 hours prior to TIGR4 inoculation to assess the effect of IFN-gamma neutralization on survival. TIGR4 LD50 values were significantly higher in Mkp-1/- mice (1.9E5 CFU) compared to wildtype mice (1.7E4 CFU). Systemic and pulmonary production of several cytokines were significantly different between Mkp-1+/+ and Mkp-1/- mice, including higher levels of IFN-gamma in wildtype mice at 48 h and IL-10, TNF-alpha, and IL-6 in Mkp-1/- mice at 24 h. At 48 h post inoculation, Mkp-1/- mice had significantly lower pulmonary and blood bacterial burdens compared to wildtype animals. Depletion of IFN-gamma via neutralizing antibody significantly increased the survival of wildtype mice compared to wildtype mice receiving an isotype control antibody. Together, these data support the model that loss of Mkp-1 is protective against primary S. pneumoniae pulmonary infection, potentially though reduced release of IFN-gamma.

Keywords: lung, pneumonia, Streptococcus pneumoniae, Mkp-1, MAPK
COMPARATIVE HOST PROTEIN INTERACTIONS WITH HTLV-1 P30 AND HTLV-2 P28: INSIGHTS INTO DIFFERENCE IN PATHOBIOLOGY OF HUMAN RETROVIRUSES. R. Anupam‡1,2, R. Doueihi‡1,2, M. Kvaratskhelia1,5, K. Green-Church6, M. D. Lairmore1,2,4,7, P. L. Green*1,2,3,4 (‡ Equal authors)

1Center for Retrovirus Research, The Ohio State University, Columbus, OH 43210, USA
2Department of Veterinary Biosciences, The Ohio State University, Columbus, OH 43210, USA
3Department of Molecular Virology, Immunology, and Medical Genetics, The Ohio State University, Columbus, OH 43210, USA
4Comprehensive Cancer Center and Solove Research Institute, The Ohio State University, Columbus, OH 43210, USA
5College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA
6Mass Spectrometry and Proteomics Facility, The Ohio State University, Columbus, OH 43210, USA
7Department of Pathology, Microbiology, and Immunology, University of California, Davis, CA 95616, USA

Human T lymphotrophic virus type-1 (HTLV-1) and type 2 (HTLV-2) are related human retroviruses. HTLV-1 is the causative agent of adult T-cell leukemia (ATL), HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP), and other inflammatory diseases. HTLV-2 infection has not been clearly associated with any disease condition. Although both viruses can transform T cells in vitro, the HTLV-1 provirus is mainly detected in CD4+ T cells whereas HTLV-2 is mainly detected in CD8+ T cells. HTLV-1 and HTLV-2 encode accessory proteins p30 and p28, respectively, which are required for viral persistence in vivo. The first and last 49 amino acids of p28 and p30 respectively share approximately 78% homology with marked differences in the remaining region. The objective of the study was to identify differences and similarities between p30 and p28 in terms of host protein interactions in order to understand their role in pathogenesis. The S-tag affinity purified p30 and p28, along with associated proteins were analyzed by mass spectrometry. The shotgun proteomics revealed a list of host proteins that interacted with p30 and p28. To further confirm the proteomic results, four proteins were selected for further analyzes by immunoblotting assays. The results confirmed the proteomic data showing that REGy and NEAF-interacting protein 30 (NIP30) interact with p30 and not with p28. In contrast heterogeneous nuclear ribonucleoprotein H1 (hnRNP H1) bound to p28 and not to p30. Whereas, protein arginine methyl transferase (PRMT5) interacted with both p30 and p28. This study compares the host protein interaction profiles of HTLV-1 p30 and HTLV-2 p28 accessory proteins. We report novel interaction of p30 with NIP30 and p28 with hnRNP H1, while both interact with PRMT5. This comparative study indicates differences and similarities of host protein interactions of p30 and p28 which could potentially contribute to the different pathobiology of HTLV-1 and HTLV-2, respectively.

Keywords: S-tag affinity purification, Shotgun proteomics
BIOLOGIC ACTIVITY OF THE NOVEL SMALL MOLECULE STAT3 INHIBITOR AGAINST CANINE OSTEOSARCOMA CELL LINES. J Couto, M Bear, J Lin, C-L Li, PHoughton, W Kisseberth, C London. Affiliations: Lin and Houghton are Nationwide Children’s Hospital, Li is at the College of Pharmacy at OSU

Signal transducer and activator of transcription 3 (STAT3) plays an important role in cancer cell proliferation, survival and metastasis. We have previously shown that STAT3 is dysregulated in canine and human osteosarcoma (OSA) and have evaluated several potential small molecule inhibitors (FLLL32, LLL3, among others) of STAT3 in OSA cell lines, although these have several limitations. The novel analog of LLL3, LLL12, exhibits significantly improved solubility over previously tested inhibitors, making it a potentially more viable candidate for future in vivo use. The purpose of this study was to characterize the biologic activity of LLL12 in canine OSA tumor cell lines and through the generation of resistant cell lines, identify mechanisms of resistance to STAT3 inhibition. Canine OSA lines (OSA 8, OSA 16, D17 and Abrams) were treated with LLL12 and effects on proliferation and apoptosis were measured using CyQuant, Caspase 3/7 assays, and Annexin-V/PI staining. Western Blots and qRT-PCR were used to analyze downstream targets of LLL12. To generate drug resistant cell lines, cells were cultured continuously in LLL12 at increasing concentrations until biologic activity of drug (i.e., cell death) was no longer apparent. LLL12 inhibited proliferation of canine OSA cell lines in a dose dependent manner and induced apoptosis of canine OSA lines as evidence by Annexin-V/PI double staining and Caspase 3/7 activity. STAT3 phosphorylation was inhibited by LLL12 in all cell lines evaluated, resulting in subsequent downregulation of survivin expression. qRT-PCR confirmed downregulation of survivin gene expression, in addition to dysregulation of cyclin D1, VEGF, BCL-XL, and MCL-1. Lastly, LLL12 exhibited synergistic inhibitory effects on OSA cell line proliferation in the presence of doxorubicin chemotherapy. Our results show that LLL12 is a promising, highly soluble small molecule inhibitor of STAT3 that exhibits biologic activity against canine OSA cell lines.

Keywords: STAT3, osteosarcoma, LLL12
COMPARISON OF HTLV-1 AND HTLV-2 ANTISENSE PROTEINS’ EFFECTS ON CELLULAR SIGNALING PATHWAYS. N. Dissinger, H. Yin, P. Green. Dept. of Veterinary Biosciences

HTLV-1 and HTLV-2 are related but distinct pathogenic complex retroviruses. HTLV-1 is the etiological agent of adult T-cell leukemia (ATL) and a chronic neurological disease termed HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). In contrast, HTLV-2 is much less pathogenic with only a few reported cases of neurological disease. In addition to the structural and enzymatic proteins encoded by all retroviruses, HTLV encodes regulatory and accessory proteins. Most of the viral proteins, including the transforming protein Tax, are encoded by the sense strand of the provirus. However, the antisense strand of both HTLV-1 and HTLV-2 encode proteins (HBZ and APH-2 respectively) that down-regulate viral transcription by repressing Tax function. Animal studies have shown that while HBZ is essential for viral persistence, APH-2 is dispensable. This lends itself to the hypothesis that the cellular interactions of HBZ and APH-2 are different and important for virus biology and pathogenesis. We examined and directly compared the effect HBZ and APH-2 had on several cellular transcription factors that have been previously shown to interact. We observed that APH-2 acted in a similar manner to HBZ in repression of classical NF-κB and IRF-1. Differences were observed, however, in the pathways for c-Jun and TGF-β. While we observe that HBZ represses c-Jun, as reported previously, we see little to no repression by APH-2. HBZ has also been reported to enhance TGF-β, but no enhancement is seen with APH-2. These differences could help explain how the two related viruses have such different disease outcomes and elucidate specific pathways to target for new therapeutics.

Keywords: HTLV-1, HTLV-2, adult T-cell leukemia, HBZ, APH-2, NF-κB, IRF-1, c-Jun, TGF-β.
CHARACTERIZATION OF MICRORNA DYSREGULATION IN CANINE MAST CELL TUMORS. Fenger, J.M.1, Harrington, B.1, Lin, T.2, Volinia, S.3,4, Kisseberth, W.C.1,4, London, C.A.1,2,4

1Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA. 2Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA. 3Department of Molecular Virology, Immunology, and Medical Genetics, The Ohio State University, Columbus, OH, USA. 4Ohio State University Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA

Introduction/Methods/Results/Conclusions: MicroRNAs (miRNAs) are non-protein coding RNAs that have fundamental roles in tumorigenesis. Canine mast cell tumors (MCT) are cutaneous tumors in dogs whose biological behavior varies from relatively benign disease to aggressive, highly metastatic disease. We hypothesize that high grade MCTs possess a miRNA expression signature distinct from benign MCTs and this contributes to their aggressive behavior.

MiRNA expression was analyzed in 12 biologically low grade and 12 biologically high grade primary MCTs using TaqMan Low Density Arrays (Applied Biosystems). MiR-9 expression in cell lines and FFPE MCTs was performed using Taqman miRNA assays. Cell proliferation, apoptosis, and invasion was evaluated in P815 cells expressing lentiviral-miR-9-GFP constructs (Systems Biosciences) using the CyQuant® Cell Proliferation Assay, Sensolyte® Caspase 3/7 Assay, and Matrigel invasion assay. Gene-expression profiling was performed with Affymetrix Mouse 4.0 GeneChips.

51 miRNAs were differentially expressed in biologically high grade versus low grade tumors (p<0.05). MiR-9 was highly expressed in aggressive primary MCTs and canine mastocytoma cell lines as compared to benign tumors or canine bone marrow cultured mast cells. An independent cohort of FFPE MCTs showed substantially higher levels of miR-9 in aggressive versus benign MCTs. MiR-9 expression in P815 cells enhanced cell invasion, but had no significant effect on cell proliferation and apoptosis. Transcriptional profiling identified 250 genes that showed altered expression in P815 cells expressing miR-9.

These findings suggest that miR-9 may play a role in regulating mast cell invasion and overexpression of miR-9 in vivo may promote the aggressive behavior of some canine MCTs.

Keywords: Mast Cell Tumor, microRNA
RNA HELICASE A INTERACTS WITH TRANSLATIONAL REGULATORY PROTEINS TO CONTROL THE EXPRESSION OF VIRAL AND CELLULAR mRNAs. S. Fritz1-3, A. Ranji2,3, K. Boris-Lawrie1-5. Integrated Biomedical Sciences Graduate Program1, Department of Veterinary Biosciences2, Center for Retrovirus Research3, Center for RNA Biology4, Comprehensive Cancer Center5

RNA helicase A (RHA) interacts with the 5' UTR of select cellular and viral mRNAs, including the protooncogene junD and HIV-1, to create a distinct translation control mechanism. Results of co-immunoprecipitation-mass spectrometry experiments identified the poly-A binding protein (PABP) as a protein cofactor of RHA. Given the role of PABP and RHA in translation initiation, we hypothesized that RHA interacts with PABP and additional translational regulatory proteins to control the expression of select mRNAs. The objectives of this study were: (1) to determine the domains of RHA and PABP that are required for their association and (2) to identify additional cofactors of RHA that are necessary for its role in regulating the translation of target transcripts. GST pull-down assays were used to test the reciprocal binding of recombinant RHA and PABP domains and additional translational regulatory proteins. Cellular immunoprecipitations were used to extend the GST pull-down findings in the presence of PCE-mRNAs and the complete translation machinery. Results demonstrate that RHA associates with PABP and the translational regulatory proteins HuR and YB-1 through its dsRBDs and its RGG domain. In addition, RHA binds the translation release factor eRF3 through its helicase core and carboxyl-terminal domain of unknown function. These results suggest that a functional interaction between RHA and select translational regulatory proteins is necessary for the stability and control of target transcript expression at all stages of translation. We hypothesize that RHA functions as the core mRNP protein to direct progression through the translation cycle, which tightly regulates protein output. Our working model is that RHA coordinates the association of the mRNA stability proteins HuR and YB-1 with target transcripts to protect against mRNA degradation and to prevent compact mRNA folding, respectively, and that RHA facilitates the appropriate interaction between PABP and eRF3 to enable translation complex release and reinitiation.

Keywords: RNA helicase A (RHA), poly-A binding protein (PABP), human antigen R (HuR), Y-box binding protein 1 (YB-1), eukaryotic release factor 3 (eRF3), translation regulation
JUNB EXHIBITS LOSS OF “PAUSED” RNA POLYMERASE II REGULATION AND PROMOTES SURVIVAL IN METASTATIC MCF10CA1A CELLS. Q. Hu, M. Hicks and J. DeWille. Department of Veterinary Biosciences

The long term objective of this research is to determine the role of “Paused” RNA Polymerase II (Pol II) regulation in breast cancer. In this study we investigated the regulation and function of JUNB in nontransformed (MCF10A) and transformed, metastatic (MCF10A^met) cells. JUNB mRNA levels were undetectable in growing MCF10A cells. However, components of the “Paused” Pol II complex, including: Pol II, Cyclin Dependent kinase9 (CDK9, a component of the positive Transcription Elongation Factorb (pTEFB) complex) and Negative Elongation Factorb (NELFB, an inhibitor of Pol II transcriptional elongation) were associated with the JUNB gene proximal promoter. In contrast, JUNB mRNA levels were constitutively elevated in MCF10A^met cells. In MCF10A^met cells the JUNB proximal promoter was associated with Pol II and CDK9 but NELFB was not detected. NELFB protein levels, however, were similar between the MCF10A and MCF10A^met cell lines. Flavopiridol (FP), a chemotherapeutic drug that functions as a CDK9 inhibitor, blocks transcription of most cellular genes. Transcriptional inhibition by FP was supported by the results from MCF10A^met cells in which the mRNA levels of CEBPD, MYC and MCL-1 significantly declined in response to FP treatment. Surprisingly, FP treatment resulted in a dramatic increase in JUNB mRNA levels in MCF10A^met cells that was not due to increased JUNB mRNA stability. JUNB siRNA treatment increased MCF10A^met cell survival in response to FP, suggesting a pro-survival role for JUNB. These results demonstrate defective “Paused” Pol II regulation of JUNB gene expression in the MCF10A^met cells. In addition, the results indicate that JUNB mRNA levels increase in response to FP and that JUNB promotes survival of FP treated MCF10A^met cells. These findings suggest that loss of “Paused” Pol regulation may play an important role in breast cancer pathogenesis and that JUNB may function in the resistance of breast cancer cells to chemotherapy.

Keywords: JUNB, “Paused” RNA Polymerase, JUNB, Cyclin Dependent kinase9, Transcription Elongation Factorb, Negative Elongation Factorb, Flavopiridol,
CELL DELIVERY ROUTE, CELL SOURCE, AND BMP2 GENE TRANSDUCTION ON ENGRAFTMENT AND BONE DENSITY IN RABBIT MODEL. A. Ishihara, K. Ohmine, S. Jump, D. Russell, S. Weisbrode, and A. Bertone. Dept. of Veterinary Clinical Science, The Ohio State University; Department of Medicine, University of Washington; The Ohio State University.

Cell-mediated gene therapy with various progenitor cells, including mesenchymal stem cells (MSC), can have a great potential to treat bone fragility disorders; however, optimal methods to increase cell engraftment in target bone tissues need to be established. Although MSC have been extensively studied for cell therapy, dermal fibroblasts (DFb) may be an alternative cell source because of their excellent plasticity and rapid cell yield. We initially examined an intra-venous or intra-medullar infusion of autologous MSC into rabbit femurs and tibiae, but these methods showed modest cell engraftment and no measurable changes in bone mineral density and microarchitecture. After we confirmed a comparable osteogenic capacity between MSC and DFb with bone morphogenetic protein-2 (BMP2) gene transduction, we then examined an application of BMP2-expressing autologous DFb into rabbit femurs and tibiae using intra-osseous injection and intra-medullar infusion. Both delivery methods of DFb-BMP2 resulted in increased bone volume and mineral density and improved bone microarchitecture in trabecular bone. The intra-osseous DFb-BMP2 injection also induced greater bone defect filling, external callus formation, and trabecular surface area. Cell engraftment within trabecular bone and bone marrow tissue was most efficiently achieved by intra-osseous injection of DFb-BMP2. Systemic biodistribution of the locally injected/infused DFb-BMP2 was not evident in distant organs or contralateral hindlimb. Our results suggested that BMP2-expressing autologous DFb may be efficiently engrafted in target bones by intra-osseous injection and intra-medullar infusion and improve bone mineral density and bone microarchitecture. It is of great interest to demonstrate an efficacy of this DFb-mediated BMP2 gene therapy to increase bone density and strength on animal models of bone fragility disorders such as osteogenesis imperfecta and osteoporosis. Use of rapidly growing DFb may induce greater therapeutic and practical benefits over MSC for an application of cell therapy in clinical setting.

Keywords: Bone fragility disorders, BMP2, Stem cells, Dermal fibroblasts, Rabbits
Department of Veterinary Biosciences1, Center for Retrovirus Research2, Integrated Biomedical Sciences Graduate Program3, Center for RNA Biology4, Comprehensive Cancer Center5, Department of Physiology and Cell Biology6
The Ohio State University, Columbus, OH 43210

RHA affects the expression of select mRNAs by recognizing a 5’ untranslated region (UTR) RNA element, post-transcriptional control element (PCE). RHA catalyzes the rearrangement of the ribonucleoprotein complex to facilitate translation initiation. The subset of RHA-responsive mRNAs has been shown to include proto-oncogenes and viruses. Our hypothesis is that RHA regulates the translation of HuR through the 5’ UTR PCE motif in the HuR transcript. HuR is a ubiquitous RNA-binding protein that stabilizes and prevents the degradation of short-lived mRNAs by binding to 3’ UTR AU-rich elements. Such mRNAs encode proteins that promote cell survival, thus implicating HuR in cancer. The HuR gene contains two promoters that are differentially controlled by transcription factors. Therefore, two HuR mRNAs are transcribed that differ in 5’UTR length: 150 or 20 nucleotides. The HuR 150 nt UTR is predicted to contain three PCEs based on an alignment with the junD UTR, a known PCE containing mRNA. To test for HuR PCE activity, the HuR 5’UTR was subcloned into the retrovirus PCE reporter plasmid and PCE activity was measured by RT-PCR and ELISA. PCE reporter assays confirmed that the 150 nt UTR exhibits PCE activity. Next, the translatability of the HuR 150 and 20 nt 5’ UTR was compared in the context of luciferase reporter mRNA. The luciferase assays indicated that the translation efficiency of the PCE-containing long form is greater than the short form. Polysome profiles created from sucrose gradients detected only the HuR extended transcript on polysomes. To determine the direct effect of RHA on the translational regulation of HuR, a siRNA knockdown of RHA was performed. A Western blot demonstrated that the knockdown of RHA decreases the level of HuR protein. Together these data indicate that RHA plays a role in regulating the translation of the HuR transcript with the long 5’UTR.

Keywords: RHA, HuR, PCE, translation
RNA helicases are multidomain RNA binding proteins that play critical roles in gene regulation, viral replication, and host innate response. They are involved in all aspects of RNA biology and encode essential gene products. RNA helicase A (RHA) is necessary for translation of selected cellular genes and viral mRNAs that contain the RHA responsive element in the 5' leader. RHA interacts with RNA structural features, designated the post-transcriptional control element (PCE), and facilitates ATPase-dependent rearrangement of the mRNA-ribonucleocomplex (mRNP) and ribosome scanning. Downregulation of RHA or mutation of PCE eliminate efficient translation of junD proto-oncogene and retroviruses, including HIV-1. My preliminary results demonstrate that RHA is subject to specific proteolytic cleavage. This cleavage truncates the lysine-rich N-terminal domain that is necessary for selective interaction with these cognate mRNAs. Moreover, the truncated form of RHA is differentially observed in normal and stressful growth conditions. Because RHA is mutated in neoplasms, RHA knockout is lethal, and PCE-encoding genes encode oncoproteins (JunD, HuR), we hypothesize that RHA/PCE activity is necessary for proper cell growth and proliferation. The long-term goal of this research is to completely understand the biological function of RHA in modulating gene expression. Herein, we examine the important open issues of defining the specific cleavage products of RHA, the biological function of these isoforms, and the stimuli that lead to their generation. The overarching hypothesis is that proteolytic processing is the gatekeeper to change RHA function. We expect that N-terminally truncated RHA is an antagonist to translation of RHA target mRNAs, either by squelching cofactors necessary for translation, participating in mRNPs with alternate partners, or forming translation-inactive complexes with residual uncleaved RHA. Proper RHA regulation is expected to be disrupted in malignant growth, contributing to the translational dysregulation of the neoplasm.

Keywords: RNA helicase A (RHA), gene expression, translation, proteolysis
**WNT SIGNALING AND PROSTATE CANCER BONE METASTASIS**

Simmons, JK; Dirksen, WP; Lanigan, LG; Rosol, TJ

**Objective:** Prostate cancer is the second most common cancer and the second leading cause of cancer death in American men. Death due to prostate cancer is largely a result of metastasis, the most common site of which is bone. Metastasis to bone is an incurable disease that is associated with significant pain and morbidity. Studies on prostate cancer metastases have shown the importance of interactions with the bone microenvironment for growth and development, but much remains unknown. As the mechanisms of tumor-bone interactions are discovered, new targets for therapeutics will be developed.

Prostate cancer bone metastases are commonly characterized as being predominantly osteoblastic (bone forming). In prostate cancer it has been proposed that tumor-derived Wnts stimulate osteoblast differentiation and the formation of osteoblastic metastases through activation of the canonical Wnt pathway in a paracrine fashion. The canonical Wnt pathway ultimately leads to the stabilization and increased levels of cytoplasmic β-catenin. At normal levels, β-catenin promotes cell adhesion and controls cell shape; up-regulation affects cell proliferation and differentiation through activation of transcription factors and alterations in gene transcription. Up-regulation of the canonical Wnt signaling pathway in osteoblasts results in an increase production of osteoprotegerin (OPG) and decreased production of receptor activator of the nuclear factor kappa-B ligand (RANKL). This increased OPG:RANKL ultimately results in decreased osteoclastogenesis and increased osteoblastogenesis. Although Wnt signaling is expected to play a significant role in bone metastasis, its role or mechanisms of action are not fully understood.

**Methodology:** We are investigating the function of the canonical Wnt pathway in the formation and development of the bone lesions in prostate cancer metastases using an in vitro model of the bone microenvironment. In this model, neonatal murine calvaria are co-cultured with a canine prostate cancer cell line, Ace-1. The Ace-1 cell line is characterized by invasive subcutaneous growth and metastasis to bone with osteoblastic lesions post-intracardiac injection in nude male mice. In the calvarial co-culture experiments, both non-transfected Ace-1 cells and Ace-1 cells that have been stably transfected with human dickkopf-1 (Ace-1-Dkk-1) are used. Dickkopf-1 (Dkk-1) is a potent inhibitor of canonical Wnt signaling, and research on prostate carcinoma cell lines has shown that Dkk-1 can change metastatic phenotype and alter tumor growth. Media from the co-cultures is collected periodically, and the calvaria are collected at the end of the study for analysis. Our hypothesis is that reduced osteoblastic canonical Wnt signaling due to the paracrine effects of Dkk-1 from prostate cancer cells will result in increased calvarial bone resorption, decreased production of OPG, and increased production of RANKL.

**Results:** Our results show that there was an 8% and 16% increase in media calcium at days 2 and 4, respectively, from Ace-1-Dkk-1 co-culture media when compared to the Ace-1 co-culture media. There was a 64% and 400% decrease of OPG on days 2 and 4, respectively, in the Ace-1-Dkk-1 co-culture media when compared to Ace-1. There was a greater than 900-fold increase of RANKL on days 2 and 4 in the Ace-1-Dkk-1 co-culture media when compared to the Ace-1. Based on these findings we conclude that Dkk-1 secreted from the neoplastic Ace-1-Dkk-1 cells have a significant effect on osteoblasts that results in a shift in the OPG:RANKL. This shift ultimately resulted in increased calvarial resorption and increased calcium released into the media.

**Significance:** These findings highlight the importance of canonical Wnt signaling pathway in the formation of osteoblastic metastases in prostate cancer. Unraveling the dialogue between of Wnt signaling between the metastatic prostate cancer cells and the bone microenvironment will help elucidate new treatment targets.

**Keywords:** Wnt, prostate, metastasis, bone
THE ROLE OF P16 IN FELINE ORAL SQUAMOUS CELL CARCINOMA. W. Supsavhad, W. Dirksen, C. Martin, S. Pillai, and T. Rosol. Department of Veterinary Biosciences

Feline oral squamous cell carcinoma (FOSCC) is an extremely aggressive head and neck cancer in cats. In humans, head and neck squamous cell carcinoma (HNSCC) can be categorized as human papilloma virus (HPV)-induced and non-HPV induced HNSCC. Non-HPV HNSCCs are associated with mutated tumor suppressor genes, especially p53 and p16. The p16 (CDKN2A/INK4A) tumor suppressor gene is one of several important genes involved in cancer progression. Increased p16 immunoreactivity is a marker of HPV infection, while decreased p16 was reported to be correlated with mutations of the p16 gene in non-HPV HNSCC. However, the relationship between p16 protein expression, p16 gene mutations and Feline Papilloma Virus (FPV) infection in FOSCC has not been investigated. In this study, we searched for Feline Domestic Papilloma Viral type II (FdPV2) DNA and p16 immunoreactivity in FOSCCs. RNA was isolated from 3 FOSCC cell lines to determine the level of p16 mRNA. FdPV2 DNA was examined in 3 FOSCC cell lines. Moreover, Immunohistochemistry of p16 was also examined in 43 FOSCC paraffin-embedded biopsy specimens and 3 FOSCC cell line xenografts. We found that 14% of FOSCC samples had increased p16 protein levels, while 40% expressed very low or decreased levels. High levels of p16 mRNA were present in the F1 cell line, which also expressed increased p16 protein by IHC. In contrast, decreased p16 immunoreactivity was found in the F2 and F3 cell lines, which also had low levels of p16 mRNA. FdPV2 DNA was not amplified in the F1, F2, or F3 cell lines. These data indicate that p16 protein levels can be used to subclassify FOSCC similar to HNSCC. p16 mRNA levels in FOSCC correlated well with p16 immunoreactivity demonstrated by IHC. FdPV2 is likely not involved in the pathogenesis of FOSCC; however, the role of other feline papillomaviruses cannot be excluded.

Keywords: p16 (CDKN2A/INK4A), Feline Oral Squamous Cell Carcinoma (FOSCC)
THE NLS AND C-TERMINUS OF PTHRP MODULATE THE WNT/β-CATENIN PATHWAY IN OSTEOBLASTS. F. Wang, B.E. Hildreth, K.M. Hernon, R.E. Toribio, Depts. of Veterinary Clinical Sciences and Veterinary Bioscience.

Parathyroid hormone-related protein (PTHrP) is essential in skeletal development. Its functions have been attributed to its N-terminus; however, there is growing evidence that the mid-region, nuclear localization sequence (NLS) and C-terminus of PTHrP also have biological actions. To further explore the roles of the NLS and C-terminus, we have developed a mouse that lacks these specific regions of PTHrP (Pthrp^Δ/Δ). These mice developed chondrodysplasia with a decreased density of osteoblasts. In addition to reduced mRNA expression of osteogenic genes (Runx2, Sox9), the expression of factors of the Wnt/β-catenin pathway was also impaired. This suggests that the NLS and C-terminus of PTHrP modulate the Wnt/β-catenin pathway. Thus, we hypothesized that PTHrP, through its NLS and C-terminus regulates osteogenesis via the Wnt/β-catenin signaling. To test this hypothesis, we compared the expression of key factors of the Wnt/β-catenin pathway (GSK-3β, β-catenin, Lrp5, Lrp6), and the expression of the PTHrP receptor (PTH1R) and Sox9, which could bridge PTHrP and the Wnt/β-catenin pathway in bone tissue and calvarial osteoblasts from Pthrp^Δ/Δ and wild type mice. We also treated osteoblasts with lithium chloride (LiCl), a specific GSK-3β inhibitor, to activate Wnt/β-catenin signaling. Results: β-catenin was decreased while GSK-3β and Lrp6 were increased in Pthrp^Δ/Δ bones comparing to wild-type samples, however, there was no difference in Lrp5, PTH1R and Sox9. In contrast, β-catenin and Sox9 were increased while GSK-3β, Lrp5, Lrp6 and PTH1R were reduced in Pthrp^Δ/Δ osteoblasts. Only in wild-type osteoblasts β-catenin was up-regulated while GSK-3β, Lrp5 and Lrp6 were down-regulated after LiCl treatment, with no difference in Pthrp^Δ/Δ osteoblasts. These results demonstrate reduced Wnt/β-catenin signaling in Pthrp^Δ/Δ bones, but enhanced signaling in cultured Pthrp^Δ/Δ osteoblasts. The opposite expression pattern of β-catenin, GSK3β and Lrp6 between tissue and cells suggests that cell-to-cell interactions may be important for PTHrP to modulate the Wnt/β-catenin pathway in vivo.

Keywords: osteoblast, PTHrP, β-catenin
EVALUATION OF EXPRESSION AND FUNCTION OF VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 2, PLATELET DERIVED GROWTH FACTOR RECEPTORS-A AND B, KIT, AND RET IN CANINE APOCRINE GLAND ANAL SAC ADENOCARCINOMAS AND THYROID CARCINOMAS.  B K Urie1, D S Russell2, W C Kisseberth1, C A London1,2*  1The Ohio State University College of Veterinary Medicine. Department of Veterinary Clinical Sciences 2The Ohio State University College of Veterinary Medicine. Department of Veterinary Biosciences

Background:  Toceranib phosphate (Palladia) has a reported objective response rate of 25% in both canine apocrine gland anal sac adenocarcinomas (AGASACA) and thyroid carcinomas (TC), with stable disease occurring in an additional 50-60% of dogs. The basis for the observed responses to toceranib is not known. The purpose of this study was to evaluate AGASACA and TC samples for the expression and activation of VEGFR2, PDGFRa, PDGFRb, KIT and RET to assess whether dysregulation of these receptor tyrosine kinases may contribute to the biologic activity of toceranib.

Methods:  Fresh frozen and formalin fixed tumor samples from 24 primary AGASACA, 11 paired metastatic lymph nodes, and 15 TC were obtained from the OSU CVM Biospecimen Repository. RNA was generated from fresh frozen tumor specimens to perform reverse-transcriptase polymerase chain reaction for detection of VEGFR2, PDGFRa/b, KIT and RET. Protein lysates from frozen tumor samples were generated and phosphorylation of RTKs was assessed using the Proteome Profiler™ Human Phospho-RTK Array Kit. Tissue microarrays were constructed using formalin fixed specimens and immunohistochemistry (IHC) was performed to assess expression of VEGFR2, PDGFRa/b, and KIT.

Results:  mRNA for VEGFR2, PDGFRa/b, KIT and RET was detected in all AGASACA samples. mRNA for VEGFR2, PDGFRa/b, and Kit was detected in all TC samples, while mRNA for Ret was amplified in 10/15 samples. No phosphorylation of VEGFR2, PDGFRa/b, or KIT was observed on the arrays. However, phosphorylation of RET was detected in 54% of the primary AGASACA and 20% of TC. VEGFR2 was present in 19/24 primary and 6/10 metastatic AGASACA and 6/15 TC samples by IHC. KIT was present in 8/24 primary and 3/10 metastatic AGASACA and 9/15 TC samples. PDGFRa expression was noted in all tumor samples. In contrast PDGFRb expression was found in only a few tumor samples but was evident in the stroma of all tumor specimens analyzed.

Conclusions:  Known targets of toceranib are expressed in both AGASAC and TC. Given the observed expression of VEGFR and PDGFRa/b and phosphorylation of RET, these RTKs merit further investigation as to their roles in the biology of AGSACA and TC and their contribution to toceranib’s activity.

Keywords: canine, toceranib, phosphorylation, growth factors, apocrine, thyroid, carcinoma
EFFECTS OF MECLOFENAMIC ACID ON LUTEAL FUNCTION OF BEEF CATTLE
C. A. Messerschmidt, F. M. Abreu, L. H. Cruppe, M. V. Biehl, M. L. Day, C. R. F. Pinto, M. A. Coutinho da Silva; Department of Veterinary Clinical Sciences and Animal Sciences

The objective of this study was to determine the effects of meclofenamic acid, a non-steroidal anti-inflammatory, on luteal function of beef cattle. A total of 18 Angus cows, aging between 2 and 3 years old, were enrolled in the experiment. All cows were synchronized using a 5-day CIDR protocol. Briefly, cows received 100 ug of gonadorelin diacetate tetrahydrate (GnRH; Cystorelin®, Merial, Athens, GA, USA) and a controlled internal drug release insert (CIDR; Eazi-BreedTM CIDR®, Pfizer Animal Health, New York, NY, USA). Five days later, the CIDR was removed and 50 mg of dinoprost (Lutalyse®, Pfizer Animal Health) was administered intramuscularly. Estrus was determined by twice daily observations of mounting behavior and tail painting scores (day of estrus = Day 0). At 72 hours after dinoprost, a second dose of gonadorelin (100 ug, IM) was administered. On Day 14, cows were randomly assigned to the following treatment groups: 1) Control: 10 ml saline solution administered IM; 2) Systemic: 2 g meclofenamic acid administered IM and 3) Oral: 2 g meclofenamic acid administered orally. Cows were treated once daily for 11 days (i.e. until Day 24) and no adverse reactions were observed. Blood sampling and ovarian ultrasonography were performed every 72 h from Day 0 until Day 12 and then every 48 h until the end of the study. Serum progesterone concentrations were determined by radioimmunoassay and were used to determine functional luteolysis (i.e. progesterone <1 ng/ml). Ovaries were evaluated for the presence of a corpus luteum and to evaluate follicular growth and subsequent ovulation. One-way ANOVA was used to compare the day of peak progesterone concentration, lifespan of the CL and the length of estrous cycle between groups. Significance was set at P<0.05 and data is presented as means ± SEM (Table 1). There were no effects of meclofenamic acid administration on any of the parameters evaluated (P>0.05). In conclusion, meclofenamic acid administration did not affect luteal function in our study. Potentially, higher doses of meclofenamic acid may be necessary to inhibit prostaglandin synthesis and prevent luteolysis.

<table>
<thead>
<tr>
<th>Group</th>
<th>Peak Progesterone (day of cycle)</th>
<th>CL Lifespan (days)</th>
<th>Estrous Cycle (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.3 ± 0.3</td>
<td>19.7 ± 0.8</td>
<td>21.0 ± 0.7</td>
</tr>
<tr>
<td>Systemic</td>
<td>13.7 ± 0.8</td>
<td>18.7 ± 0.7</td>
<td>21.0 ± 0.7</td>
</tr>
<tr>
<td>Oral</td>
<td>14.0 ± 0.9</td>
<td>17.7 ± 1.1</td>
<td>20.7 ± 0.4</td>
</tr>
</tbody>
</table>

P>0.05

Keywords: cattle, luteal function, Meclofenamic
EFFECTS OF HEMATOCRIT AND RED BLOOD CELL-INDEPENDENT VISCOSITY MANIPULATION ON THROMBOELASTOGRAPHIC VARIABLES IN DOGS.  A.C. Brooks, J. Guillaumin, E. Cooper, G. Couto.  Department of Veterinary Clinical Sciences.

Thromboelastography (TEG) is a viscoelastic method to assess whole blood clotting in vitro. It assess primary and secondary hemostasis, fibrinolysis and can be used to diagnose hypercoagulability. Previous studies using TEG in dogs have suggested a hypercoagulable tendency in patients with anemia. We hypothesize that these TEG changes are an in vitro phenomenon related to alterations in blood viscosity created by reduction in hematocrit (Hct), rather than in vivo changes in hemostasis.

Twenty-one samples were collected from 7 dogs. Each blood sample was manipulated to produce 1 of 3 Hct conditions: 45%, 20%, or 10% while standardizing platelet count between 150,000-300,000/µL. Each Hct condition was tested in two situations: viscosity adjusted to approximately 4 cP (normal canine blood viscosity) by adding alginate (ALG), or dilution with an equal volume of saline (SAL). Both samples were analyzed with TEG simultaneously. Viscosity was measured with a cone-and-plate viscometer. Variables were compared using paired sample t-tests with Bonferroni correction. Adjusted P <0.05 were considered significant.

Viscosity of 20% and 10% ALG samples was not different than the 45% ALG sample. Viscosity of 20% and 10% SAL samples was significantly lower than the 45% SAL sample (P<0.001). TEG variables of 20% and 10% ALG showed significantly decreased alpha angle, MA, and G compared to the 20% and 10% SAL (P< 0.006). 20% and 10% SAL samples showed significantly higher alpha angle, MA, and G compared to 45% SAL (P<0.05).

TEG variables of the 20% and 10% ALG showed hypocoagulability, whereas those of the 20% and 10% SAL showed hypercoagulability. This suggests that that TEG variables are influenced by blood viscosity independent of red cell mass. Therefore the effects of Hct on TEG variables are likely an in vitro phenomenon related to changes in viscosity, making TEG variables difficult to interpret when Hct is low.

Keywords: Thromboelastography, viscosity, hematocrit, alginate
THE USE OF RADIOGRAPHS DUAL-ENERGY X-RAY ABSORPTIOMETRY, QUANTITATIVE COMPUTED TOMOGRAPHY AND MICRO COMPUTED TOMOGRAPHY TO DETERMINE LOCAL CANCELLOUS BONE QUALITY IN THE PROXIMAL FEMUR. Townsend KL¹, Hart R², Samii V¹, Motta T¹, Noble G², Dyce J¹ and Allen MJ¹. Department of Veterinary Clinical Sciences¹, Department of Biomedical Engineering²

Proximal femoral bone quality and quantity are two important variables to provide long-term stability in cementless total hip arthroplasty (THA). The goal of this study is to determine the feasibility of using non-invasive imaging data to predict the quality and quantity of cancellous bone in the canine proximal femur and directly compare this with bone microstructure and mechanical analysis. Twelve cadaveric canine femora were used. Radiographs were taken, along with DEXA scans and quantitative CT. Bone Mineral Content (BMC) and Bone Mineral Density (BMD) were measured from DEXA scans and CT, and radiographs were scored. Cores of cancellous bone were harvested from the proximal femur. High-resolution micro computed tomography (uCT) was performed to assess bone microstructure (fractional bone volume BV/TV, polar moment of inertia). Direct mechanical testing of the bone core was performed to assess bone strength (break load, stiffness, energy at break). Pearson correlation was used to compare the biomechanical parameters with the imaging modalities. uCT values (BV/TV and AMI) had the best predictor of mechanical properties of cancellous bone in the proximal femur, while clinical imaging modalities such as radiographs, DEXA BMD and BMC and qCT had only mild correlation with predicting bone strength. uCT has the best predictor of bone strength, suggesting that bone architecture plays a significant role in determining bone strength. CT, DEXA and radiographs all showed only mild correlation of bone strength, indicating that clinical parameters are not useful in predicting proximal femoral bone strength.

Keywords: Radiographs, quantitative CT, DEXA, MicroCT, bone quality.
BIOMECHANICAL EVALUATION OF MEDIAL FEMORAL CONDYLAR
SUBCHONDRAL CYSTIC LESIONS AND THE EFFECTS OF TREATMENT WITH
INTERNAL FIXATION. Williams JM¹, Santschi EM¹, Allen MJ¹, and Litsky AS²
¹Department of Clinical Sciences, The Ohio State University, College of Veterinary
Medicine, Columbus, OH; ²The Orthopaedic BioMaterials Laboratory, The Ohio State
University, Columbus, OH

Purpose: Subchondral bone cysts (SBCs) of the medial femoral condyle cause
lameness in the horse. The goal of treatment is to abolish lameness by healing the
subchondral and trabecular bone, and promote a resilient cartilage surface. The
objective of this study was to measure the strain in the stifle before and after the
creation of a “cyst-like” defect, and to evaluate the changes in strain following internal
fixation. Our hypothesis is that strain in the bone and meniscus of the stifle would be
altered by the defect, and that internal fixation would return the strain towards predefect
measurements.

Methods: Cadaveric stifle joints were collected from horses that were euthanized for
reasons unrelated to musculoskeletal disease. The limbs were dissected leaving the
joint intact and maintained in extension. Strain gages were placed on the medial
femoral condyle, medial meniscus, and proximal tibia. Axial compression was applied
incrementally from 440-3960 N and strain recorded. A subchondral bone defect was
created on the medial condyle and the limbs retested. A 4.5mm cortical bone screw
was placed in lag fashion across the defect and a final test was performed. The data
was tested for normal distribution and a 2-way repeated measure ANOVA used to
determine significance (p<0.05).

Results: Following creation of the subchondral defect, strain on the medial femoral
condyle decreased, and strain on the medial meniscus increased. After insertion of the
transcondylar screw, the strain on the medial femoral condyle increased to levels above
those measured prior to the creation of the defect, and the strain on the medial
meniscus decreased to pre-defect measurements.

Implications: This study provides the first biomechanical assessment of the equine
distal femur, meniscus, and proximal tibia, and offers experimental data suggesting that
SBC alter the strain environment of the stifle and that internal fixation can normalize
those values.

Keywords: equine, stifle, subchondral bone cyst, internal fixation
The objective of the Comparative Orthopedics Research Laboratory (PI: Alicia Bertone) is to investigate musculoskeletal biology and molecular medicine therapies that are associated with orthopedic disease conditions in horses and humans. Under this umbrella, we study gene therapy systems and tissue engineering, as well as utilize a comparative approach in our research. Our five main research focuses include: (1) Enhancement of articular cartilage healing with emphasis on the cartilage/bone interface, (2) Acceleration of bone repair, (3) Identification of genetic markers of orthopedic disease, (4) Physiology and pharmacology of medications for joint disease, (5) Optimizing gene therapy protocols for use in patients with orthopedic disease.

The Surgical Research Laboratory (PI: Matthew Allen) has expertise in the design, execution and analysis of preclinical and clinical studies related to orthopedic, oncologic and spinal surgery. Ongoing activities in the laboratory include: (1) Assessment of a novel surgical implant for treating osteoarthritis, (2) Evaluation of total knee replacement in the dog, (3) Quantitative assessment of the morphologic and mechanical characteristics of the canine proximal femur, (4) 3-dimensional kinematics of the canine cervical spine, (5) Novel biomaterials for spinal fusion, (6) Rodent models of musculoskeletal tumors, (7) Noninvasive measurement of functional recovery after stifle joint surgery in dogs.

Toribio Laboratory (PI: Ramiro Toribio) investigates genes involved in the transcriptional regulation of bone development, specifically on how mesenchymal stem cells commit to various cell lineages (osteogenic, chondrogenic hematopoietic) to affect bone mass. We have engineered mice lacking various domains of PTHrP and demonstrated that this protein is a pleiotropic factor necessary for bone development. We have also shown that domains of PTHrP are involved in the communication between the bone and bone marrow compartments. Our work has been presented in national and international meetings and is funded by the National Institutes of Health. Areas of interest include: (1) Bone development, (2) Stem cells, (3) Regulation of osteogenesis, (4) Interactions between bone and bone marrow cells, (5) Skeletal image acquisition and reconstruction.

The Oncology Service at the Ohio State University.Veterinary Teaching Hospital (OSU-VTH) is a major referral center for the diagnosis and treatment of osteosarcoma (OSA) and other primary and metastatic bone tumors in pet animals. A close and coordinated collaboration between the Oncology, Surgery, and Radiology Services is fundamental to the correct diagnosis (i.e cytology, image) and treatment (i.e. amputation, limb-sparing, radiotherapy, chemotherapy) of bone tumors. Our clinical trials have included studies of new combinations of established chemotherapy drugs, e.g. carboplatin and gemcitabine; new applications of old drugs to enhance chemosensitization without enhancing toxicity, e.g. combined low-dose suramin and doxorubicin); and novel targeted experimental therapeutic strategies for metastatic disease, e.g. rapamycin therapy.

Canine Ortho-Spine Program (PI: Bianca Hettlich): Spinal column stabilization is an emerging procedure for a variety of conditions in dogs, fractures and luxation, congenital instability, and the treatment of canine cervical spondylomyelopathy ('Wobbler’s Syndrome'). Current research involves: (1) Biomechanical assessment and safety of different stabilization constructs in the canine cervical spine, (2) Improvement of cervical implant longevity, (3) Assessment of modern imaging technology for instrumented spines, (4) Development of a novel disc replacement for the canine cervical and thoracolumbar spine.

Equine stifle project (PI: Elizabeth Santschi): Long-term goal of this project is to better understand the biomechanics of the equine stifle to understand pathophysiology and treatment of stifle disease. Current research include: (1) Strain gages on medial stifle joint: intact, with defect and with transcondylar screw, (2) Pressure sensor on medial tibial plateau to determine area of peak pressure, (3) Construct a FEM of the equine stifle to assist in understanding biomechanics.
PLATELET ENHANCEMENT THERAPY FOR CANINE OSTEOARTHRITIS. A. Bertone, M. Fahie, V. Guercio, J. Schaffer, G. Johnston, J. Au, B. Hettlich, T. Phillips, M. Allen, and G. Ortolano. Dept. of Veterinary Clinical Sciences, The Ohio State University; College of Veterinary Medicine, Western University of Health Sciences.

Dog-owning clientele are becoming aware of regenerative biologic treatments for joint pain and are inquiring about alternatives for their lame pets. As the techniques for canine joint injection become more mainstream in companion animal veterinary practice, the use of intra-articular therapies for joint disease in dogs will grow. Platelet-derived growth factors can affect regenerative processes in joints that may be of value in treating osteoarthritis (OA). The goal of this study was to determine if a single intra-articular injection of C-PET [Pall Corp] platelet concentrate improved lameness and joint pain in dogs with natural-occurring OA compared with saline-injected control OA dogs. 20 client-owned dogs with OA were enrolled at either The Ohio State University (OSU; n=10) or Western University (WU; n=10). At week 0, dogs were examined, sedated, radiographed, and administered a 3ml intra-articular injection of saline (OSU n=5, WU n=5) or C-PET (OSU n=5, WU n=5) into the affected joint. Saline control dogs crossed over to C-PET treatment at 12 weeks (n=7), and were reassessed 12 weeks later resulting in n=10 saline and n=17 C-PET evaluation points. At weeks 0 and 12, and 12 weeks post-crossover treatment, radiographic OA scores and surveys of pain and lameness were completed by owners using the Hudson Visual Analog Scale (HVAS) and Canine Brief Pain Inventory (CBPI). In addition, OSU measured force plate-derived peak vertical force (PVF) using an average velocity of 1.7 m/s. Combined C-PET treatment data from both study sites (n=17) showed a significant improvement in treatment scores 12 weeks after C-PET treatment compared to before C-PET treatment (HVAS p=0.0186, CBPI p=0.0087, PVF p=0.0307), that was not seen in saline controls. C-PET can be considered a safe, rapid (~8min), point-of-use treatment option for canine OA with clinical sign-modifying effects on lameness and joint pain in a majority of dogs.

Keywords: Dog, Osteoarthritis, Platelet therapy, Clinical trial, Gait analysis
NON-INVASIVE MEASURE OF BONE DENSITY TO PREDICT MECHANICAL PROPERTIES OF THE VERTEBRAL ENDPLATE IN THE CANINE CERVICAL SPINE.

Bertran J., Fitzpatrick N., Allen MJ.

Implant subsidence is a clinically significant problem in humans and dogs with cervical interbody cages, grafts or disc replacements. A reliable and predictive method of endplate fracture risk is required to further minimize postoperative complications. We hypothesized that the structural properties (stiffness and peak load) of the endplate would correlate to the endplate bone mineral density (BMD) measured on computed tomography (CT) and dual-energy x-ray absorptiometry (DEXA). Ten skeletally mature cervical spines (C3-C6) underwent quantitative CT scan, DEXA and indentation testing of the cranial endplate. No correlation was found between trabecular BMD measured by DEXA and QCT. Mean (± SD) initial stiffness and peak load of the endplate were 776.85±32.2 N/mm and 537.4 ± 47.94 N, respectively. Mean (± SD) area of the endplate was 157.2 ± 4.5 mm². No significant difference was found across the cervical levels for any variable. Endplate BMD was weakly but significantly correlated to initial stiffness ($r^2=0.14$, $p=0.027$). A stronger relationship was identified between endplate BMD and peak load ($r^2=0.65$, $p=0.0001$). There was no association between initial stiffness ($p=0.57$) and peak load ($p=0.71$) when compared to endplate area. The results from this study demonstrate that endplate BMD measured by QCT predicts the mechanical properties of the endplate. These findings suggest that preoperative assessment of BMD may be useful as a guide to patient and surgical technique selection in dogs that are being evaluated as candidates for interbody fusion or total disc replacement.

Keywords: cervical spine, mechanical testing, bone mineral density, implant subsidence
MEDIAL TIBIAL PLATEAU CONTACT PRESSURE IN HORSES
A. Bonilla¹, J. Williams¹, A. Litsky² and E. Santschi³.
¹Department of Clinical Sciences, The Ohio State University, College of Veterinary Medicine, Columbus, OH; ²The Orthopaedic BioMaterials Laboratory, The Ohio State University, Columbus, OH

Lameness in the horse due to injury in the medial femoro-tibial joint is common, but little is known about biomechanics of the stifle (knee) joint. The objective of this study was to determine the load pattern on the equine medial tibial plateau during axial loading with an intact patellar extensor mechanism and medial collateral ligament. An equine limb from femur to mid-tibia was mounted in a servohydraulic testing machine. The limb was loaded by a transverse pin in the proximal femur, and the transected tibia was placed in a holding jig that allowed rotation. Flexion of the stifle joint during loading was opposed by cables attached to the proximal femur and patella connected by a turnbuckle and an in-line tensometer. Pressure maps of the medial tibial plateau were acquired using an electronic pressure sensor (Tekscan) placed between the medial meniscus and the tibial plateau. Stifle joints were tested in duplicate at starting angles of 160° (full extension), 145° and 130°, and were loaded to 1800N. At maximum load, the joint angles of stifles in flexion decreased 11° and 16°. Patellar tension increased with load and decreasing stifle angle. Peak contact pressure and area was determined at maximal load. Total contact area was similar (mean of 9.2 cm²) for all trials. The peak contact pressure was on the cartilage of the medial tibial condyle not covered by meniscus at 160° and 145°. Lower contact pressures were measured under the meniscus, and decreased centrifugally from the area of peak pressure. At 130°, the area of peak tibial pressure enlarged caudally to include the axial aspect of the caudal meniscus, and pressure on the cranial meniscus also increased. This data confirms that the medial meniscus dissipates load, and suggests that both cranial and caudal meniscal injuries can occur during stifle flexion.

Keywords: Equine, Stifle, Biomechanics, Femoro-tibial joint, Orthopaedics, Pressure
The use of warmed peritoneal lavage solutions has been shown to reduce the incidence and severity of postoperative hypothermia. In veterinary practice, conventional microwave ovens are commonly used to warm peritoneal lavage solutions prior to application in surgery, although the results of this technique have not been reported and wide variations in temperature are possible. An alternative technology has been developed, using novel temperature-controlled fluid delivery systems. We hypothesized that the conventional fluid warming technique would produce higher variations in temperature than an on-demand system and that microwaved fluids would cool rapidly in a simulated surgical environment. Four experimental groups were used. Group 1 consisted of ten 500 ml bottles of 0.9% NaCl warmed in a microwave for 1 minute each; the temperature of each saline bottle was tested using a thermometer probe inserted into the bottle. Group 2 consisted of 10-500 ml bottles of 0.9% NaCl warmed in a microwave for 1 minute each, then poured into a stainless steel bowl; the temperature of the saline was measured in the bowl. Group 3 consisted measuring the temperature 0.9% NaCl warmed using the on-demand fluid warming unit at a flow rate of 600 ml/min. Group 4 consisted of measuring the temperature of 0.9% NaCl warmed using the on-demand fluid warming unit at a flow rate of 800 ml/min. Fluid temperature data were analyzed over time using repeated-measures ANOVA. Comparisons of temperatures at specific time points were performed using post-hoc Tukey tests. Results showed that the use of an on-demand fluid warming system was more consistent than warming bottles of saline in a microwave. The next step of this study will involve using the device in a clinical setting to determine whether or not a consistent fluid temperature will have an impact on patient body temperature.

Keywords: peritoneal lavage, peritonitis, hypothermia
Sepsis is the leading cause of foal mortality in the first week of life. Septic foals are often hypovolemic and hypotensive from decreased fluid intake, systemic inflammation, electrolyte imbalances and ongoing losses. The interplay between the RAAS and HPAA (Hypothalamic-Pituitary-Adrenal Axis) maintains organ perfusion and function during hypotension, inflammation, and sepsis. HPAA dysfunction and relative adrenal insufficiency (RAI) are common in septic foals. Data is lacking on the RAAS response to disease in newborn foals. The purpose of this study was to investigate the RAAS and factors of the HPAA that interact with the RAAS, in septic, sick non-septic (SNS) and healthy foals, and to determine their association with clinical findings and mortality. We hypothesized that critical illness will result in RAAS activation that will be associated with clinical findings, and that an inappropriately low aldosterone response to sepsis is part of RAI syndrome of sick foals. Blood samples were collected on admission from 74 septic (sepsis score >12), 59 sick non-septic, and 34 healthy foals of <7 days of age. Blood concentrations of angiotensin-II (ANG-II), adrenocorticotropin (ACTH), cortisol, aldosterone, and plasma renin activity (PRA) were determined by radioimmunoassays. ANG-II, aldosterone, ACTH and cortisol concentrations, as well as ACTH/aldosterone and ACTH/cortisol ratios were higher in septic than healthy foals (P<0.05). Cortisol concentrations were higher in SNS than healthy foals. No difference in PRA between groups was found. Septic foals which died had lower cortisol concentrations and higher ACTH/cortisol ratios than septic surviving foals. High potassium and low chloride concentrations were associated with hyperaldosteronemia in septic foals. RAAS activation in critically ill foals is characterized by increased ANG-II and aldosterone concentrations. Inadequate aldosterone and cortisol response to stress in septic foals indicates that RAI is not restricted to the zona fasciculata, but also affects the zona glomerulosa in equine severe illness.

Keywords: sepsis, hypovolemia, adrenal insufficiency, foal, equine
KINEMATIC GAIT ANALYSIS USING 3-D MOTION CAPTURE IN DOBERMAN PINSCHERS WITH AND WITHOUT CERVICAL SPONDYLOMYELOPATHY
K Foss, R C. da Costa, S Moore. Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA.

The most widely used method for determining the response to treatment in patients with cervical spondylomyelopathy (CSM) is based off of the owner and clinician’s perception of the gait. This form of evaluation is highly subjective and can suffer from observer bias. The purpose of this study was to utilize digital video motion capture to compare multiple kinematic parameters between Doberman Pinscher dogs with and without CSM.

Nineteen Doberman Pinschers were prospectively studied; 10 clinically normal dogs and 9 with CSM. Neurologic examinations were performed in all dogs prior to enrollment. All CSM-affected dogs had their diagnosis confirmed with a 3.0 T MRI. All dogs were fitted with a lycra bodysuit and 32 reflective markers were applied. 3-D motion capture was performed and a 3-D stick diagram representation was reconstructed of each dog from which several parameters of interest were measured. Parameters evaluated included stride (duration, length, height), maximal and minimal spinal angles, elbow and stifle flexion and extension, and maximum and minimum distances between the thoracic and pelvic limbs. Using a random effects linear regression model, all parameters were compared between groups.

Significant differences between groups included smaller minimum thoracic limb distance ($P=0.024$) and maximum thoracic limb distance ($P=0.001$) in CSM-affected dogs. Additionally, stride duration in the thoracic limbs was also significantly smaller ($P=0.009$) in CSM-affected dogs. In the pelvic limbs, the average stifle flexion ($P=0.048$) and extension ($P=0.009$) were different between groups.

In conclusion, the thoracic limb distances and thoracic limb stride duration were found to be significantly smaller in CSM-affected Doberman Pinschers when compared to normal Dobermans. Interestingly, these findings suggest that when using computerized kinematic gait analysis to compare the gait between normal and CSM-affected Dobermans, the thoracic limbs appear to reveal more consistent differences than the pelvic limbs.

Keywords: Doberman Pinscher, gait analysis, digital motion capture
THE EFFECT OF KETOCONAZOLE ON BLOOD AND SKIN CONCENTRATIONS IN CANINES. L. L. Gray, A. Hillier, L. K. Cole, P. J. Rajala-Schultz. Departments of Veterinary Clinical Sciences and Veterinary Biosciences

**Background:** Canine atopic dermatitis (CAD) is a common pruritic allergic skin disease for which cyclosporine (CSA) is currently the only FDA approved therapeutic. Treatment of CAD is life-long, as it is a disease that is controlled rather than cured. Administration of CSA can be cost prohibitive, particularly for medium to large sized dogs. CSA is metabolized by the liver cytochrome P450 enzymes, a process which is inhibited by ketoconazole (KTZ) thereby increasing CSA concentrations. **Hypothesis/Objectives:** The aims of this study were to determine skin and blood CSA concentrations when CSA was administered alone at 5.0 mg/kg (Treatment 1: T1) or 2.5 mg/kg (Treatment 2; T2); and when CSA was administered at 2.5 mg/kg concurrently with KTZ at 5 mg/kg (Treatment 3; T3) or 2.5 mg/kg (Treatment 4; T4). We hypothesized that skin and blood CSA concentrations in T1 would not differ from those obtained with T3 or T4. **Animals:** In a randomized cross-over study, six healthy research dogs received each of the treatments (T1, T2, T3, T4) once daily for 7 days. **Methods:** After the first, fourth, and seventh dose for each treatment a peak and trough skin punch biopsy sample and whole blood sample were collected and analyzed with high-performance liquid chromatography tandem mass spectrometry. Data were analyzed using a repeated measures approach with PROC MIXED in SAS. Pairwise comparisons were performed with least squares means and Tukey-Kramer adjustment for multiple comparisons. **Results:** Mean blood CSA concentrations in T1 were not different from T2 or T4, but were less than T3. Mean skin CSA concentrations in T1 were greater than T2, not different from T4, and less than T3. **Conclusions and Clinical Relevance:** Administration of CSA and KTZ concurrently at 2.5 mg/kg each may be as effective as CSA alone at 5.0 mg/kg for treatment of CAD.

Keywords: Cyclosporine, Ketoconazole, Skin, Blood, Concentration, Canine

Introduction: Laminitis is a devastating cause of equine lameness in which injury to the digital laminae commonly results in distal displacement of the distal phalanx and solar compression. Radiography is standard-of-care for diagnosis, prognosis and evaluation of treatment response, but is limited regarding laminar imaging because of tissue superimposition and poor soft tissue contrast resolution. Magnetic Resonance Imaging (MRI) has superior soft tissue contrast resolution. Our purpose was to establish normal hoof wall and sole measurements for digital radiography (DR) and MRI, to correlate and compare DR/MRI measurements and to evaluate inter- and intraobserver variability.

Materials and Methods: The front feet of 25 horses, euthanized for non-lameness conditions, were disarticulated. Lateromedial and horizontal dorsopalmar radiographs were made. For MRI, a 3 Tesla magnet and knee coil were used. T2*, 3-D gradient echo and proton density sequences were acquired in sagittal and dorsal planes. Image review and measurements were made by two board-certified radiologists, one board-certified equine surgeon and a radiology resident. DR/MRI measurements were compared. Statistical analysis and inter/intraobserver methods were performed using ANOVA, Student’s T-test and Fleiss’ Kappa.

Results: Normal overall DR/MR sole thickness, epidermis and dermis are 13.5mm/12.0mm, 8.0mm/7.6mm and 5.5mm/4.5mm. MR lamina and corium sole thickness are 1.4mm and 3.1mm. There was mild difference between DR and MR measurements (P<0.001); average DR measurements were 2 mm greater than average MR measurements. There was good overall inter/intraobserver correlation (P>0.98/0.85) between DR (P>0.98) and MR (P>0.99) measurements.

Conclusions: Normal hoof measurements for DR/MR are presented. There is good correlation between DR/MR, with measurement differences of ≤ 2 mm (DR ≥ MR). DR/MR measurements are reproducible diagnostic tests, with better observer correlation for MR. Future studies using laminitic feet and this protocol are needed to determine measurement changes and accuracy to guide treatment and prognosis.

Keywords: horse, radiography, laminitis, MRI, 3T

**Purpose:** To determine the effects of modified Cyclosporine A (CsA) on canine lens epithelial cell (LEC) and corneal endothelial cell viability *in vitro*. **Methods:** Lens capsules and corneas were harvested from canine cadaver eyes. Lens capsules were randomly assigned to one of three treatment groups: 0 µg/mL CsA (culture media only; n=6), 20 µg/mL CsA (n=9), or 40 µg/mL CsA (n=9) and were treated for 7 days before processing for routine H&E staining. Additional lens capsules were treated for 7 days with 0 µg/mL CsA (n=6) or 40 µg/mL CsA (n=6), subsequently incubated in culture media without CsA for 21 additional days, and were processed for routine H&E staining. Corneas (n=7 per group) were randomly assigned to one of three control groups (no treatment, BSS, mitomycin-C) or to one of three treatment groups (20, 40, 100 µg/mL CsA). Corneas were treated for 180 minutes prior to staining and histologic evaluation. **Results:** Capsules that received culture media with no CsA had complete PCO formation by 7 days; capsules that were maintained for 28 days were wrinkled and confluent with LEC. Comparatively, capsules that received 40 µg/mL CsA had few to no LEC present by 7 days. Lens capsules initially treated with 40 µg/mL CsA and maintained for 28 days had progressive clearing of LEC without any regrowth by 28 days. Endothelial cell viability was not decreased in corneas acutely exposed to 20, 40, or 100 µg/mL of CsA. **Conclusions:** CsA results in decreased LEC viability in a dose-dependent fashion *in vitro*, with greater doses causing greater cytotoxicity. 40 µg/mL of CsA was most effective at clearing LEC and preventing long-term regrowth. CsA was not acutely toxic to the corneal endothelium at concentrations up to 100 µg/mL. CsA may be a safe and effective tool for the treatment of canine PCO. **Keywords:** Cyclosporine A, lens epithelial cell, corneal endothelium, posterior capsule opacification, canine
FACTORS AFFECTING IN VITRO MATURATION OF ALPACA OOCYTES
C.A. Messerschmidt, M.A. Coutinho da Silva, B.S. Forshey, E.A. Coffman, C.R.F. Pinto. Department of Veterinary Clinical Sciences

A 2 x 2 x 2 factorial design investigating age (old vs. young), oocyte collection method (aspiration vs. slicing) and media with or without fetal bovine serum (FBS) was utilized to determine optimum in vitro maturation rates of alpaca oocytes. We hypothesized that oocytes aspirated from young alpacas and placed in maturation media supplemented with FBS would have greater maturation rates than those incubated in any other factorial combination. Oocytes were collected from the ovaries of 6 young alpacas and 5 old alpacas. Once all follicles ≥2 mm were aspirated, a scalpel blade was used to recover oocytes ≤1 mm by sequential slicing. Morphologically normal oocytes (MNO) were kept separate and halves of each group were randomly divided and incubated 24 h in chemically defined in vitro maturation media with or without 10% FBS. Maturation was defined by the observation of a polar body at the end of the incubation period. The proportions of collected and matured oocytes were analyzed by Chi-Square or Fisher Exact Test, whenever appropriate. Significance was set at P ≤ 0.05 and probabilities between P > 0.05 and ≤0.1 indicated a tendency for significance. A total of 276 oocytes were collected and 118 (42.7%) were classified as MNO. Overall, aspiration yielded a greater percent of MNO than that obtained following slicing, 61.6% (53/86) vs. 36.8% (70/190) respectively (P<0.001). There were no significant differences in the percent of MNO recovered following aspiration or slicing of the ovaries between young and old alpacas. The overall oocyte maturation rate was 17.8% (21/118) and no differences were observed between age groups regardless of collection method or media used. Within the old group, there was a trend (P=0.056) for greater maturation rates when oocytes were incubated in media supplemented with FBS 50% (5/10) than without it 8.3% (1/12). In conclusion, a greater proportion of MNO can be collected via aspiration of antral follicles ≥2 mm and are more likely to reach maturation than those obtained via ovary slicing. Addition of FBS to the maturation media may improve in vitro maturation rates of oocytes obtained from older alpacas.

Keywords: Alpaca, oocyte, in vitro maturation, fetal bovine serum, Camelid
EFFECT OF BODY POSITION ON ABDOMINAL PRESSURES IN ADULT HORSES.

**Reasons for performing study:** Intra-abdominal hypertension (IAH) is a significant cause of morbidity in critically ill humans and has also been described in horses with colic. Diagnosis requires measurement of intra-abdominal pressure (IAP). Standardized acquisition methodologies are developed in people because body position is known to alter IAP values obtained.

**Objectives:** To investigate the effect of body position on direct IAP (dIAP) and abdominal perfusion pressure (APP) in normal horses.

**Methods:** Nine healthy adult horses underwent a standardized total intravenous anesthetic protocol to control patient positioning. dIAP via abdominal cannulation and electronic sphygmomanometry was measured from the left flank (LFl), right flank (RFl) and ventral (V) abdomen with horses randomly positioned twice into left lateral (LLR), right lateral (RLR) and dorsal recumbencies (DR). Direct mean arterial blood pressure (MAP) was obtained concurrently to calculate APP (MAP - IAP). Differences in mean IAP/MAP/APP at each site and body position were assessed by paired t-tests or repeated measures ANOVA. P<0.05 was significant.

**Results:** Ventrum dIAP was lower with horses in DR (-7 mmHg) than LLR or RLR (11 mmHg and 13 mmHg respectively); P<0.0001. Ventrum APP was not different in all recumbencies (DR = 76 mmHg, LLR = 80 mmHg, RLR = 72 mmHg). MAP obtained from DR (69 mmHg) was lower than LLR or RLR (83 mmHg and 94 mmHg respectively); P<0.0001. LFl dIAP was lower (-5 mmHg) and APP higher (100 mmHg) with horses in RLR compared to DR (15 mmHg and 54 mmHg respectively); P<0.0001 for both. RFl dIAP was lower (-8 mmHg; P<0.001) and APP higher (91 mmHg; P=0.002) with horses in LLR compared to DR (15 mmHg and 55 mmHg respectively).

**Conclusions and potential relevance:** Body position directly affects intra-abdominal and hemodynamic pressures. This should be considered when evaluating abdominal pressure profiles and visceral perfusion in horses.

Keywords: abdominal, pressure, recumbency, horse
NOVEL PUBLIC HEALTH RISK FROM SUBCLINICAL INFLUENZA A VIRUS INFECTIONS AT SWINE SHOWS. A. Bowman, J. Nolting, S. Nelson, R. Slemons. Department of Veterinary Preventive Medicine

Rationale: Pigs play a critical role in the ecology and epidemiology of influenza A viruses by serving as hosts for viruses originating from avian and mammalian species and by serving as the source for novel reassortant viruses infecting humans. The swine-human interface at agricultural exhibitions has not been substantially investigated despite being repeatedly associated with bi-directional interspecies virus transmission.

Hypothesis: Influenza A virus infections in swine at agricultural exhibitions are occurring more frequently than documented. With the goal of protecting public health, this study was initiated to actively monitor the antigenic and genomic properties of influenza A viruses in pigs at Ohio agricultural exhibitions.

Methods: In this prospective epidemiological study, 15, 16, and 22 Ohio fairs were strategically recruited to participate during 2009, 2010, and 2011, respectively. At the time of visit, the pigs at each exhibition were visually examined for signs of respiratory illness and individual nasal swabs were collected from twenty selected pigs. Influenza A viruses were isolated in MDCK cells and subtyped with RRT-PCR.

Results: Influenza A viruses were recovered from pigs at 3 of 15 (20%) exhibitions in 2009, 3 of 16 (18.8%) exhibitions in 2010, and 6 of 22 (27.3%) exhibitions in 2011. Pigs with clinical signs of respiratory illness were only observed at 2 of 53 (3.7%) exhibitions. The hemagglutinin and neuraminidase subtypes of the influenza A viruses recovered were consistent with influenza A viruses concurrently circulating in the U.S. swine herd.

Conclusions: Subclinical influenza A virus infections, common among exhibition swine in Ohio, are going unreported in current national swine influenza virus surveillance programs. These subclinical infections at the swine-human interface likely play a role in interspecies virus transmission, thus explaining swine-origin influenza in humans only exposed to apparently health swine. This investigation highlights the need for expanded risk assessments at agricultural exhibitions.

Keywords: surveillance, swine-human interface, pigs, zoonotic, fairs
CHARACTERIZATION OF AN ORTHOTOPIC MOUSE MODEL OF OSTEOSARCOMA. B.K. Chaffee, F. Xu, M.J. Allen, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH.

Osteosarcoma (OSA) is the most common primary malignancy of bone in humans and dogs, and the second most frequent cause of cancer related deaths in children. Despite improvements in the management of the primary disease, many patients eventually succumb to distant metastasis, most often involving the lungs. We developed an orthotopic xenograft model that recapitulates the growth of the primary bone tumor followed by metastasis to the lung. Nude mice underwent intra-tibial implantation of solid osteosarcoma tumor tissue. Mice were monitored by digital radiography. Beginning one week post-implantation, two mice were euthanized every week to evaluate the time course of the tumor growth in the tibia. At 5 weeks post-implantation, remaining mice underwent hind limb amputation at the coxofemoral joint to completely remove the primary tumor. Micro-CT imaging of the tibias was performed, followed by routine histologic processing. At 12 weeks post-implantation, the mice were euthanized and the lungs harvested for histology. By 5 weeks post-implantation, 11 of 16 mice developed lytic and proliferative radiographic lesions in the proximal tibia and the diagnosis of osteosarcoma was confirmed by histology. At the 12 week time point, 10 of 11 mice had lung metastasis with a mean percent of affected lung of 33.4%. The successful growth of the osteosarcoma at the primary site in bone, followed by a relatively high rate of metastasis, demonstrates that this is a promising model that can be used to further characterize the disease and investigate therapeutic interventions. In addition, the model has been used to implant primary OSA tissues from canine patients successfully, demonstrating additional possibilities for utilizing the model to predict therapeutic responses or rate of metastasis in canine or human patients.

Keywords: osteosarcoma, metastasis, canine, mouse model
GENOTYPIC VARIATION OF *STAPHYLOCOCCUS AUREUS* IN DAIRY CATTLE

L. da Costa, P. Rajala-Schultz. Department of Preventive Medicine

*Staphylococcus aureus* (SA) still the most common cause of intramammary infection (IMI) suggesting that different sources other than the udder possibly play a role in the transmission. Skin colonization has been reported as an important risk factor. Studies have used Pulsed-field gel electrophoresis (PFGE) to characterize isolates from dairy cows. The aim of the study was to compare the genotypic relatedness of SA isolates from teat skin and milk of infected cows using PFGE. Quarter milk samples and teat skin swabs were collected from 12-15 cows in each of the four positive herds. A total of 228 teat skin samples were collected using sterile cotton swabs and 228 quarter milk samples were cultured on blood agar plates. These were incubated overnight at 37°C and growth identified as *SA*. PFGE was performed following the modified version of Mulvey et al. 2001. Results were analyzed by Bionumerics Software. *Staphylococcus aureus* was detected from 20 cows and 43 quarters. Of these quarters, ten were positive both for milk and skin, 18 only for milk and 15 only for teat skin. All twenty-eight SA milk isolates and twenty-five from teat skin were typed using PFGE. Three main clusters, A, B and C, were identified, using a cutoff at 80% similarity. All three contained isolates from both milk and teat skin. In general, 72% belonged to cluster B, with 58% originating from milk and 42% from teat skin. Clusters A and B contained isolates from all herds, while cluster C was exclusively restricted to one herd. The results of the current study indicate that within herds SA isolates from milk are indistinguishable from those on teat skin. Based on the genotypic similarity of isolates from milk and skin, our results reinforce the theory that teat skin can be an important source of SA causing IMI.

Keywords: *Staphylococcus aureus*, PFGE, milk, teat skin
BIOMECHANICAL COMPARISON OF 3 FIXATION CONSTRUCTS IN THE CANINE CADAVERIC CERVICAL VERTEBRAL COLUMN

B. Hettlich, M. Allen¹, D. Pascetta¹, G. Fosgate³, and A. Litsky². Departments of Veterinary Clinical Sciences, College of Veterinary Medicine¹, Orthopaedics and Biomedical Engineering, College of Medicine², Ohio State University, and Production Animal Studies³, University of Pretoria, South Africa.

Objective: To compare biomechanical stiffness of bicortical stainless steel pin to monocortical stainless steel and titanium screw/polymethylmethacrylate constructs in a cadaveric canine cervical spinal model.

Study Design: Biomechanical cadaver study.

Animals: Eighteen canine vertebral columns (C2-C7) were collected from skeletally mature dogs (weight range 22 to 32kg) euthanized for reasons unrelated to this study.

Methods: Specimens were radiographed to rule out prior injuries or deformities and sorted into three balanced groups on the basis of bone density values (by dual energy x-ray absorptiometry). Stiffness of the unaltered C4-C5 intervertebral articulation was determined in extension, flexion and lateral bending using non-destructive 4-point bend testing. Specimens were then stabilized according to group: 1) bicortical stainless steel pins/PMMA, 2) monocortical stainless steel screws/PMMA, and 3) monocortical titanium screws/PMMA. Mechanical testing was repeated and stiffness data from unaltered specimens and 3 treatment groups were compared.

Results: All 3 surgical methods significantly increased stiffness of the C4-C5 articulation compared to the unaltered specimen (p<0.001 for all treatments), but stiffness was not significantly different among the 3 fixation groups (p=0.578).

Conclusions: In this model, monocortical screw fixation (with stainless steel or titanium screws) was biomechanically comparable to bicortical fixation.

Clinical Relevance: Monocortical screw fixation with either stainless steel or titanium screws is a viable alternative to bicortical pin placement and may decrease the risk for neurologic complications associated with aberrant pin placement in clinical patients undergoing cervical vertebral column stabilization.

Keywords: Cervical vertebral column, dog, titanium, stainless steel, monocortical screws, bicortical pins
PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF METHICILLIN-RESISTANT \textit{STAPHYLOCOCCUS AUREUS} IN DOGS, CATS, AND HORSES AT A VETERINARY TEACHING HOSPITAL FROM 2007 TO 2010

J. Mathews$^1$, N. Tiao$^1$, P. Patchanee$^2$, W. Gebreyes$^1$

$^1$Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH

$^2$Veterinary Public Health Centre for Asia Pacific, Faculty of Veterinary Medicine, Chiang Mai University, Thailand

Prevalence of methicillin-resistant \textit{Staphylococcus aureus} (MRSA) in veterinary hospitals is variable. The Ohio State University Veterinary Medical Center and Infectious Diseases Molecular Epidemiology Laboratory instituted a passive surveillance system to improve hospital biosecurity and infection control, veterinary patient care, and overall public health. Aims include determining the frequency of MRSA, characterizing \textit{Staphylococcal Cassette Chromosome mec} (SCC\textit{mec}) types, and determining the level of clonality among \textit{S. aureus} isolates. Antimicrobial susceptibility profiles were determined for patient samples submitted to the Clinical Microbiology Laboratory (\(n = 4,684\)) using Kirby-Bauer disc diffusion and Sensititre. Multiplex PCR was used to identify the \textit{mecA} gene and SCC\textit{mec} types. Pulsed-field gel electrophoresis was used to assess clonality. Of the 132 \textit{S. aureus} isolates collected from canine (\(n = 83\)), equine (\(n = 33\)), and feline patients (\(n = 20\)), 124 isolates were available for genotypic analysis. Phenotypic (\(n = 62\)) and genotypic (\(n = 56\)) MRSA comprised 1.2-1.3\% of bacterial isolates. Samples were primarily from musculoskeletal tissue (35\%) or soft tissue injury/infection (30\%) in dogs, soft tissue injury/infection (70\%) in horses, and skin (45\%) in cats. Phenotypic oxacillin resistance was 53\% in dogs, 49\% in horses, and 26\% cats, while genotypic oxacillin resistance was found in 52\% of dogs, 44\% of horses and 18\% of cats. SCC\textit{mec} Type II predominated among canine and feline isolates, while equine isolates were mainly SCC\textit{mec} type IV. PFGE revealed a high level of clonality among isolates from apparently unrelated canine patients. Overall, frequency of MRSA was low. Canine and equine isolates had similar origin and level of oxacillin resistance but differed in SCC\textit{mec} types. Oxacillin resistance in cats was lower than in dogs and horses. It is unclear if the level of clonality indicates persistent or recurrent environmental contamination or reflects a feature of MRSA in the overall patient population.

Keywords: methicillin, \textit{Staphylococcus aureus}, oxacillin, \textit{mecA}, SCC\textit{mec}
VALIDATING METHODS FOR ISOLATING CONTEMPORARY INFLUENZA A VIRUSES FROM SWINE.  S. Nelson, A. Bowman, J. Nolting, J. Edwards, R. Slemons. Department of Veterinary Preventive Medicine

Surveillance for influenza A viruses in swine is crucial to elucidating the ecology and epidemiology of emerging influenza A viruses which may threaten human health. Isolation of swine-origin influenza A viruses from nasal swabs collected from pigs is routinely performed in either embryonated chicken eggs (ECE) or Madin-Darby canine kidney (MDCK) cells. The continuous genetic drift and frequent genetic reassortment seen in swine-origin influenza A viruses since 1998 requires repeated validation of detection methods with contemporary strains. The purpose of this study was to validate these virus isolation methods for use in an ongoing surveillance program at the swine-human interface. Based on previous studies, it was hypothesized that the use of MDCK cell culture would prove superior to ECE for the recovery of contemporary influenza A viruses from swine. During 2009, nasal swabs were collected from 221 clinically healthy pigs at 12 Ohio county fairs. Swabs were tested in parallel with two passages through MDCK cells, two passages through ECE, and RRT-PCR targeted at a conserved region of the influenza A virus genome. The results were analyzed with the McNemar chi-squared test. Subtype specific RRT-PCRs were used to determine hemagglutinin and neuraminidase combinations of the isolated viruses. Of the 221 samples, 40 (18.1%) were positive in MDCK cell culture, while only 13 (5.9%) were positive in ECE (p<0.0001). All samples positive in ECE were also positive in MDCK cell culture. Results from virus isolation in MDCK cells were in perfect agreement with results of the pan-influenza A virus RRT-PCR. Subtypes of the recovered isolates were consistent with viruses contemporaneously circulating in U.S. pigs. The use of MDCK cell culture is more appropriate than ECE for the isolation of contemporary swine-origin influenza A viruses, and thus is selected as the primary isolation method for current swine-origin influenza A virus surveillance.

Keywords: MDCK, cell culture, embryonated chicken eggs, pigs

It is well established that wild, free-ranging waterfowl, shorebirds, and gulls are natural reservoirs for antigenically diverse populations of influenza A viruses. Today, most significance given to wild bird-origin influenza A viruses stems from the accumulating evidence that incriminates this pool of viruses as the ancestral origins of influenza A viruses infecting domestic birds, lower mammals, and humans. Of the thousands of wild bird-origin influenza A virus isolates recovered from around the world over the last 40 years and which represent 16 of the 17 hemagglutinin and all 9 of the neuraminidase subtypes of influenza A viruses, only three possess the H14 hemagglutinin subtype. These three rare isolates were recovered in 1982 from two mallards and a herring gull sampled along the north coast of the Caspian Sea - the border region between Eastern Europe and Western Asia. During the fall waterfowl migration of 2010 our virus surveillance efforts in the Mississippi Migratory Bird Flyway resulted in the recovery of three additional H14 influenza A virus isolates, two from long-tailed ducks (Clangula hyemalis) and one from a white-winged scoter (Melanitta fusca) sampled in Wisconsin. Genomic sequence analysis confirmed the antigenic subtyping results. Ongoing sequence analysis will hopefully provide further insight into where and how the H14 genomic segment persisted and provide clues for identifying the natural reservoir host(s) for these rarely detected viruses. This surprising recovery of three H14 influenza A virus isolates in Wisconsin demonstrated that current concerns over inter-continental spread of a rare wild bird-origin influenza A virus genomic segment is justified and that temporal and spatial gaps clearly exist in the general understanding of the natural history of the H14 AIVs. Finally, these findings reinforce the position that other ecological niches in the natural history of influenza A viruses may be going undetected.

Key Words: H14, type A influenza viruses, inter-continental, sea ducks
ENVIRONMENTAL FACTORS THAT AFFECT THE BEHAVIOR AND WELFARE OF DOMESTIC CATS (FELIS SYLVESTRIS CATUS) HOUSED IN CAGES.  

aJ. Stella, bC. Croney, PhD, cT. Buffington, DVM, PhD.  

aVeterinary Preventative Medicine, cVeterinary Clinical Sciences, The Ohio State University, Columbus, Ohio  
bAnimal Sciences, Purdue University, West Lafayette, Indiana

To improve the behavior and overall welfare of cats housed in cages in shelters, veterinary hospitals or laboratories, it is important to understand how to optimize their environments. The aim of this study was to examine the effects of the macro- (room) and micro- (cage) environments on cat behavior. Seventy six cats were singly housed in cages at the Ohio State University Veterinary Medical Center vivarium for 48 hours. Cats were randomly assigned to one of four treatment groups: M+m+, M+m-, M-m+, M-m-. (M+) was a managed macro-environment (minimal noise, disruption, consistent schedule); (m+) was an enriched micro-environment (hide area, perch, consistent cage set-up); (M-) was an unmanaged macro-environment (recorded dog barks, frequent disruptions, unpredictable schedule); (m-) was an unenriched micro-environment (no hide area or perch, inconsistent cage set-up). Cats were observed for 8 hrs daily for maintenance behaviors (e.g., eating, drinking, elimination), agonistic and avoidant behaviors (e.g., growling, hissing, hiding) and affiliative behaviors (e.g., soliciting caretaker interaction) using hourly scan sampling and 5 minute focal sampling. A stranger approach test was conducted at the end of day two. Data analysis by 2-way ANOVA revealed fewer (P <0.0001) and shorter duration (P<0.0001) agonistic behaviors in M+ vs. M- whereas m+ vs. m- revealed more avoidant behaviors (P<0.0001) of longer duration (P<0.0001). Food intake was higher in M+ vs. M- and m+ vs. m- (both P<0.0001). Unpaired t-tests of the effect of treatment during the approach test revealed shorter latency to interact (P=0.03), longer duration of interaction (P=0.03) and more cats approaching (P=0.008) in M+ vs. M-. These preliminary results suggest that the macro-environment appears to be at least as relevant to the cat as the micro-environment. This indicates that attending to cage enrichment without consideration for the effects of the room may be insufficient to protect captive cat welfare.

Key words: Cats, behavior, environment, enrichment
RESEARCH PATHOLOGY SUPPORT FOR EXPERIMENTAL ANIMAL MODELS PROVIDED BY THE COMPARATIVE PATHOLOGY & MOUSE PHENOTYPING SHARED RESOURCE. K. La Perle and B. Bolon. Department of Veterinary Biosciences.

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

Keywords: animal model, genetically engineered mice, histology, pathology, pre-clinical
The Clinical Trials Office (CTO) at The Ohio State University College of Veterinary Medicine is the largest and most comprehensive center for clinical trials involving client-owned animals in the country. The CTO works closely with the Ohio State Center for Clinical and Translational Sciences, Ohio State Comprehensive Cancer Center, and Nationwide Children’s Hospital as well as pharmaceutical companies across the country to bring advanced diagnostic techniques and new treatments for animals with a variety of diseases, with the ultimate goal of translating these findings into human medicine.

The CTO provides assistance in the design, execution, and evaluation of veterinary clinical trials of client-owned animals, as well as familiarizes those involved in the principles of Good Clinical Practice/Good Laboratory Practice (GCP/GLP) guidelines. The CTO facilitates the conduct of such studies through input into clinical trial design, networking with regional veterinarians to insure timely enrollment, assistance in collection and coordination of data, and establishment of standard operating procedures (SOPs).

There are several advantages for patients and clients to participate in a clinical trial. In many cases all of the costs associated with the study are covered. Clinical trials frequently provide a financial credit at the Veterinary Medical Center for future treatments and patients enrolled in clinical trials often receive advanced therapies not currently available. The CTO has established and maintained a network of regional specialists and veterinarians to assist with patient enrollment, through newsletters and periodic updates. Plans are also underway to set up satellite locations in Cincinnati, Cleveland, and Pittsburgh areas to enhance recruitment into various studies. Clients may participate knowing that these studies provide critical new information that helps advance the treatment of humans with similar diseases.

Over the past three years the CTO has supervised more than 60 clinical trials involving over 1500 client-owned animals. These studies have provided an unparalleled opportunity for animals and their owners to participate in state-of-the-art treatments that have helped to shape the future of both veterinary and human medicine. The ability to perform effective and well-executed clinical trials also enhances the regional, national and international recognition of the OSU CVM as a center for veterinary research, contributing to the advancement of both veterinary and human health.

Keywords: clinical trials, treatment, veterinary medicine, human health
VETERINARY BIOSPECIMEN REPOSITORY (TISSUE BANK)
H.L. Borghese, N. Ruffin, W.C. Kisseberth, C.A. London, M.L. Wellman. Departments of Veterinary Biosciences and Veterinary Clinical Sciences

The Biospecimen Repository (BR), also known as a tissue bank, collects normal and affected tissues from animals presented to the OSU Veterinary Medical Center and stores these for use by investigators across the OSU and Nationwide Children’s Hospital communities. These samples consist of tumors, blood samples, urine, etc that are critical in helping researchers to understand the molecular biology of diseases in animals. Such banking efforts in human medicine have been critical to the recent advances in the treatment of cancer and heart disease, among others, and will likely impact veterinary medicine in the same manner. The overriding purpose of the BR is to assist researchers is translating findings of regarding the biology of disease in animals and humans into novel therapeutic approaches to treat these diseases.

The BR has been providing samples to researches at OSU and Nationwide Children's Hospital over the past 2 years resulting in the identification of new targets for therapy in osteosarcoma, melanoma and lymphoma common to both the human and canine diseases. As a result, these efforts have generated several new grant/funding opportunities that will ultimately benefit human and animal patients.

The banking effort at OSU is supported by the National Cancer Institute’s Comparative Oncology Program that selected the OSU CVM as one of three veterinary institutions to initially begin populating a multi-institutional tissue bank that will soon be available to researchers nationwide. The BR represents a remarkable resource that will assist investigators as they strive to develop new prevention and treatment strategies for both animals and people with a variety of illnesses.

Keywords: tissue bank, samples, animals, humans, diseases
ECHOCARDIOGRAPHIC ASSESSMENT OF RIGHT VENTRICULAR FUNCTION IN 150 DOGS WITH PULMONIC STENOSIS. A.T. Amburgy, and B.A. Scansen.
Department of Veterinary Clinical Sciences

Congenital pulmonary valve stenosis (PS) is among the most common congenital heart defects of the dog. Dogs with pulmonic stenosis commonly progress to signs of lethargy, collapse, right heart failure, or sudden death. The purpose of this study was to retrospectively evaluate cases of PS to determine signalment, disease severity, treatment, and to evaluate the feasibility of echocardiography to estimate RV function. 203 cases of PS were screened, of which 150 fit the entry criteria. A single trained observer measured each echocardiogram and averaged the following variables over 3-4 cardiac cycles: the maximum trans-pulmonary pressure gradient (PGmax), the RV freewall thickness in diastole (RVFWd), the RV fractional shortening (%FSRV), the RV fractional area change (%FACRV), and the tricuspid annular plane systolic excursion (TAPSE). The English Bulldog was the most common breed, comprising 17% of the cases. 71 dogs underwent balloon pulmonary valvuloplasty (BPV), while 93 dogs were started on medical therapy with a beta-adrenergic blocker (BB). Dogs receiving BB did not show a significant change in PGmax, while dogs that had BPV showed a significant decrease in PS pressure gradient. 78 dogs had sufficient images to calculate variables of RV systolic function. The mean %FSRV was 38% ± 15%, the median %FACRV was 63% (range, 37% to 82%), and the mean TAPSE was 1.7 cm ± 0.5 cm. There was no correlation between %FSRV, %FACRV, or TAPSE and the PGmax. The %FSRV and %FACRV were positively correlated (r = 0.66); however, neither were correlated to TAPSE. There was a weak correlation (r = 0.41) between RVFWd and PGmax.

Echocardiography to assess the severity of PS showed that BPV provided a significant reduction in pulmonary gradient, while BB did not. Echocardiographic estimates of RV function were obtainable, but not well-correlated. Prospective studies evaluating RV function in dogs with PS are warranted.

Keywords: pulmonic stenosis, balloon valvuloplasty, echocardiography
Encouraging clients to bring cats to the veterinarian more frequently is an important means by which to expand feline health and welfare, veterinary practice effectiveness and profitability.\(^1\) Anecdotally, one reason owners avoid veterinary care is the stress involved with carrier transport of cats\(^2\) although to our knowledge no controlled study on this subject has yet been reported. The purpose of this study was to determine if carrier training and/or simulated car rides habituate cats to transport and therefore increase the ease of getting the cat into the carrier and/or reduce stress-related behaviors upon presentation at the clinic. A randomized trial of 20 cats owned by veterinary faculty students and staff showed that after completing the basic carrier training protocol, both the difficulty of getting a cat into the carrier and the time until the cat was inside decreased significantly. Stress-related behaviors upon presentation to the clinic were not substantially decreased, and the introduction of simulated car rides into the protocol increased entry time and difficulty of carrier entry. Therefore this study shows that the basic protocol in the absence of simulated car rides can allow owners to train their cats to more easily enter carriers for transport to the vet.

Keywords: Cat, Carrier, Transport
In recent years, the use of frozen semen has gained increasing popularity in dog breeding. However, not all sperm will be able to withstand the stress put on it during the freezing and thawing process. All current protocols include removal of seminal plasma as a critical step in semen preparation as its presence is widely known to negatively affect sperm survival. The most commonly used method is centrifugation, which can in itself have a negative effect on sperm. A new filtration method for removal of seminal plasma has been developed. This study was designed to determine whether filtration would be superior to the traditional centrifugation method. To evaluate this, eight ejaculates (two from each of four dogs) were split into two equal aliquots and a different method was used for each. The viability and motility of the sperm were assessed before and after treatments, and then again after thawing. There was no significant difference in any of the measured sperm parameters of recovery, total motility, progressive motility, or viability as a result of method. Only progressive motility experienced a significant decrease after treatment, but this occurred in both centrifuged and filtered samples. Both filtered and centrifuged samples experienced a significant decrease in progressive and total motility, and viability from pre-freeze to post-thaw. However, there was not a significant change in motility from time 0 post-thaw to time 30 post-thaw. There was no significant difference in the method’s impact on the sperm success over time. A difference was considered significant if \( P < 0.05 \). Filtration was found to be statistically equivalent to centrifugation, yet with further research, filtration could yet be proven useful for dogs whose semen does not withstand centrifugation.

Keywords: Filtration, Centrifugation, Semen, Cryopreservation, Seminal Plasma

a The Ohio State University, College of Veterinary Medicine, Department of Clinical Sciences
b University of Florida, College of Veterinary Medicine, Department of LACS

Serum amyloid A (SAA) is a major acute phase protein and its concentrations have been shown to increase quickly and with larger amplitude in response to inflammation than other acute phase proteins (e.g. fibrinogen) in horses. Moreover, SAA concentrations return to baseline levels shortly after resolution of the inflammatory process. Therefore, our hypothesis was that onset of placentitis would result in a rapid increase in SAA in pregnant mares. Moreover, a significant decline in SAA following treatment would correlate with a positive outcome of pregnancy (i.e. live foal). Our objectives were: 1) to determine if placentitis affects SAA concentration; 2) to determine changes in SAA relative to initiation of treatment; and 3) to determine the correlation between changes in SAA concentration and the outcome of pregnancy. Mares were inoculated with *Streptococcus equi* subspecies *zooepidemicus* at 280-295 days of pregnancy and randomly allocated to control (5) and treatment (9) groups. Mares were monitored daily by physical exam and ultrasonography per rectum for 7 days following inoculation and then three times per week until abortion or delivery. Blood samples were collected prior to inoculation and then at the time of physical examination. Treatment was administered upon the onset of clinical signs. SAA levels were determined using a commercially available ELISA assay. SAA levels increased approximately 2 to 4 days after inoculation in most mares. All control mares aborted after the rise in SAA levels. Therapy was effective (P<0.05) in preventing the rise in SAA in 66% (6/9) of mares; and only 1 out of 3 mares with elevated SAA aborted. A decrease in SAA concentration coincided with a decrease in clinical signs in treated mares which lead to positive outcomes of pregnancy. In summary, SAA concentrations could potentially be used to monitor the efficacy of treatment in cases of placentitis in mares.

Keywords: equine, placentitis, serum amyloid A, ELISA, inflammation
Cranial cruciate ligament (CrCL) disease is a common orthopedic problem in dogs that incurs costs of more than $1 billion annually to pet-owners. Surgical repair of CrCl disease is frequently performed and usually preceded by arthrotomy or arthroscopy. Meniscal injury can occur secondary to cranial cruciate ligament disease in up to 70% of cases, due to residual instability after CrCl surgery. Meniscal release is commonly performed during CrCl repair to allow the medial meniscus to adapt to the pressure of the femoral condyle during tibial translation. This study compared three different approaches to medial meniscal release by: objective evaluation of complete vs. incomplete meniscotibial ligament transection, objective measurement of iatrogenic articular cartilage damage, and subjective evaluation of iatrogenic ligament and meniscal damage. We hypothesize that medial meniscal release efficacy and damage to the articular surface and surrounding soft tissue structures is significantly different following three different methods of transection of the caudal meniscotibial ligament. Animals included in this study weighed 20 to 40 kg, were skeletally mature, and showed no signs of notable orthopedic disease. Twenty-six limbs were included in the study. Each limb was randomly assigned to one of three experimental groups: (I) arthrotomy, retraction with a Hohmann & meniscal release with an #11 scalpel blade, (II) arthroscopy, retraction with a Hohmann & meniscal release with a hook knife, (III) arthroscopy, no retraction & meniscal release with a hook knife. The iatrogenic damage to the articular surface of the femur was analyzed using image analysis software (BioQuant®). Completeness of meniscal transection and iatrogenic damage was compared between the groups using a Fisher's Exact test. Cartilage damage was compared between groups I and II and between groups II and III using t-test. Data analysis was completed using SAS software. The use of a Hohmann retractor resulted in a significant increase in the damaged articular cartilage area of specimens in group II, compared to no retraction in group III. No other significant differences were found. Arthrotomy is more invasive and offers less visualization of the caudal aspect of the stifle joint resulting in more notable damage to the joint surface and soft tissue structures in the vicinity. Damage to the caudal cruciate was not expected and can significantly impact joint stability. This damage could be a consequence of meniscotibial ligament transection or cranial cruciate transection. Due the anatomical location of the meniscotibial ligament and its proximity to the tibial attachment of the caudal cruciate ligament, likely the caudal cruciate ligament was damaged while the meniscotibial ligament was transected.

Keywords: arthroscopy, arthrotomy, meniscal release, cruciate disease
EFFECTS OF PREADOPPTION COUNSELING FOR OWNERS ON SEPARATION ANXIETY IN SHELTER DOGS. M. Herron, L. Lord, and S. Husseini. Depts. of Veterinary Clinical Sciences (Herron) and Veterinary Preventive Medicine (Lord).

The efficacy of preadoption counseling in the education and prevention of separation anxiety problems was tested in a prospective, randomized, parallel-group study. Participants included 133 new owners of dogs 6 months of age and older. Prior to adoption, 66 of these owners were chosen at random, according to a computerized random number generator, to receive five minutes of counseling on separation anxiety. The remaining 67 owners that did not receive counseling served as the control group. A follow-up survey was conducted one month post adoption. 19 dogs in the total population were reported to have separation anxiety. There was no significant correlation for counseling to decrease the incidence of separation anxiety. Data shows that owners in both groups were performing most recommendations given during counseling. Of the 6 dogs relinquished, 3 were returned with the primary complaint of having separation anxiety. Dogs that were reported to have separation anxiety were more likely to show nervous or panicked behavior as the owner prepared to leave (p=0.00) and signs of neediness (p=0.031). Individual symptoms of separation anxiety such as destructive behavior, house-soiling, barking, and escaping had no significant variations between the two groups. Having another dog in the home was not protective for separation anxiety. There was a slight trend for putting food in a toy to be protective of separation anxiety(p=0.129). Owners in the treatment group were more likely than control to put food inside a toy (p=0.00); this suggests that preadoption counseling was implemented by the owners in the home. Owner compliance supports the idea that counseling is a useful tool for owners. Further investigation should be done to find more specific, effective prevention tools for owners to use in the home to minimize the development of separation anxiety.

Keywords: Separation anxiety, behavior, shelter dogs, relinquishment, owner education

Type 1 diabetes mellitus (T1DM) is one of the most frequently diagnosed endocrinopathies in dogs, requiring life-long management. Pancreatic islet transplantation, a novel method of cell-based therapy for treatment of human diabetics, is the only non-invasive, curative treatment for T1DM. Our goal was to establish a canine pancreatic islet isolation method yielding sufficient islet mass for clinical transplantation in a medium-sized (20kg) dog (6,000-10,000 islets/kg). We hypothesized that clinically acceptable islet yield and purity could be achieved using canine cadaveric donors and standard centrifugation equipment. Pancreata were procured from canine cadavers euthanized for reasons unrelated to this study. Collagenase digestion was performed using a Ricordi chamber and temperature-controlled perfusion circuit. Islets were separated from the exocrine tissue using a discontinuous density gradient and standard laboratory centrifuge. After isolation, islet yield was calculated and viability was assessed using dual fluorescent staining techniques. Islet isolation was completed in 6 dogs. Median (interquartile range) islet yield was 36,756 IEq (28,527 IEq) per pancreas. A high degree of islet purity [87.5%(10%)], and viability [87.4%(12.4%)] were achieved. The islet yield achieved using this technique would be adequate for transplantation into a 6kg dog. Larger dogs would require use of multiple pancreatic donors. Purity and viability of the isolated islets was excellent and would be sufficient for clinical transplantation. Based on initial results, clinically relevant islet yield and quality can be obtained from canine cadavers using standard laboratory equipment.

Keywords: pancreas, canine islets, digestion, isolation, Ricordi chamber
HEMATOLOGY AND CHEMISTRY REFERENCE RANGES IN RACING GREYHOUNDS.  K. Kontur¹, OSU CVM Class of 2014; G. Couto¹, DVM, DACVIM; L. Bohenko², DVM; S. J. Horvath¹, K. Yant¹, J. Chase³, BS; M. Frye³, MS, DVM; D. DeNicola³, DVM, PhD, DACVP.
¹The Ohio State University College of Veterinary Medicine; ²The West Virginia Racing Commission; ³IDEXX Laboratories.

Greyhounds differ from other dog breeds in many of their hematological values. Previous studies using mostly retired racers document high HCT, hemoglobin concentration, and erythrocyte counts, and low leukocyte and platelet counts in Greyhounds. High BUN and creatinine concentrations and low total protein and globulin concentrations have also been reported.

In this study, we collected blood samples via jugular venipuncture from 125 actively racing Greyhounds at the Wheeling Island Racetrack in West Virginia. Samples in EDTA were analyzed using a ProCyteDx (IDEXX Laboratories), and chemistry samples (heparinized plasma) were analyzed using an Olympus Analyzer. To generate reference intervals, values outside of the 3% IQR were deemed outliers and were excluded from analysis. Reference intervals were generated using 5th and 95th percentiles. Results are displayed below.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>4.7-10.9 X10⁹/L</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>0-0.5%</td>
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<tr>
<td>Albumin</td>
<td>2.9-3.8 G/dL</td>
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<tr>
<td>GGT</td>
<td>0-7 U/L</td>
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<td>Lymphocytes</td>
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<td>MCV</td>
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<tr>
<td>ALP</td>
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<tr>
<td>Glucose</td>
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<tr>
<td>Monocytes</td>
<td>0.2-0.7 X10⁹/L</td>
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<tr>
<td>MCHC</td>
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<tr>
<td>Total bilirubin</td>
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<tr>
<td>Phosphorus</td>
<td>2.5-4.9 mg/dL</td>
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<tr>
<td>Neutrophils</td>
<td>2.7-6.4 X10⁹/L</td>
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<tr>
<td>MCH</td>
<td>22.4-24.5 pG</td>
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<tr>
<td>BUN</td>
<td>17-39 mg/dL</td>
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<tr>
<td>Protein</td>
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<tr>
<td>Eosinophils</td>
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<tr>
<td>RDW</td>
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<td>Calcium</td>
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<td>Basophils</td>
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<tr>
<td>Platelets</td>
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<td>Bicarbonate</td>
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<td>ALT</td>
<td>40-106 U/L</td>
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<tr>
<td>HCT</td>
<td>55.7-66.9%</td>
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<tr>
<td>MPV</td>
<td>8.9-11.5 fL</td>
</tr>
<tr>
<td>Chloride</td>
<td>109-116 mEq/L</td>
</tr>
<tr>
<td>Sodium</td>
<td>144-152 mEq/L</td>
</tr>
<tr>
<td>RBC</td>
<td>8.0-9.8 X10⁹/L</td>
</tr>
<tr>
<td>PCT</td>
<td>0.08-0.2%</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>84-166 mg/dL</td>
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<tr>
<td>Potassium</td>
<td>4-5.2 mEq/L</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>18.8-22.8 G/dL</td>
</tr>
<tr>
<td>PDW</td>
<td>9.2-13.9%</td>
</tr>
<tr>
<td>CK</td>
<td>92.7-381 U/L</td>
</tr>
<tr>
<td>Anion gap</td>
<td>12-24 mEq/L</td>
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<tr>
<td>Reticulocytes</td>
<td>5.9-41.5 X10⁹/L</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.1-1.8 mg/dL</td>
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</table>

Keywords: greyhounds, hematology
THE EFFECTS OF AUTOLOGOUS PROTEIN SOLUTION ON LAMENESS SCORES AND FORCE PLATE GAIT ANALYSES IN EQUINE OSTEOARTHRITIS. K. Lewis, A. Ishihara, BVSc, PhD, L. Zekas DVM, ACVR, AVBP, A.L. Bertone, DVM, DACVS, PhD. Dept. of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University

Advances in regenerative medicine currently focus on blood products to aid healing of certain diseases, including equine osteoarthritis (OA). The objective of this randomized, placebo-controlled, double-blinded, prospective clinical trial using client-owned horses with naturally-occurring OA was to assess the potential improvement of lameness using an autologous protein solution (APS). APS is created by mixing platelet-rich plasma (PRP) with polyacrylamide beads that absorb water, effectively concentrating platelets, WBC, plasma and soluble proteins. APS has greater concentrations of platelet and plasma-derived growth factors and cytokines compared to PRP (O’Shaughnessey 2011; Woodell-May 2011; Eppley 2004), and thus may have superior anti-inflammatory and anabolic effects in joints with OA. Forty horses with naturally-occurring OA received an intra-articular injection of APS (n=20) or saline (n=20) at Day1, were evaluated by subjective lameness grading by a blinded evaluator (ALB) and objectively by force plate gait analyses at Day0, Day7 and Day14. Horses were exercised twice weekly by treadmill. The APS group showed significant improvement in subjective lameness scoring at Day7 and Day14 compared to the saline group, and from baseline (Day0). The APS group also showed significantly lower Asymmetry Index of Vertical Force Peak (AI-VFP) at Day7 and Day14 compared to the saline group and Day0, which corresponded to improvement in lameness. The AI-VFP values were significantly higher in the saline group, which shows a worsening lameness with no treatment. Lameness scoring and force plate data had significant positive correlation and validated using force plate analyses. These results suggest intra-articular APS therapy may improve lameness caused by OA without observed short-term detrimental effects. Supplemental data obtained in conjunction with these lameness data focused on the safety of APS by measuring CBCs, venous blood profiles, joint fluid cytology and joint fluid cytokine analyses. Advances in regenerative medicine biotherapies may hold promise for debilitating orthopedic diseases like OA.

Keywords: Equine, osteoarthritis, regenerative medicine, APS, subjective, objective, lameness score, intra-articular injection
The objective of this study was to compare the serum concentrations of total calcium, phosphorus, and vitamin D (25-hydroxyvitamin D3; 25-(OH)D3) in healthy horses and foals in Thailand and the USA. In Thailand, blood samples were collected from ponies (n=21; 1.5 months -20 years old) in Chiang Rai, and from horses (n=46; 2 days-15 years old) in Kanchanaburi. In the USA, samples were collected from adult horses (n=17; 5-12 years old) and newborn foals (n=10; <1 week old) in Ohio and Kentucky. Equids in Thailand were stratified by age (foals, yearlings, and >3 years old) within each location. Serum 25-(OH)D3 concentrations in both countries were measured using a 25-(OH)D3 ELISA. Calcium and phosphorus concentrations were measured by colorimetry. Data was normally distributed and comparisons between age groups and geography were carried out via unpaired two-sided t-tests and one-way ANOVA (Prism 4.0).

In Kanchanaburi, foals had significantly lower concentrations of 25-(OH)D3 than adult horses (P<.0001) and yearlings (P<.0001). There was no difference in 25-(OH)D3 between adults and yearlings. Total calcium concentrations were significantly higher in yearlings than in adults (P=.024), but no significant differences were found between adults and foals nor between yearlings and foals. Phosphorus concentrations were significantly higher in foals than yearlings (P<.0001) and adults (P<.0001), and higher in yearlings than adults (P=.0035). In Thailand, horses >3 years old had significantly higher 25-(OH)D3 concentrations than ponies of similar age (P<.0001). In the USA, serum 25-(OH)D3 was significantly lower in foals than horses (P=.0047). No differences were detected between adult equids from the USA and Thailand.

This is the first time vitamin D has been measured and reported in the same season, in similar equine populations, at different latitudes. This work supports that, regardless of geography, there are age differences in vitamin D in horses and ponies. Research to explain these differences is ongoing.

Keywords: equine, Vitamin D, Calcium, Phosphorus, Thailand
EVALUATION OF THE ARTICULAR ANTI-INFLAMMATORY EFFECTS OF INTRA-ARTICULAR AUTOLOGOUS PROTEIN SOLUTION IN HORSES WITH OSTEOARTHRITIS.  R. Schwarze, A. Ishihara, A. Barnaba, L. Zekas, M. Wellman, A. Bertone. Department of Veterinary Clinical Sciences, The Ohio State University, College of Veterinary Medicine, 1900 Coffey Road, Columbus, Ohio 43210

Osteoarthritis (OA) has been implicated as the most prevalent cause of reduced performance in the athletic horse. Interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNFα) are the main pro-inflammatory cytokines elevated in OA. Interleukin-1 receptor antagonist (IL-1RA) is an anti-inflammatory cytokine that decreases as severity of OA increases. Down regulation of one inflammatory cytokine can lead to up-regulation of another, making OA a disease refractory to a single treatment. Autologous Protein Solution (APS) (Biomet, Inc.) is a therapeutic blood product with a greater concentration of platelets, plasma-derived growth factors, and cytokines compared to conventional platelet-rich plasma. Research indicates APS has been successful in preventing cartilage breakdown in humans by increasing multiple anti-inflammatory cytokines. The presence of IL-1RA in APS can block IL-1β induced TNFα production, thus we chose to measure all three cytokine concentrations in synovial fluid of horses with OA before and after treatment with APS (IL-1β, TNFα, and IL-1RA). The purpose of this study was to evaluate synovial inflammation and pain by correlating changes in synovial cell counts, cell differentiation, proteins, and cytokines, as well as joint soreness, before and after intra-articular APS treatment. We hypothesized APS treatment of horses with OA of a high motion joint would improve clinical signs of joint soreness and synovial fluid composition in comparison to the saline-control group. After treatment, pain-free range of joint motion and pain score on flexion improved significantly in the APS group compared to the control group by a blinded observer. Joint circumference was not significantly different between APS and saline-injected groups. The joint fluid total protein concentration was significantly elevated in the placebo group on day 14 compared to day 0 and correlated to worsening clinical signs of joint soreness. Total synovial fluid WBC count and % neutrophils did not significantly differ between groups. Synovial fluid concentrations or ratios of IL-1β, TNFα, and IL-1RA did not vary significantly between groups at baseline or following treatment. Although signs of joint soreness improved with APS treatment, and synovial protein worsened in the placebo group, this did not correspond to changes in synovial fluid mediators of inflammation and cartilage degradation. Intra-articular APS therapy can be considered an alternative treatment option for equine OA based upon improvement in pain-free range of joint motion and pain score on flexion.

Keywords: Equine, osteoarthritis, autologous protein solution, blood product, cytokines, synovial fluid
USE OF A BLOOD BIOMARKER FOR PAINFUL BLADDER SYNDROME/INTERSTITIAL CYSTITIS. Sesemann, K L, Rodriguez-Saona, R E, Food Science and Technology and Buffington C. A. T Veterinary Clinical Sciences

Painful bladder Syndrome/Interstitial Cystitis (IC) is a chronic visceral pain syndrome of cats and humans. No reliable diagnostic tests for IC currently are available. We recently have identified a biomarker in the blood of patients with IC. This study examined different methods for preparation of blood spots to enhance differentiation between cats with IC and healthy controls. Blood spots were collected from 17 healthy control cats and 12 cats with confirmed IC. Punch samples from the bloodspot cards were extracted with buffer and filtered using a 10 kDa filter. Infrared spectra were obtained from the filtrate and analyzed using the Soft Independent Modeling by Class Analogy (SIMCA). The SIMCA model was able to successfully classify IC cats versus Healthy Control cats with an interclass difference was 21.3, compared to 3.4 for spots analyzed without filtration. The classification required information from 1200-1450 cm\(^{-1}\) of the spectral region to discriminate between IC and healthy. The highest spectral peaks were identified at 1294 cm\(^{-1}\), 1344 cm\(^{-1}\) and 1419 cm\(^{-1}\), most likely associated with amino acid components. These results indicate that use of filtered samples can improve the reliability of differentiation of cats with IC from healthy cats.

Keywords: Painful bladder Syndrome, Interstitial Cystitis, Infrared Microscopy, Blood spot
EFFECT OF ASSISTED AND UNASSISTED BIRTHS ON COW BEHAVIOR AT CALVING IN HOLSTEIN DAIRY COWS. M. Titler, S. Bas and G.M. Schuenemann. Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University

Dystocia increases the incidence of stillbirth and calf mortality within 30 days post-calving. Effects on the dam include increased incidences of trauma, uterine disorders, decreased milk yield, and reduced reproductive performance. The objective of the present study was to assess the effect of calving ease (assisted or unassisted births) on cow behavior 48 hours prior to- and after calving. A total of 37 Holstein cows from 1 commercial dairy herd where used. Electronic data loggers (IceQuebe, Scotland, UK) were placed on periparturient dairy cows at approximately 10 days prior to the expected calving date and removed 10 days after birth. The calving ease of cows [assisted (n=5) or unassisted (n=32)] and calving date and time were recorded. The number of steps (min), number of lying bouts, and mean duration of lying/standing bouts (min) were recorded. Data were analyzed using MIXED procedures of SAS. Overall, the mean duration of lying bout during labor stage was 40.4 min for unassisted and 60.8 min for assisted cows. Cows with assisted births spent more time (min/d) standing 24 hours prior to and after calving than unassisted cows. Cows with assisted births had fewer steps and lying bouts, spent more time standing with lying bouts of longer duration. These findings provided evidence that cows experiencing difficult births showed distinct behavior 24 hours prior to calving. The use of electronic data loggers to monitor cow activity may allow calving personnel to identify those cows at risk for dystocia 24 hours around the clock; thus, improving the overall calf-cow survival and welfare.

Keywords: Dystocia, calving, behavior, dairy cow
ACID-BASE AND ELECTROLYTE CONCENTRATIONS IN GREYHOUNDS AFTER RACING. K. Yant¹, C. G. Couto¹, L. Bohenko², S. Horvath¹, K. Kontur¹, L. Marin¹, J. Chase³, M. Frye³, D. DeNicola³;
¹The Ohio State University College of Veterinary Medicine, Department of Veterinary Clinical Sciences; ²The West Virginia Racing Commission; ³IDEXX Laboratories.

Greyhounds experience profound changes in homeostasis immediately after a race. We analyzed venous blood gas, acid base, and electrolyte concentrations before racing (A), immediately after racing (B), and one hour after racing (C) using a VetStat Electrolyte and Blood Gas Analyzer (IDEXX laboratories, Westbrook, ME).

We collected blood samples (N=37) from the external jugular vein using a 20 G Vacutainer butterfly into heparinized tubes and analyzed them immediately.

All parameters measured by the analyzer had statistically significant differences between times A, B, and C (p<0.0001). The anion gap (A, 19.8±2.0; B, 43.5±2.7; C, 22.0±2.9 mEq/L), Na+ (A, 156±1.5; B, 167.4±3.1; C, 155.3±1.7 mEq/L), K+, (A, 4.5±0.5; B, 4.6±0.4; C, 4.2±0.2 mEq/L), and Cl- (A, 115.5±2.2; B, 121.6±2.2; C, 116.2±1.5 mEq/L) increased post-race, then decreased at 1 hour post-race; while the pH (A, 7.4±0.04; B, 7.16±0.05; C, 7.45±0.02), pCO2 (A, 43.4±4.3 mmHg; B, 20.7±3.3; C, 33.5±2.2 mmHg), HCO3- (A, 25.4±1.3; B, 6.8±1.2; C, 21.6±1.5 mEq/L), and tCO2 (A, 26.7±1.3; B, 7.4±1.3; C, 22.6±1.5 mmHg) decreased post-race, then increased at 1 hour post-race. All the parameters returned to the reference range at 1 hour post-race.

The racing Greyhound has a unique ability to reestablish homeostasis shortly after a race. All the values returned to the pre-race range after just 1 hour post-race. We hypothesize that the rapid buffering is primarily carried out by hemoglobin. The VetStat was a user friendly point-of-care device that can be easily used in the racetrack.

Keywords: Racing Greyhounds, blood gas, acid base, electrolyte values
Hypertrophic cardiomyopathy (HCM) is the most common heart disease in cats with little documentation of the effects of treatment on outcome in cats with preclinical disease. Therefore, this prospective study was undertaken to evaluate the effects of treatment with atenolol on outcome in cats with asymptomatic HCM. We hypothesized that administration of atenolol would reduce 5-year mortality.

Cats were enrolled over a 5-year time period (2003 to 2007) in a prospective, open-label, observational study. Diagnosis of HCM was based on transthoracic echocardiography. Cats were either treated with atenolol (6.25 or 12.5 mg/kg, q12h, PO) or did not receive treatment. Baseline echocardiograms were analyzed, and morbidity and mortality were monitored at 3 and 6 months and thereafter by annual rechecks and phone interviews (rDVMs and owners) until reaching study end point. Groups were compared by standard statistical procedures. Kaplan-Meier survival curves were constructed and compared using the Log-Rank test with right censoring. Predictors of 5-year survival were identified by multivariate logistic regression analysis.

63 cats with occult HCM (42 treated, 21 not treated) and 31 matched healthy control cats were identified. 27 (43%, 14 cardiac death, 13 non-cardiac death) HCM cats and 10 (32%, all non-cardiac death) control cats reached the study end-point (death). Statistical analyses (HCM vs. control; HCM treated vs. HCM not treated; HCM without obstruction treated [n=16] vs. HCM without obstruction not treated [n=17]) failed to demonstrate a significant difference (P>0.05) in 5-year all cause and cardiac mortality between groups. Only age and left atrial size at diagnosis were independent predictors of outcome but not treatment with atenolol, severity of hypertrophy, presence of diastolic dysfunction, and presence of dynamic outflow tract obstruction (all P > 0.10).

This study did not demonstrate any beneficial (or detrimental) effect of treatment with atenolol on 5-year outcome in cats with occult HCM.

Keywords: Feline, Heart disease, HCM, Echocardiography, Outcome

The dry period is critical for the health and production of the cow in the next lactation. The most common method to dry-off dairy cows is abrupt cessation of milking. Previous studies have indicated reducing milking frequency alters lying behavior because of milk accumulation, but have not examined abrupt cessation of milking. The objective of the present study was to evaluate if abrupt cessation of milking was associated with alterations in dairy cow lying behavior. Lying behavior is an important and objective indicator of the health and welfare of cows.

Eighteen cows in three dairies were enrolled in the study. IceQube™ activity sensors (IceRobotics Ltd, Edinburgh, Scotland) tracked steps taken, standing and lying times, and number of lying bouts for seven days before and after dry-off. The duration of lying bouts was calculated from the data. The activity sensors calculated a motion index based on the recorded parameters. Daily averages were used as the outcomes and analyzed using PROC MIXED of SAS (SAS Inst. Inc. Cary, NC). The effects of lactation number (1 vs. 2+) and milk yield on the day prior to dry-off on cow activity were also assessed.

The motion index increased across herds after dry-off (p<0.0001). But, behavioral patterns varied between herds. Cows in Herd 2 had significantly more (p<0.02) but significantly shorter (p<0.01) lying bouts after dry-off than before. However, cows in Herds 1 and 3 took shorter lying bouts with no change in the number of lying bouts after dry-off (p<0.28 and p<0.69, respectively). Regardless of the pattern, cows in all three herds showed significantly alterations in lying behavior. Both patterns have previously been associated with decreased welfare in dairy cows. Neither milk yield prior to dry-off nor parity was significantly associated with the activity parameters.

Keywords: dairy cows, dry-off, lying behavior
EMERGENCE OF THE LYME DISEASE VECTOR AND AGENT IN OHIO
P. Wang¹, M. Glowacki¹, D. Acosta¹, M. Wellman¹, C. G. Couto¹, A. Hoet¹,², R. Gary³, K. Smith³, G. Needham⁴,⁵,⁶, and X. Li¹
Colleges of ¹Veterinary Medicine, ²Public Health, ⁴Natural and Mathematical Sciences, ⁵Food, Agricultural, and Environmental Sciences of The Ohio State University, ⁶The Ohio Department of Health, OSU Extension

Lyme disease, the most common vector-borne disease in the United States, is caused by the spirochete Borrelia burgdorferi transmitted by the blacklegged tick Ixodes scapularis. Currently, Ohio is considered by the Centers for Disease Control and Prevention to be non-endemic for Lyme disease. Here, we present evidence for the emergence of both the vector and the bacterium in Ohio. Since 2009, there has been a sharp increase in the number of submissions of the blacklegged tick to a passive surveillance program at Ohio Department of Health. From March to November of 2010, all 3 active stages (larva, nymph, and adult) of the blacklegged tick have been found in a central Ohio area surveyed in this study. Furthermore, 7 of 52 (13.5%) nymphal and 8 of 221 (3.6%) adult I. scapularis ticks tested positive for B. burgdorferi DNA by quantitative polymerase chain reactions. In September 2010, 10 white-footed mice (Peromyscus leucopus, a reservoir host for B. burgdorferi) were captured in the area where the Ixodes ticks were found, and 2 of these mice tested positive for antibodies against B. burgdorferi antigens by the enzyme-linked immunoabsorbent assay (ELISA) and by Western blot analysis. In a seroprevalence study, 41 of 355 (11.5%) dogs with Ohio residency tested positive for antibodies against B. burgdorferi antigens by ELISA. Collectively, these data indicate that the enzootic life cycle of B. burgdorferi is beginning to establish in Ohio, which could pose an increased risk of Lyme disease for humans and domestic animals.

Keywords: Lyme disease, Ohio, Peromyscus leucopus, Ixodes scapularis, Borrelia burgdorferi, seroprevalence, canine
BOVINE ABORTIONS DUE TO ZOONOTIC ETIOLOGY IN OHIO, 2001-2008
S. Greenbaum, P.J. Rajala-Schultz¹, J. Hayes²
¹Department of Veterinary Preventive Medicine, The Ohio State University College of Veterinary Medicine. ²Ohio Department of Agriculture Animal Disease and Diagnostic Laboratory

Occupational exposure to farm animals is associated with increased seroprevalence to zoonotic agents in veterinarians and farm employees (Whitney et al. 2009a; Whitney et al. 2009b). Cows often receive assistance or intervention from human caretakers during parturition and reproductive examinations. Bovine abortions are events where multiple people including farm employees and veterinarians come into contact with potentially infectious dams, fetus(es), or reproductive fluids. Electronic records from the Ohio Department of Agriculture-Animal Disease and Diagnostic Laboratory were searched for bovine abortion submissions between 2001 and 2008. There were 829 submissions between 2001-2008. Of all the submissions, 651 had a complete tissue set (at least four of the following submitted – lung, liver, kidney, stomach contents, spleen, placenta) and 188 of those (29%) received a diagnosis such as abortion due to bacterial, viral, or mycotic etiology. The most common zoonotic diagnoses were *Salmonella* sp. and *Listeria monocytogenes*. Data were analyzed by dividing the number of abortions due to zoonotic etiology by the total number of abortions with complete tissue sets submitted. The proportion of abortions due to zoonotic etiology was 5% of submissions with complete tissue sets. The proportion of organisms with zoonotic potential that were not associated with lesions was 13%, stressing the critical importance of biosecurity and personal protection when handling aborted fetuses and associated tissues or fluids.

Keywords: bovine abortion, diagnosis, zoonotic

Sources:


DETECTION AND SURVEILLANCE OF TUBERCULOSIS IN RAW MILK SAMPLES FROM THE SEMI-ARID REGION OF BRAZIL. W. Gebreyes, A. Guimarães, The Ohio State University, Department of Veterinary Preventive Medicine and M. Matiuzzi, Universidade Federal do Vale do São Francisco

Mycobacterium tuberculosis (TB) continues to be a major public health issue globally including Brazil, not only due to its affect in the food animal industry but also because of its zoonotic potential. Tuberculosis in Brazil accounts for 31% of human tuberculosis cases as reported by the World Health Organization in their Latin American Region. Contamination issues in raw milk above the minimum microbiological standards have led to the development of this project; with the objective detect TB from raw milk samples taken from goat and cattle dairy farms in the semi-arid region of Brazil. In-lab training prior to collection of DNA extraction and PCR was performed using previously grown cultures of TB. Samples were taken at 2 goat farms and 1 cattle farm. Milk from each teat was tested using the California Mastitis Test and if it tested positive, milk samples were taken for inoculation, DNA extraction, and testing using PCR. Using the new protocol, any growth in the cultures will be able to be tested with PCR and gel electrophoresis analysis and used in implementing plans to improve food safety in milk-borne diseases in this region of Brazil.

Keywords: Mycobacterium Tuberculosis, Brazil, Milk
NUTRITIONAL PROFILE COMPARISONS OF VARIOUS POPULAR PET FOODS.
A. Hanthorn, T. Buffington. Department of Veterinary Clinical Sciences, The Ohio State University.

The growth of the pet food industry in recent years has been tremendous, both in sales and appearance of new brands. With clever marketing, confusing claims, and conflicting opinions, pet owners and veterinarians alike can often be confused about product value and pets’ actual needs. This study looked at ten popular dog foods on the market from several categories including conventional, specialty, holistic, organic, and raw brands, and compared them based on macronutrient content, composition, and price. Nutrient values provided by the manufacturer were used to calculate values for protein, fat, carbohydrates, and fiber on a dry matter basis, and in grams of nutrient per 100 kilo calories. Values were compared to the nutrient requirement ranges established by veterinary nutritionists in published references. The cost per 100 kilo calories was also calculated for each based on the current price of the comparable size units. Results showed diets on the less expensive end of the spectrum were within the required ranges necessary to satisfy the nutritional levels established and still meet the consumer need for affordability, while diets on the higher end of the price scale far exceeded the necessary level of nutrients to provide adequate nutrition while carrying a much higher price. A survey of pet owners was conducted to better appreciate the level of awareness of general nutrient requirements and popular pet food definitions and labeling lingo. This information revealed a weakness in the average owners understanding of common terms and marketing strategies used today, as well as misdirected feeding practices. It is clear from this information there is a need for better veterinary student education as well as client support and instruction to help them understand true nutritional needs and interpret labels so they can choose a pet food that fits their and their clients’ needs and wants.

Keywords: nutrition, pet food, nutrient requirements
CONSEQUENCES OF CUTANEOUS INJURIES FROM RADIO TRANSMITTER ANTENNAS ON REINTRODUCED EASTERN PLAINS GARTER SNAKES (\textit{Thamnophis radix radix}). R. Lauer, D. Wynn, J. McKinley, and N. Reichenbach.

College of Veterinary Medicine, Department of Evolution, Ecology, and Organismal Biology, and Liberty University, Dept. of Biology and Chemistry

The Eastern plains garter snake is endangered in Ohio. Efforts are being made by the Cleveland Metroparks and Columbus Zoos and Ohio Division of Wildlife to help restore populations of this species. In order to monitor the snakes, some are implanted with radio transmitters to allow researchers to track their movements and habitat use. Twenty snakes were implanted with radio transmitters at the Columbus Zoo using a modified version of the Reinert and Cundall (1982) method. On the day of release, twelve snakes were found with cutaneous injuries from the antennas and four of these snakes had the antenna protrude through the skin. The protruding wires were cut and the snakes were released the following day. Some snakes that initially had non-protruding injuries were found with the antenna protruding later in the study. Since the antennas can collect bacteria and lead into the abdominal cavity, the chance for local and systemic infection is increased when the antennas protrude through the skin. Injuries could also affect behavior. Reptiles use behavioral fever to kill invasive microorganisms by selecting areas with higher temperature. Distances traveled, substrate and soil temperature in areas found, and mortality were compared between those with good surgical outcomes, those with injuries from the antenna but no protrusion, and those that had the antennas protrude through the skin. Investigators tracked the snakes three times per week. GPS coordinates and ambient, substrate, and soil temperatures were recorded where snakes were found. GPS coordinates were used to create maps of the snakes movements and measure distances using Google Earth. GraphPad Prism program was used for statistical analysis between the three groups. The injuries from the antennas did not appear to have a significant effect on distances traveled, substrate and soil temperature selected, and survival between the three groups.

Keywords: Eastern plains garter snake, radio transmitters, cutaneous injuries, behavioral fever
INVESTIGATION OF EPIDEMIOLOGICAL AND NUTRITIONAL FACTORS ASSOCIATED WITH THE HIGH PREVALENCE OF TRANSITIONAL CELL CARCINOMA IN FISHING CATS (*PRIONAILURUS VIVERRINUS*)

E. Marshall¹, W. Swanson², R. Kelley³, T. Vennard³ and T. Buffington¹

¹College of Veterinary Medicine, Ohio State University, Columbus, OH 43210; ²Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden, Cincinnati, OH 45220; ³Procter & Gamble Pet Care, Mason, OH 45040

A high prevalence of transitional cell carcinoma (TCC) has been identified in the small fishing cat population maintained in North American zoos. TCC in fishing cats has become a major concern for zoos attempting to maintain a genetically-viable population as a conservation priority. A more thorough assessment of TCC prevalence in North American and international zoos is required, and a better understanding of the correlative and causative factors associated with TCC is essential for long-term survival of fishing cats in zoos. We hypothesize that epidemiological and dietary factors will be strongly associated with TCC occurrence in captive fishing cats.

To investigate TCC epidemiology, the Fishing Cat International Studbook was accessed using PopLink 2.1 software, and data analyzed using the SPSS Statistics program to evaluate relative risk of various factors. To assess nutritional effects, serum samples (n=58) from 42 fishing cats at 17 zoos and representative diets (n = 9) are being analyzed at the P&G Pet Care/IAMS nutrition laboratories. Diets are being assessed for proximates (ash, gross energy, moisture, protein and fat) as well as amino acid, mineral, vitamin, and fatty acid levels while serum is being analyzed for fatty acids and vitamins A and E. Using the International Studbook and direct communication with multiple zoos, we have identified 27 TCC cases from the 82 total fishing cat deaths in North American zoos over the past 15 years. TCC also has been diagnosed in fishing cats in Australia (>2 cases) and Europe (>7 cases) during this time span. Preliminary results indicate that genetic relatedness, geographic region, number of different exhibit locations and gender are not correlative factors with TCC. Dietary analyses are ongoing. These findings suggest that TCC is a global disease concern for captive fishing cats with a complex etiology that will require further investigation to resolve.

Key words: fishing cat; transitional cell carcinoma; *Prionailurus viverrinus*
IMPACT OF CEFTIOFUR USE ON THE DISSEMINATION OF EXTENDED-SPECTRUM CEPHALOSPORIN RESISTANT ENTERIC BACTERIA IN FINISHING SWINE POPULATIONS. J. McGintee, D. Mollenkopf, T. Wittum, PhD. Veterinary Preventive Medicine. The Ohio State University

The use of ceftiofur in food animal populations may lead to widespread dissemination of important cephalosporin resistance genes including $\text{bla}_{\text{CMY-2}}$ and $\text{bla}_{\text{CTX-M}}$. These genes confer bacteria the ability to produce powerful $\beta$-lactamase enzymes that inactivate important drugs such as third and fourth generation cephalosporins. The objective of this study is to quantify the relationship between ceftiofur use in finishing swine with the dissemination of $\text{Escherichia coli}$ and $\text{Salmonella}$ spp. carrying plasmid-born $\text{bla}_{\text{CMY-2}}$ and $\text{bla}_{\text{CTX-M}}$. Fecal samples from a total of 50 swine finishing barns in multiple states will be collected. Thirty fecal samples will be obtained from each barn and cultured for the presence of $\text{Salmonella}$ spp. and $\text{E. coli}$ resistant to third or fourth generation cephalosporins. Detailed antimicrobial treatment records will also be obtained for each barn. Our results for the 34 finishing barns that have been sampled to date indicate the presence of $\text{bla}_{\text{CMY-2}}$ in 100% of barns and in 74% of individual fecal samples. $\text{bla}_{\text{CTX-M}}$ is present in approximately 15% of barns, but in 1% of individual fecal samples. $\text{Salmonella}$ spp. are present in 41% of barns and in 6% of individual fecal samples. $\text{Salmonella}$ isolates from one barn were resistant to second and third generation cephalosporins, a phenotype that is consistent with a $\text{bla}_{\text{CMY}}$ genotype. We expect that our results will provide important data to characterize the selection pressure of ceftiofur use on bacterial flora of finishing swine populations.

Keywords: Ceftiofur, cephalosporin, resistant, swine
INTESTINAL PARASITE PREVALENCE IN HUMANS AND DOMESTIC ANIMAL SPECIES: AN EXPLORATORY STUDY IN THE FAR NORTH REGION OF CAMEROON. V. Nesser and Dr. R. Garabed. Department of Veterinary Preventive Medicine.

A total of 108 fecal samples were collected from humans, bovine, ovine, caprine, equine, equid (donkey), porcine, and canine species in the Far North Region of Cameroon to assess the prevalence of intestinal parasites in each of four geographic regions. Samples were collected from three villages in each of the floodplains, mountain, and plains regions, and samples from two villages were collected from the river region. Each fecal sample was prepared via zinc sulfate flotation and the Kato-Katz technique, and analyzed with microscopy. 48.1% of all samples were positive for at least one species of intestinal parasite. Prevalence of Strongyle spp. was highest (35%), followed by Eimeria spp., (15.7%), and Schistosoma spp. (6.5%). Strongyle spp. were present in each of the four geographic regions sampled, were found in all species except for humans, and accounted for the highest parasite prevalence in each region. Schistosoma spp. were recorded in all regions except the plains, and were the only parasites found in humans. Absence of intestinal parasites in humans (other than Schistosoma spp.) suggests that people living in the regions studied are not at risk of contracting zoonoses, despite living in close proximity to their animals. The intestinal parasite burden of non-human animals suggests that animals sold at markets should be de-wormed to prevent the spread of new parasite species to different locations.

Keywords: Intestinal parasites, Schistosoma spp., Strongyle spp., Eimeria spp., Ascarid spp., the Far North Region of Cameroon, Helminth, Nematode, Cestode.
BIOMECHANICAL COMPARISON BETWEEN 2 CONSTRUCTS IN THE CANINE CERVICAL SPINE. D. Pascetta, A. Litsky, M. Allen, and B. Hettlich. Departments of Veterinary Clinical Sciences, College of Veterinary Medicine, and Orthopaedics and Biomedical Engineering, College of Medicine, The Ohio State University

Objective: To compare biomechanical properties of a traditional bicortical stainless steel pin/polymethylmethacrylate (PMMA) construct to a novel monocortical stainless steel screw/PMMA construct used to stabilize the C4-C5 articulation of cadaveric canine cervical vertebral columns.

Study Design: Biomechanical cadaver study.

Animals: Twelve canine vertebral columns (C1-T1) were collected from skeletally mature dogs (weight range 22 to 32kg) euthanized for reasons unrelated to this study.

Methods: Specimens were radiographed to rule out prior injuries or deformities and sorted into two balanced groups on the basis of bone density values (by dual energy x-ray absorptiometry). The stiffness of the unaltered C4-C5 intervertebral articulation was determined in extension using non-destructive 4-point bend testing. Specimens were then stabilized with bicortical stainless steel positive-profile pins with PMMA (Group 1, n=6) or monocortical stainless steel cortical screws with PMMA (Group 2, n=6). The mechanical testing was then repeated. Stiffness data from unaltered specimens and the two surgically stabilized groups were compared using one-way analysis of variance with post-hoc testing.

Results: Both surgical methods significantly increased the stiffness of the C4-C5 articulation compared to the unaltered specimen (p<0.001 for both treatments). The stiffness was not significantly different between the two fixation groups (p=0.344).

Conclusions: The C4-C5 articulation can be successfully stabilized using monocortical screw fixation with PMMA.

Clinical Relevance: Monocortical screw fixation with PMMA is a viable alternative to bicortical pin placement in the canine cervical vertebral column and avoids the potential for severe neurologic complications associated with aberrant pin placement.

Keywords: Vertebral column, dog, implants, stainless steel, bicortical pins, monocortical screws

Black rhinoceros are critically endangered, and captive breeding programs are not self-sustaining due to metabolic disorders (including hemolytic anemia, necrolytic dermatopathy, iron storage disorder, and rhabdomyolysis) not usually seen in free-ranging populations. Researchers hypothesize that these diseases are due to obesity-mediated chronic inflammation that contributes to iron overload, insulin resistance, and hypophosphatemia. Preliminary data from ongoing projects at Cleveland Metroparks Zoo (CMZ) indicate measurable differences between markers of insulin resistance and inflammation in captive versus free-ranging black rhinos. Rhinos are hind-gut fermenters, similar to domestic horses. Original rhino diets were formulated at CMZ based on the Rhino SSP Husbandry Manual and National Research Council domestic horse diet recommendations. Low-starch diets have been shown to manage insulin resistance in horses. Working within the parameters of the previously established diets, we replaced high-starch grain pellets (Mazuri® ADF#16) with low-starch grain pellets (Mazuri® 5V05). Total quantities (with similar caloric content) of all aspects of the diet remained unaltered. All animals will be weighed regularly. Baseline blood samples were collected prior to the diet change, and further blood samples will be collected bi-weekly. The black rhinos will be maintained on the low-starch diet for six months, then serum samples will be analyzed for serum insulin, glucose, TNFα, and serum amyloid A using enzyme-linked immunosorbent assays (ELISAs) previously validated at the CMZ endocrinology lab for use with black rhino serum. We expect to see improvements in insulin sensitivity as measured by insulin and glucose and decreases in inflammatory markers (TNFα and serum amyloid A).

Keywords: black rhinoceros, insulin resistance, low-starch diet
IN VITRO CULTURE OF FRESHWATER MUSSELS
M. Shoemaker, B. Wolfe. The Ohio State University College of Veterinary Medicine, The Wilds, Columbus Zoo Freshwater Mussel Conservation and Research Facility

The freshwater mussel, superfamily Unionoidea, is the most endangered taxon in North America due in part to habitat destruction, competition from invasive species, and a decline in host fish populations. Freshwater mussels have a unique life cycle, in which the larvae (glochidia) are temporarily ectoparasitic on fish, using serum as a nutrient source. After this parasitic phase, the glochidia transform into juvenile mussels, detach from the host fish and burrow into the substrate. The objective of this study was to investigate the use of in vitro culture methods to support the development of glochidia to the juvenile stage in the absence of a fish host. Our specific aim was to compare the effects of different environmental conditions on transformation of glochidia following in vitro culture. Species included in the study were the native Ohio River mussels Amblema plicata, Quadrula pustulosa, Plethobasus cyphyus, and Quadrula metanevra. The culture medium consisted of Medium 199 (Sigma Aldrich Co., St. Louis, MO), horse serum, and an antibiotic/antimycotic cocktail at a ratio of 2:1:0.5 respectively. Environmental conditions compared included temperature (23°C vs. 20°C), buffer (5% CO₂ vs. 25mM HEPES), pH (7.3 vs. 8.0), and gas exchange (medium with or without mineral oil overlay). Contamination was controlled by multiple washings prior to culture and frequent medium changes. Our preliminary data indicate that: 1) lower temperatures decrease contamination; 2) the use of HEPES buffer does not have an adverse effect on growth as found in previous studies; and 3) inter-species survival rates vary significantly depending on the combination of conditions with no one medium component having a significant effect on survival. With the development of an efficient in vitro culture protocol, glochidia successfully transformed into juveniles can be reintroduced into Midwestern freshwater bodies in an effort to slow the populations’ steep decline.

Keywords: Glochidia, Artificial culture, Unionoidea
HIGH SEROPREVALENCE OF *TOXOPLASMA GONDII* IN FERAL CATS IN ADDIS ABABA, ETHIOPIA. N. Tiao¹, C. Darrington¹, B. Molla¹, W.J.A. Saville¹, G. Tilahun², O.C.H. Kwok³, W.A. Gebreyes¹, M.R. Lappin⁴, J.L. Jones⁵, and J.P. Dubey³

¹Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210-1092, USA
²Aklilu Lema Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia
³United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Parasite Biology, Epidemiology and Systematics Laboratory, Beltsville, Maryland 20705-2350, USA
⁴Department of Clinical Sciences, College of Veterinary Medicine, Colorado State University, Fort Collins, Colorado 80523, USA.
⁵Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, 4770 Buford Highway, MS: F22, Chamblee, GA 30341, USA.

*Toxoplasma gondii* is a parasitic protozoan of global public health concern. Domesticated cats and other felines in the family Felidae are the definitive hosts of *T. gondii* shedding infectious oocysts in their feces. This parasite can infect any warm-blooded mammal. Humans, specifically those that are immunocompromised, become infected by consuming undercooked meat infected with tissue cysts or by ingestion of food or water contaminated with oocysts from feces of infected cats.

In this study, 48 feral cats from Addis Ababa, Ethiopia were examined for *T. gondii* infection serologically. Using a modified agglutination test, IgG antibodies against *T. gondii* were found in 85.4% (n=41) of the cats with titers of 1:25 in 1, 1:50 in 1, 1:200 in 6, 1:4000 in 6, and 1:800 in 6, 1:1600 in 8 and 1:3200 in 13 cats. IgM antibodies were found in 11 cats (23%) by an enzyme-linked immunosorbent assay (ELISA). All IgM-positive cats also were positive for IgG antibodies. About one-third (n=13) of the titer-positive cats achieved an IgG titer >3200 indicating an active infectious process. Younger cats (56%) generally did not have antibodies against *T. gondii* compared to older cats (3%).

The high proportion of cats with antibodies against *Toxoplasma gondii* and the very high titers suggests a heavy oocyst contamination load in the environment in Addis Ababa, Ethiopia. This finding in conjunction with commonly practiced raw meat consumption, the high HIV/AIDS rate in Addis Ababa and the lack of animal control is a public health concern.

Keywords: *Toxoplasma gondii*, Ethiopia, epidemiology, cats, public health
HEALTH ASSESSMENTS OF FOUR TURTLE SPECIES WITHIN THE OHIO ERIE CANAL. D. Vajda, S. Koeth, O. Lockhart, P. Dennis. Ohio State University College of Veterinary Medicine, Cleveland Metroparks Zoo and Cleveland State University

Turtle populations have been monitored for several years in marshes around the CanalWay Center in the Ohio Erie Canal (OEC) in an attempt to assess annual survival rates and estimate population size in species, including the common snapping turtle (*Chelydra serpentina*), Blanding’s turtle (*Emydoidea blandingii*), midland painted turtle (*Chrysemys picta marginata*) and eastern musk turtle (*Sternotherus odoratus*). However, infectious disease and health parameters have not previously been investigated. While we hypothesize that infectious disease agents are not a primary cause of decline within turtle populations, evaluating the influence of infectious disease will contribute valuable information to assist in determining factors affecting turtle survival in the OEC. Turtles were captured using hoop nets and the turtles sampled were examined for general physical appearance, gross lesions, abnormal secretions, external parasites and behavioral status. Each turtle was weighed and measured along four parameters. Blood samples of ≤ 0.5% individual body weight were collected from the subcarapacial sinus or tail vein. Blood smears were made following sample collection and analyzed for cell morphology as well as the presence of infectious agents and/or inclusion bodies. A complete blood count was run on samples collected from snapping turtles, Blanding’s turtles and musk turtles. We sampled 89 turtles: 62 midland painted, 11 Blanding’s, 7 eastern musk and 9 snapping turtles. Thus far, we have found blood parasites in 53.3% of these samples, which we believe to be *Haemoproteus* spp. Plasma and oropharyngeal swabs were banked for future use in the investigation of infectious diseases. We are currently investigating testing options for herpesvirus, mycoplasmosis and iridovirus. This project will allow us to assess health parameters and determine whether infectious disease is an important variable to consider as part of turtle preservation in northeast Ohio.

Keywords: turtle, Ohio Erie Canal, health parameters, epidemiology, infectious disease
COMPARING ENVIRONMENTAL FACTORS OF TSETSE (GLOSSINA SPP) FLY POPULATIONS TO MODEL TRYPANOSOMIASIS RISK IN THE FAR NORTH REGION OF CAMEROON. S. Valerius and R. Garabed. Dept. of Veterinary Preventive Medicine.

Trypanosomiasis is a deadly parasitic disease transmitted to animals and people by tsetse flies across much of Africa. A study identifying the environmental risk factors that influence tsetse fly exposure in the Far North Region of Cameroon was conducted by using bi-conical fly traps in the early rainy seasons of 2010 and 2011. Two species of tsetse flies, Glossina tachinoides and Glossina morsitans submorsitans, were caught. In 2011 traps were placed in four different environments at each village: near a water source, a human road, a large tree, and an animal pen. Odds ratios were calculated for both species comparing the four trap environments. G. tachinoides had 8.8 times higher odds of being found near water than any other environment mentioned (OR=8.8, 95% CI-1.5-51.56, p-value=0.02), but no other environment was found to have statistical significance in finding either fly species. Trypanosomiasis results from trapped flies are still pending.

Keywords: Tsetse fly, trypanosomiasis, Cameroon
THE EFFECT OF HABITAT BURNING ON TSETSE FLY POPULATIONS IN CAMEROON, L. Wagner and R. Garabed, Department of Veterinary Preventive Medicine

Parasitic diseases are a major cause of illness and economic loss in many parts of the world. In the Far North Region of Cameroon, Trypanosomiasis can result in severe anemia, weight loss, and death in agriculturally important cattle. Trypanosomes are protozoan species spread between mammalian hosts in Africa primarily by the tsetse fly (*Glossina spp*). To prevent disease spread, animals can be treated directly with preventative medication or the tsetse fly vector populations can be eradicated. One of the current anecdotal methods used against Trypanosomiasis spread in the Far North Region is controlled burning of vegetation. Limited burning is thought to reduce tsetse fly populations by removing vegetation that the flies require for resting; however, the effectiveness of this practice has not been proven. It was hypothesized that controlled burning reduces fly numbers in a local area, but is not effective as a permanent eradication method. To test this hypothesis, a computer model was constructed in NetLogo using published data on tsetse fly population dynamics and maps of the ecological habitats in the Far North Region. The model simulations of tsetse flies were assessed for differences in population number and fly distribution for three years after initiation of habitat burning. The number of fires, size of burned areas, and location of habitat burning did not significantly affect the fly population \( p = 0.058 - 0.24 \). The frequency of burning (annual and biannual burning) did significantly decrease the fly population and limited the distribution of flies \( p = 0.00034 - 0.0047 \). These results may help cattle herders in the Far North Region of Cameroon understand how best to manage tsetse fly populations and stop the spread of trypanosomiasis.

Keywords: tsetse fly, trypanosomiasis, parasite vector control, burning, computer model
CHANGES IN ANTIMICROBIAL RESISTANCE ON OHIO DAIRY HERDS
M. Weeman, D. Mollenkopf, T. Wittum. Veterinary Preventative Medicine

Antimicrobial drug use within food animal production systems has been implicated as a potential risk factor for the dissemination of antimicrobial resistance in both human and animal populations. We investigated the antimicrobial resistance patterns of commensal *Escherichia coli* recovered from feces of healthy dairy cattle populations and compared them to similar data generated in the same herds approximately 10 years prior. Fecal samples from sixteen Ohio dairy herds that participated in the original surveillance program were collected from 25 randomly selected cows at each farm and cultured on non-selective media. A single *Escherichia coli* isolate recovered from each sample was then used to determine the minimum inhibitory concentrations (MIC) by micro broth dilution to a panel of antimicrobial drugs important in human and veterinary medicine.

Antimicrobial resistance phenotypes among *Escherichia coli* fecal isolates recovered in this study are most commonly pansusceptible, as they were 10 years ago. Some increases in resistance to specific drugs are apparent, but the overall resistance to important antibiotics has remained very low. We are continuing to identify the resistance genes present among the *Escherichia coli* fecal isolates and their association with antimicrobial drug use on the farms. These results may be used to develop future monitoring and intervention programs focused on minimizing the development and spread of antimicrobial resistant microorganisms.

Keywords: Dairy Cows, antibiotic resistance, extended-spectrum cephalosporin
EPIDEMIOLOGY AND GENOTYPIC DIVERSITY OF CAMPYLOBACTER SPP. FROM BROILER FLOCKS IN CHIANG MAI, THAILAND IN COMPARISON TO ISOLATES FOUND IN THE MIDWEST, UNITED STATES. C. Chokboonmongkol1, W. Gebreyes2, K-H Zessin3, T. Alter4, C. Widmann2, P. Patchanee1
1Veterinary Public Health Center for Asia Pacific (VPHCAP), Faculty of Veterinary Medicine, Chiang Mai University, Thailand; 2 Ohio State University, College of Veterinary Medicine; 3Department Panel, Veterinary Public Health, Freie Universitat Berlin, Germany; 4Institute of Food Hygiene, Freie Universitat Berlin, Germany

*Correspondence email address: patprapas@gmail.com

Campylobacter spp is a major cause of gastroenteritis throughout the world, in underdeveloped and developed countries alike. Oral contraction is the primary route of infection chiefly occurring by consumption of contaminated water and undercooked meat products. Campylobacter isolates were gathered from 98 broiler flocks in the Chiang Mai Province, Thailand from a private slaughter house from both whole carcasses and intact cecas at the time of evisceration. Sixteen isolates confirmed to be C.jejuni were genotypically characterized by using Multi Locus Sequencing Types (MLST). Isolates in the United States were investigated using the database provided by pubmlst.org and were chosen based upon their location in the Midwest. Sources included humans, poultry, pigs and cattle. Clonal relationships from the Thailand and US isolates were investigated by running Minimal Spanning Tree (MST) analysis and grouping isolates in clonal complexes to compare the genetic diversity and evolution of the investigated strains. The prevalence of Campylobacter in broiler flocks was 11.22% (11/98) while on broiler carcasses, 51.02% (50/98) at 95% CI (4.97-17.47) and (41.12-60.92). The lowest level average of Campylobacter contaminated chicken carcasses was 145.11 cfu/g. Concentration of Campylobacter contamination on broiler carcass was relatively high which is a risk to human health. Nine different STs were identified among the samples taken from Thailand. Three new additional STs in Thailand were firstly reported, C.jejuni STs 305, STs 5213, and STs 1075 C.jejuni isolates from Thailand and USA demonstrate large different allelic profiles which are considered to have resulted by major diversification from a recent common ancestor. More isolates to analyze from Thailand and USA to identify a genotypic vs. phenotypic resistance patterns are needed.

Keywords: genotypic diversity, campylobacter, Thailand, United States
The major inducible 70 kDa heat shock protein is a cellular chaperone that up-regulates viral gene expression but also has potential to enhance innate immune responses when released from virus-infected cells. Focus of our work is to establish how virus-hsp70 interaction influences outcome of infection in an animal host, using the neonatal mouse model of neuronal infection by measles virus (MeV). Previous studies showed that transgenic constitutive expression of hsp70 in neurons protects mice MeV neurovirulence. This Hsp70 mediated protection has an early innate component based upon studies using T-cell depletion and results of transcript analysis in total brain RNA at 5 days post infection, where markers of macrophage activation were increased in brains of transgenic (TG) relative to non-transgenic (NT) mice. The hypothesis tested in the current work is that enhanced activation of macrophages in TG mice at 5 days post infection reflects a response by resident microglia and not blood origin monocytes. The approach was based upon immunohistochemical staining of infected brains. CD45 staining was negative on all accounts, ruling out infiltrating monocytic origin. F4/80, a marker specific to microglia and upregulated upon activation, demonstrated a stronger response in the TG mice when compared to NT mice. MHCII, whose expression indicates macrophage activation in the brain, was significantly increased in TG compared to NT mice, and staining conformed to a location and morphology that was characteristic of microglia. Evidence of enhanced microglial activation in infected TG mice is important because activated microglia are the major source of type 1 interferon (IFN-β) during neuron tropic viral infection (not neurons). IFN-β induces antigen presentation in brain macrophages and supports activation of virus specific T cells during the adaptive component of the immune response. Collectively these results support an important role for Hsp70 in the immune response to neurotropic viral infections.

Keywords: HSP70, measles virus, mice
IN VITRO REPLICATION OF PORCINE TORQUE TENO VIRUS.
E. Ihms, S. Ringler, R. Jackwood, S. Krakowka. OSU College of Veterinary Medicine, Department of Veterinary Biosciences

Torque Teno Virus (TTV) is a novel circular single-strand DNA virus belonging to the Circovirus family. This virus is unique among DNA viruses because of its remarkably high genotypic variability (approximately 40%). TTVs have been identified in numerous species including humans, with prevalence in some populations approaching 100%. Although TTVs alone have not been shown to cause primary disease, they have been implicated in having a synergistic role in several important diseases - namely Postweaning Multisystemic Wasting Syndrome and Porcine Reproductive and Respiratory Syndrome of swine. However, due to the lack of a reliable culture system, the pathogenesis of TTV infection remains unknown. Recently, a commercial porcine cell line has been discovered that harbors two genogroups of TTV. We propose to use this permissive cell line to optimize viral production in vitro. Cells will be cleared of virus by passaging with limiting dilution, and treating with interferon alpha. Virus-free cells will then be reinfected by co-culturing with supernatant from infected cells. Cells will be stimulated with Phytohemagglutinin (PHA) and Lipopolysaccharide (LPS) in order to maximize viral production. Secondly, this experiment seeks to elucidate the means of TTV replication within the cell. We hypothesize that one mode of TTV replication makes use of the low-fidelity mitochondrial DNA polymerase. To investigate this, mitochondria will be tagged with a magnetically-labeled antibody and purified in a magnetic field. Isolated mitochondria will then be evaluated for the presence of virus by electron microscopy and PCR.

Keywords: Torque Teno Virus, single-strand DNA virus, viral replication

Viral hemorrhagic septicemia virus (VHSV) is a rhabdovirus affecting freshwater fish, specifically rainbow trout and salmon. This virus causes severe destruction of the kidney and liver along with hemorrhaging and widespread necrosis of surrounding tissue. In Europe, VHSV has had a severe impact on aquaculture. A new 4b genotype of VHSV was first isolated in the Great Lakes in 2005. While it is widely known that freshwater fish are hosts and transmitters of VHSV, little is known about the ability of invertebrates to take up the virus. As filter feeders, freshwater mussels bioaccumulate many organisms in their environment. In other studies, they have been proven to accumulate infectious salmon anemia virus (ISAV) and infectious pancreatic necrosis virus. Two species of mussels were used in these experiments to investigate their ability to accumulate the VHS virus and serve as bioindicators of viral presence in the water. Corbicula fluminea is an invasive species of bivalves that originated in Asia and was first found in 2002. Amblema plicata is an indigenous species found naturally in rivers and streams in the Eastern parts of the United States. Each group of mussels was separately inoculated with both 100 and 200 Tissue Culture Infective Dose (TCID50) of VHSV and held in a 15°C incubator. Mussel tissues were tested for VHS 72h, 120h, and 168h post-exposure using rt-PCR. Initial results indicate that freshwater mussels have the ability to harbor the VHS virus when it is present in the water, especially in higher concentrations. Mussels can thereby serve as valuable indicators of infectious diseases and pathogens of fresh water in the U.S. Mussels are not likely to maintain VHS long term within their tissues, and are therefore not likely vectors of the disease. These results show that mussels may also be used where other methods of monitoring the water for infectious diseases would be prohibitively costly.

Keywords: Viral Hemorrhagic Septicemia Virus (VHSV), Mussels, infectious disease, water monitoring, rt-PCR, filter feeders, Corbicula fluminea, Amblema plicata
RETINOIC ACID FOR REGULATION OF HOST RESPONSE TO SUBLINGUAL VACCINE AGAINST RESPIRATORY PATHOGENS. J. Morrison, J. Jee, M. Fial, H. Steiner, A. Bonnegarde and P. Boyaka. Department of Veterinary Biosciences, The Ohio State University, Columbus, Ohio, USA

Mucosal immune responses induced by vaccines are critically important because the mucosal surface is a major portal of entry for infectious agents. Especially in the gut-microenvironment, retinoic acid (RA) plays an important role in inducing gut-migratory molecules on lymphocytes and secreting antibodies (Abs) to neutralize pathogens. Sublingual immunization with Bacillus anthracis edema toxin (EdTx) has been studied as a non-invasive alternative to avoid previously issued adverse effects, e.g., tolerance in oral and facial paralysis in nasal immunization. It is hypothesized that sublingually-administered RA modulates activity of antigen-presenting cells (APCs) in cervical lymph nodes (CLNs) and improves Ab-responses to attenuated H1N1 influenza virus (attH1N1). Our studies aim to demonstrate the effect of RA on immune cell-population, expression of co-stimulatory and gut-migratory molecules in CLNs in vitro, and systematic as well as mucosal Ab-responses to sublingually-administered attH1N1 in vivo. As a method, flow-cytometry and real-time quantitative PCR will be used to analyze surface-molecules expressed on immune cells and the expression of pro-inflammatory mRNA level in CLNs, respectively. Serum, vaginal washes, fecal extracts, and saliva samples from immunized C57BL/6 mice will be subject to indirect Ab-ELISA to quantify systematic and mucosal isotype Ab-responses, i.e., IgG, IgG1, IgG2a and IgA. The co-administered RA with attH1N1 is expected to induce more gut-migratory molecules, e.g., α4β7 and CCR9 on immune cells in CLNs, and elevate more systematic and mucosal Ab-titters, especially secretory IgA in the gut. Finally, the immunized mice in the RA group will be challenged with a mouse-adapted influenza virus, possibly resulting in ameliorating protection level, compared with the control group. Our experimental design will shed new light on the strategies for preventing infectious agents from animal as well as human population.

Keywords: retinoic acid, vaccines, PCR, pathogens
A COMPARISON OF THREE ANTIBODIES FOR THE IMMUNOPURIFICATION OF EQUINE MONOCYTES.  M. O’Brien¹, W.M. Yeo², and T. Stokol²

¹The Ohio State University College of Veterinary Medicine. ²Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University

Monocytes are immune cells that likely have important roles in equine disease pathogenesis. Isolation of purified monocytes would improve research conducted with these cells. Antibody-facilitated immunopurification is a rapid and efficient technique for monocyte isolation. Various antibodies have been used for this purpose in horses, but no direct comparisons of purification efficiency have been performed. Our goal was to compare the yield and purity of monocytes isolated from equine peripheral blood with three antibodies. Peripheral blood mononuclear cells (PBMC) were derived by gravity sedimentation followed by density gradient centrifugation of heparinized equine blood. PMBC were then incubated with one of three antibodies: an anti-human CD14 (biG 10, Biometec GmbH), an anti-equine CD14 (clone 105, a gift from Dr. Wagner at Cornell University) and an anti-human CD163 (BerMac3, MBL International). Antibody-bound cells were separated from PBMC by employing magnetic column sorting (MACS™) and monocyte yield and purity were determined using flow cytometry and cytologic examination of cytospin smears. We found the anti-CD163 antibody yielded maximum monocyte purity (90 ± 5%) versus the anti-CD14 antibodies, biG10 and 105 (80 ± 5% and 82 ± 2%, respectively). Differences between antibodies were noted in the types of non-monocytic cells in the sorted fraction; lymphocytes dominated for biG10, whereas neutrophils and lymphocytes were present with 105. Despite higher purity, monocyte yield was lower with the anti-CD163 antibody, which labeled a subset of CD14-positive cells. Our results indicate that purity and yield of immunomagnetic bead-isolated monocytes varies substantially between antibodies. There are also different phenotypic monocyte subsets, which may translate into functional differences in vivo. Isolation of specific monocyte subsets may affect the outcome of studies designed at evaluating monocyte responses to equine diseases.

Keywords: Equine, Monocytes, Immunopurification, CD14, CD163
BROAD RELEVANCE OF HSP70 MEDIATED INNATE IMMUNITY IN THE VIRUS INFECTED BRAIN. Peterson, C., Kim, M.Y., Dell Armelina Rocha, P.R., Shu, Y., Oglesbee, M. Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, USA.

Cellular heat shock proteins have been shown to stimulate viral gene expression and infectious progeny release within virus infected cells, but the consequence may be to release heat shock proteins into the extracellular environment where they can stimulate innate immune responses. The significance of cellular heat shock protein-virus interaction has been previously demonstrated in both in vitro and in vivo using measles virus (MeV). In vitro, the major inducible 70 kDa heat shock protein (hsp70) enhances MeV gene expression and cytopathic effect. The in vivo consequences of the virus-hsp70 interaction was shown using transgenic mice that express human hsp70 in neurons, the cellular target of MeV infection. Virus-hsp70 interaction confers protection against MeV neurovirulence by enhancing innate immune responses that include: increased expression of type 1 interferon (IFN), which in the brain is IFN-β; activation of brain macrophages; induction of antigen presenting molecules. The hypothesis tested in the current work is that the paradoxical relationship between (a) hsp70-dependent stimulation of viral gene expression in vitro and (b) protective effects in vivo has more broad virological relevance. The hypothesis will be tested using vesicular stomatitis virus (VSV), a virus also having a tropism for neurons. In vitro, murine neuroblastoma cells were infected with VSV and cellular levels of hsp70 were increased by stable transfection. Here, hsp70 stimulated viral antigen expression, progeny release and cytopathic effect. In vivo, hsp70 transgenic and non-transgenic mice, with or without disruption of the type I IFN receptor (Ifnar-/-), were challenged with VSV via the intranasal route. Survival analysis showed hsp70 mediated protection and that protection was lost in the Ifnar-/- mice. Collectively, results support the more broad virological relevance of a novel axis of innate antiviral immunity in which hsp70 mediated increases in viral gene expression are associated with a protective innate immune response that is IFN-β dependent.

Keywords: heat shock proteins, neurovirulence, type I interferon, innate immunity
EFFECT OF AUTOANTIBODY-MEDIATED INFLAMMATION ON Borrelia burgdorferi SURVIVAL AND GENE EXPRESSION IN VIVO

L. Ramos, D. Acosta, and X. Li. Department of Veterinary Biosciences

Borrelia burgdorferi (Bb) is a spirochetal pathogen that causes Lyme disease, a tick-borne infection of public health concern. In approximately 10% of US patients with Lyme arthritis (LA), a late stage of the disease, joint inflammation does not resolve even with aggressive antibiotics. The underlying causes for these antibiotic-refractory LA cases can be infection-induced autoimmunity and/or bacterial persistence. Previous studies have shown that patients with LA develop high-titer IgG response to Dps, a protein that helps bacteria survive stress by entering dormancy. We hypothesize that the Dps-mediated stress response allows bacteria become more resistant to antibiotic killing. However, Dps-specific IgG response is absent in the current murine model of Lyme arthritis. Here, we utilized the K/BxN serum transfer model to determine the effect of autoantibody-induced joint inflammation on Dps expression and Bb survival in C3H mice. Four experimental groups were examined in this study: 4 mice not infected with Bb or treated with the serum, 4 mice treated with the serum alone, 18 mice infected with Bb, and 18 mice infected with Bb and treated with the serum. During necropsy tissues including heart, skin, and joint were collected for culture and nucleic acid analyses. The results indicate that Bb infection resulted in much milder pathology in the joint compared to the K/BxN serum treatment. Also, mice treated with the serum, regardless of whether or not they were infected with Bb, developed marked arthritis and had significant reduction in body weight (<15%) at the peak of arthritis. However, the joint inflammation induced by autoantibody failed to kill the resident spirochete or to induce the Dps-mediated stress response. On the contrary, preexisting Bb infection in the joint alleviated the symptoms of autoantibody-induced arthritis. More specifically targeted immune response to Bb may be required to induce the Dps-mediated stress response in the spirochete.

Keywords: Borrelia burgdorferi, Lyme arthritis, murine model, bacterial stress response
CHARACTERIZATION OF A MODEL OF HTLV-1 ORAL TRANSMISSION IN THE RABBIT MODEL.  E. M. Simpson,¹ R. A. Haines,¹ M. D. Lairmore¹ ²
¹Center for Retrovirus Research and Department of Veterinary Biosciences, ²Comprehensive Cancer Center, Arthur G. James Cancer Hospital and Solove Research Institute

A primary route of transmission of human T-lymphotrophic virus 1 (HTLV-1) is from mother-to-child via breast milk, but knowledge of the early immunologic events in orally acquired HTLV-1 infection is limited. To characterize normal rabbit gut-associated lymphoid tissues (GALT) and perform studies to develop an oral model of HTLV-1 infection, key GALT inductive and effector sites will be characterized using immunohistochemistry and laser capture microscopy. Our data indicate that unexposed rabbits GALT have a predominant CD4+ lymphocyte population similar to humans. To establish a HTLV-1 oral model 12 week old female New Zealand White rabbits were previously intravenously or orally inoculated with CD3+CD4+CD25+ rabbit lymphocyte cell line immortalized with the HTLV-1 molecular clone ACH (R-49) cells or control Jurkat T-cells. The rabbits were monitored for hematologic and virologic parameters prior to serial sacrifice. Collective, 66 to 100% of HTLV-1 orally exposed rabbits became persistently infected. HTLV-1 orally exposed and infected rabbits during early time points (1-4 weeks post exposure) had delayed and often less intense anti-HTLV-1 antibody response, variable leukocytosis, and a delayed p19 matrix antigen production and proviral DNA amounts in peripheral blood leukocytes compared to the IV infected rabbits. Interestingly, by 8 weeks post exposure orally exposed rabbits had established similar systemic spread of the virus compared to IV exposure rabbits. The goal of this summer project is to develop protocols for laser capture microscopy so that it can be used to isolate tissue compartments that harbor HTLV-1 in GALT. These tissue compartments will be analyzed for HTLV-1 proviral load. Immunohistochemistry is being used to characterize regions of proliferation throughout the GALT in normal rabbits and eventually these regions will be compared to the regions of proliferation in HTLV-1 orally infected rabbits.

Keywords: HTLV-1, Rabbit, GALT, Immunohistochemistry, Laser Capture Microdissection
SUPPRESSION OF CANINE MYELOID CELLS BY SOLUBLE FACTORS FROM CULTURED CANINE TUMOR CELLS. J. Wasserman, T. Papenfuss. Department of Veterinary Biosciences

Cancer profoundly affects immunity and causes immunosuppression that contributes to tumor escape, metastases and resistance to therapy. The mechanisms by which cancer cells influence immune cells are not fully known but both innate and adaptive immune cells can be altered by cancer. Myeloid cells are innate immune cells that comprise the mononuclear phagocytic system (MPS) and include monocytes, macrophages, dendritic cells (DCs) and their progenitors. Myeloid cells play important roles in both the promotion and regulation of immune responses. Dysregulated myeloid cells are increasingly being recognized as contributing to cancer-related immunosuppression. This study investigated whether soluble factors produced by canine tumor cells inhibited canine myeloid cell function. Methods: These studies investigated the utility of using the canine DH82 cell line for assessment of canine myeloid responses to tumor-derived soluble factors (TDSFs). Phenotypic comparisons to canine bone marrow-derived DCs (BM-DCs) and bone marrow-derived macrophages (BM-MFs) were performed and expression of myeloid cell markers CD11b, CD11c, CD80, and major histocompatibility complex (MHC) class II were evaluated by flow cytometry. Phenotypic and functional changes of DC populations were then determined following exposure to tumor-conditioned media (TCM) from canine osteosarcoma, melanoma and mammary carcinoma cell lines. Results: We found that the canine BM-DCs and the DH82 cell line shared similar CD11b, CD11c and MHC II expression and morphologic characteristics that were distinct from canine BM-MFs. Myeloid cells exposed to TDSFs showed decreased expression of MHC class II and CD80, had reduced phagocytic activity and suppressed the proliferation of responder immune cells. TDSF exposure during differentiation generated immunosuppressive immature myeloid cells that shared characteristics of myeloid derived suppressor cells (MDSCs). Conclusion: These results show that soluble factors secreted from canine tumor cells suppress the activation and function of canine myeloid cells and we provide the first description of a method to produce immunosuppressive canine MDSCs. Our results suggest that, similar to humans, dysregulated myeloid cells may contribute to immunosuppression in dogs with cancer.

Keywords: canine, myeloid, dendritic cells, macrophages, DH82, cancer, immunology
CROSS-REACTIVITY OF HUMAN FOXP3 ANTIBODIES: IDENTIFICATION OF REGULATORY T CELLS IN RABBIT GALT. T. Wyszynski1, R. Haines1, M. Lairmore1,2, K. Landes1, and E. Simpson1. 1. Center for Retrovirus Research and Department of Veterinary Biosciences, College of Veterinary Medicine, 2. Comprehensive Cancer Center, The Arthur G. James Cancer Hospital and Solove Research Institute, The Ohio State University, Columbus Ohio

FOXP3, a 50 kD transcription factor, is highly conserved among mammalian species and widely recognized as the best single marker of CD4+ CD25+ regulatory T cells, which are actively involved in numerous diseases such as autoimmune disorders and cancer. A disease in which the role of Tregs is unknown is Adult T cell Leukemia/lymphoma (ATL), a cancer developed from the retroviral causative agent, HTLV-1. The rabbit can be used as a model of oral infection of HTLV-1, the mode of transmission most common in endemic disease regions which consistently results in the development of ATL. In our study we look to further characterize normal rabbit gut-associated lymphoid tissues (GALT), using immunohistochemistry, to better develop the oral model of HTLV-1 infection. Unfortunately, there is a lack of commercially available reagents which specifically recognize FOXP3+ Treg cells in rabbit tissue. Archived formalin fixed paraffin embedded and frozen lymphoid tissues from twelve week old New Zealand White female rabbits were analyzed using Avidin-Biotin Complex IHC methodology. Four anti-human FOXP3 monoclonal antibodies (206D PE, 259D, 86D, 157B) were tested for rabbit cross-reactivity, in appendix and spleen, using thirty minute and two hour incubation times. A stain was labeled as positive by visualization of nuclear stain of a lymphocytic cell subset in T cell rich areas. 259D, 86D, and 157B positively stained a FFPE human tonsil control, yet displayed no positive reactivity in either formalin-fixed or frozen rabbit tissue; 206D PE was negative on all samples. Future research should utilize other techniques, namely flow cytometry and western blotting, to exhaustively test the reactivity of the 259D, 86D, and 157B FOXP3 clones in rabbit GALT. Knowledge of regulatory T cell dynamics in the rabbit model may provide valuable insight into the pathogenesis of HTLV-1/ ATL, aiding in the development of better diagnostics and treatment.

Keywords: FOXP3, regulatory T cell, immunohistochemistry, mucosal immunology, rabbit, HTLV-1, ATL
EXPRESSION OF MIR-21 AND MIR-720 IN CANINE MELANOMA AND TRANSITIONAL CELL CARCINOMA. D.L.H. Smith, S. Murahari, J.M. Fenger, and W.C. Kisseberth. Department of Veterinary Clinical Science, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio

MicroRNAs (miRs) are small (~22 nt) non-coding RNAs that regulate gene expression primarily by targeting complementary sites in the mRNA 3′ untranslated region (UTR), resulting in mRNA degradation, translational repression, or gene activation. MiRs have been shown to be involved in many different cellular processes including cell cycle control, differentiation, and apoptosis. MiRs often are dysregulated in cancer, suggesting they can play a role in tumorigenesis. We determined miR expression profiles of 23 canine melanoma (11 tumors, 12 cell lines) and 13 canine transitional cell carcinoma (TCC) samples (6 tumors, 7 cell lines) using the Nanostring Technologies nCounter miRNA Expression Assay Kit. Of the most highly expressed miRs, we selected two for further investigation, miR-21 and miR-720, which were found to be highly expressed in malignant melanoma and TCC cells, respectively. In order to begin to elucidate the functions of these highly expressed miRs in canine tumor cells, we transduced canine melanoma and TCC cell lines with lentiviral constructs expressing miR silencing transcripts specific for miR-21, miR-720, or a scrambled construct. Transduced cells were flow cytometry sorted, expanded, and then assayed for changes in miR-21 and miR-720 expression, growth and apoptosis. Preliminary data suggests that miR-21 plays an important role in melanoma cell survival and miRNA-720 in cell proliferation. TCC cell proliferation was unchanged when transduced with miRZip-21 and miRZip-720 constructs. Caspase activity was not significantly altered by transduction with any of the constructs. These data must be interpreted cautiously, as miR-21 and miR-720 expression levels were not significantly altered in transduced cells, despite high GFP expression in transduced cells. Elucidating the role of these miRs in canine melanoma and TCC pathogenesis may provide future opportunities for therapeutic intervention.

Keywords: microRNA, melanoma, transitional cell carcinoma
CALCITRIOL AND CURCUMIN POTENTIATE ANTIPROLIFERATIVE EFFECTS OF GEMCITABINE AND CARBOPLATIN ON CANINE TRANSITIONAL CELL CARCINOMA. K Bakewell and N Inpanbutr. Department of Veterinary Biosciences

Transitional cell carcinoma of the urinary bladder in dogs (cbTCC) is a challenging disease. Only 12 to 25% of affected dogs receiving chemotherapy have at least a partial response to treatment. Nonsteroidal anti-inflammatory drugs, piroxicam and mitoxantrone have become the most common treatment. Carboplatin and Gemcitabine, a current standard therapy for cbTCC have shown little activity as single agents for the treatment of canine TCC. We have shown better results of a combination Carboplatin and Gemcitabine. The known synergistic effects of calcitriol and Gemcitabine in human TCC, may be potentiated by the combination of these compounds providing maximum anti-cancer effects on canine TCC. We hope to improve considerably the treatment of transitional cell carcinoma in dogs by adding calcitriol and curcumin to Gemcitabine and Carboplatin without causing major hypercalcemic side-effects.

Therefore, we hypothesized that the combination of calcitriol and curcumin with Gemcitabine and Carboplatin potentiate antiproliferative effects of these two drugs on cbTCC cells growth compared to using the single compound alone. As a result, Carboplatin was shown to be synergistic when combined with 1uM Calcitriol (25uM and 100uM) and antagonistic when combined with Curcumin. The combination of calcitriol and gemcitabine was shown to be antagonistic at most concentrations.

Keywords: Calcitriol, Transitional cell carcinoma, Gemcitabine, Carboplatin, Bladder Cancer, Vitamin D
LENGTH CHANGES OF THE COLLATERAL LIGAMENTS OF THE CANINE STIFLE JOINT: A COMPARISON OF IMAGING VERSUS SURGICAL NAVIGATION. C. Clark, M. Allen. The Ohio State University, College of Veterinary Medicine. Dept. of Veterinary Clinical Sciences

Appropriate tensioning of the collateral ligaments is critical to joint stability during activities of daily living. In humans, the lengths of the medial (MCL) and lateral (LCL) collateral ligaments vary less than 2% during motion and the ligaments are considered isometric. Understanding ligament length changes during knee motion is vital to successful reconstruction after ligament trauma. The purpose of this study was to compare a radiographic method for measuring ligament length against a computer assisted tracking technique that can be used intra-operatively to quantify knee motion. We hypothesized that the computerized tracking method, performed without the need for implanted markers, would produce results that are comparable to those obtained by direct radiographic assessment. A calibrated computer assisted stylus, linked to a 6-degrees of freedom motion capture system (Polaris Vicra; Northern Digital, Inc.), was used to pinpoint the femoral and tibial insertions of the MCL and LCL in six cadaveric canine stifle joints. The distance between insertion points was measured at 15° intervals from full extension (150°) to full flexion (45°). Spherical metal markers were then implanted at the insertion points and fluoroscopy used to measure the distance between markers. The Vicra system was found to be highly reproducible, with an average coefficient of variation of 2.91% for repeated measurements. Both MCL and LCL demonstrated isometry from full extension (150°) to mid-flexion (75°) but began to shorten at flexion angles less than 75°. These results are consistent with clinical experience of collateral ligament reconstruction. In the next stage of this work, we will use the computerized tracking technique to map locations on the femur and tibia at which it is possible to achieve isometric collateral ligament reconstruction. These maps will aid surgeons in identifying the optimal locations for implanting ligament repair devices such as suture anchors or screws and spiked washers.

Keywords: canine, stifle joint, collateral ligament, isometry, motion capture
THE EFFECT OF OSMOLALITY ON SNAKE SEMEN STORAGE AT TWO DIFFERENT TEMPERATURES. E. Ferris, M. Cox, A. Santas, B. Wolfe, The Ohio State University College of Veterinary Medicine, Muskingum University Department of Biology, The Wilds Department of Conservation Medicine

Long-term storage of reptile semen has not yet been successful. Preliminary data from this lab have shown that physiologic osmolality of snake seminal plasma ranges from 500-1200 mosm/l, leading to our hypothesis that in-vivo semen storage in snakes may involve a physiologic hyperosmolar state. We studied the effects of 4 different medium osmolalities (300, 600, 900 and 1200mOsm) on motility and viability of snake semen over time. Ham’s F10 medium was reconstituted to 10x then diluted to 300mOsm, 600mOsm, 900mOsm and 1200mOsm. Each sample was extended in Ham’s F10 at each osmolality, then stored at ambient temperature (24°C) or refrigerated (4°C). A small amount of extended semen was evaluated hourly for motility and viability for up to 6 hours. Viability was determined using SYBR-PI live/dead stain (Molecular Probes, Invirogen) and evaluation under a fluorescent microscope. At both temperatures, motility was highest in 300mOsm media, although it decreased faster over time at 24°C than at 4°C. Viability was highest in both 600mOsm and 900mOsm media at 24°C, and 900mOsm at 4°C. In 600mOsm media, motility did not cease immediately after dilution, but decreased over time while viability remained higher than motility. In both 900mOsm and 1200mOsm media, motility ceased immediately upon dilution but viability remained high over the 6 hour time period. Based on these results, we concluded that snake spermatozoa become quiescent in high osmolality media but remain viable under these conditions. With further study, this phenomenon may lead to a better method of long term reptile semen preservation.

Keywords: Snake, semen, spermatozoa, osmolality
Greyhounds have numerous hematological values that differ from those of other dog breeds. In addition, increases in packed cell volume, total protein and hemoglobin concentration, erythrocyte counts, and leukocyte counts occur immediately after exercise; these values return to resting values within a few hours after racing. This study evaluated the effects of exercise on the circulating concentration of reticulocytes in racing Greyhounds. We hypothesized that reticulocyte numbers would increase significantly immediately after a race, and would return to baseline within 1-2 hours post-race. Fifty actively racing Greyhounds at the Wheeling Island Racetrack and Casino were included in the study. Samples were collected by jugular venipuncture one day prior to racing at the kennel (resting), immediately after racing, and 1-2 hours after the race (recovery), and analyzed using a IDEXX ProCyte Dx® Hematology Analyzer (IDEXX Laboratories, Inc., Westbrook, ME, USA); results were compared statistically using the Friedman test due to non-parametric distribution. Reticulocyte concentrations were significantly different among the 3 sample collection times (p<0.0001). There was a significant increase in reticulocyte concentration immediately after racing (p<0.001); the reticulocyte numbers then decreased significantly 1-2 hours after racing (p<0.001). There were no significant differences between the resting and recovery samples. This increase in reticulocyte concentration is likely related to splenic contraction secondary to the release of catecholamines. Thus, it is important to consider a Greyhound’s activity and degree of excitement when interpreting selected hematological data in a clinical setting.

Keywords: Exercise, Red Blood Cells, Spleen
EFFECTS OF LACTOFERRIN ON STALLION SPERM SURVIVAL AND FUNCTION IN VITRO. D.G. Howell, E.E. Clark, C.R.F. Pinto, and M.A. Coutinho da Silva.
Department of Veterinary Clinical Sciences

Our laboratory has recently demonstrated that lactoferrin reduces post-breeding uterine inflammation in mares. Potentially, lactoferrin could be incorporated into commercially available semen extenders to modulate this uterine inflammation post-breeding. Our study aimed at determining the effects of lactoferrin on sperm survival and function in vitro. Our hypothesis was that addition of lactoferrin to semen extender would not affect sperm motility and viability. Four ejaculates from four stallions were collected and seminal plasma was removed by centrifugation. Sperm were resuspended in extender containing 0 (control), 10, 20, and 30 mg/mL of lactoferrin. Samples were stored at 5°C and evaluated at 0, 24, and 48 h for motility (Computer-Assisted Semen Analysis and subjective readings) and viability (NucleoCounter®). At 24 h of incubation, an aliquot of each sample was cultured under aerobic conditions to determine the growth of microorganisms. Data were analyzed by ANOVA for repeated measures and significance set at P<0.05. Total and progressive motility were similar between control and lactoferrin groups at times 0 and 24 h post-incubation. However, a significant decrease in total and progressive motility was observed at 48 h for 20 mg/ml (47% & 24%) and 30 mg/ml (44% & 22%) lactoferrin compared to control (61% & 38%), respectively. No significant changes in sperm survival were observed between groups. Growth of microorganisms was significantly reduced when lactoferrin was present at 20 mg/ml and 30 mg/ml. In conclusion, higher concentrations of lactoferrin (20 and 30 mg/ml) reduced sperm motility after 48 h of incubation. However, lactoferrin did not affect sperm viability over time and was effective in reducing the growth of microorganisms.

Keywords: Lactoferrin, stallion, semen, spermatozoa, horse
SKIN LEVELS OF CYCLOSPORINE IN VARIOUS TOPOGRAPHIC LOCATIONS IN NORMAL DOGS. K. Moning, A. Hillier DVM Dept. of Veterinary Dermatology

Cyclosporine (CSA) at 5mg/kg PO once daily is one of the few treatments with good evidence for efficacy in the control of atopic dermatitis (AD). We evaluated the effect of duration of treatment, body location, and timing of sampling on skin CSA concentrations. We hypothesized that when sampled from multiple topographic locations, the skin would have similar CSA concentrations in all 4 areas of interest: dorsal neck, lateral neck, axilla, and ventral abdomen.

This study was performed with 2 groups of healthy class A research dogs with 3 dogs in each group. Group 1 (G1) was given 5 mg/kg daily throughout 28 days and Group 2 (G2) given 2.5 mg/kg CSA concurrent with 2.5 mg/kg KTZ daily throughout 28 days. Blood and skin peak and trough samples were collected on a weekly basis and sent to The University of Colorado, Denver. All skin samples were collected by 8-mm skin punch biopsy and were analyzed via HPLC assay. There was no difference between G1 and G2 in the weekly mean skin (2.03 and 2.32 ng/mg respectively) and whole blood concentrations (313 and 338 ng/ml respectively) of CSA.

On days 1 and 28 skin samples were collected at hours 2-6 post medication administration. Skin samples were also collected from 4 locations including the dorsal neck, lateral neck, axilla, and ventral abdomen on days 15, and 21. Skin CSA concentrations were highly variable (0.29 – 8.59 ng/mg). Highest 4-hour post administration skin CSA concentrations were detected on days 14 or 21 in G1 and day 7 in all 3 dogs in G2. Highest skin concentrations varied and no clear time of peak skin concentration could be determined. Mean CSA skin concentrations by location showed dorsal neck＞axilla＞lateral neck＞ventral abdomen. Due to individual variability, monitoring skin CSA concentrations may not be clinically useful.

Keywords: Cyclosporine A, Ketoconazole, Atopic Dermatitis
While the Indian rhino captive breeding program has been rather successful, improperly timed introductions for mating can lead to severe aggression between the male and female. To minimize this risk, pairings or artificial insemination (AI) must be timed to ovulation as accurately as possible. Currently, urinary concentrations of estrogen and progesterone metabolites are used to monitor ovarian function and time breeding or AI. Collecting clean urine samples consistently can be a major challenge with some rhinos, and has limited the number of individuals for which urinary hormone monitoring can be used. Reproductive hormones can also be detected in saliva, and have been previously measured in a female Indian rhino. However, before saliva can be used as the sole biological sample for monitoring ovarian function in this species, it must be proven to be reliable, accurate and reflective of urinary hormone profiles.

Matched saliva and urine samples were collected from 3 adult female Indian rhinos and analyzed using enzyme immunoassays (EIA) for testosterone, estrogen conjugate (EC), progesterone metabolite (PdG) progesterone (P4) and cortisol. Rectal ultrasound exams were conducted on 2 females to monitor follicular growth and verify ovulation. Serial dilutions of pooled saliva samples produced displacement curves parallel to the testosterone (r = .98), progesterone (r = .99) and cortisol (r = .99) standard curves. Preliminary results indicate matched values of salivary testosterone and urinary EC were correlated throughout the follicular phase of the estrous cycle. Profiles of salivary P4 and urinary PdG were also positively associated and reflected the luteal phase. Finally, it appears a cyclic variation in salivary and urine cortisol levels may occur during the estrous cycle. However, additional data is warranted. Salivary hormone analysis can serve as an alternative method to monitor estrous cycle dynamics in the Indian rhino.

Keywords: Indian rhino, Rhinoceros unicornis, salivary hormones, estrous cycle

Blood is carried to the rectum by the Cranial Rectal artery (a.), and may also be delivered by the Median Sacral a. and the Internal Pudendal a. These arteries were examined in cats and dogs that were selected from a sample population which included a range of ages, weights, and both genders. Analysis of rectal blood vessels in dissected specimens revealed some differences in the branching pattern of arteries between cats and dogs. The cat was found to have a more elaborate network of primary and secondary branches from the Cranial Rectal a. to the rectum. Our analysis also included the calculation of a ratio between the Caudal Mesenteric a. length and the Cranial Rectal a. length. From this ratio we found that the Cranial Rectal a. was proportionally longer in the cat, indicating that the Cranial Rectal a. branches from the Caudal Mesenteric a. closer to the abdominal aorta. We concluded that a longer Cranial Rectal a. is associated with more arterial branches to the colon, and thus cats have more numerous Cranial Rectal a. branches to the colon than dogs. Our findings on rectal artery branching patterns are relevant to current surgery practices, as cats typically recover more quickly than dogs following subtotal colectomy for megacolon. Though more dissections are needed to confirm our hypothesis, our research suggests that the more intricate arterial pattern in the feline rectum, as well as the ratio of the Caudal Mesenteric a. length to the Cranial Rectal a. length, may be the reason for more rapid healing time in cats than dogs following rectal surgery. Knowledge generated from this and future studies will be applicable to veterinary medicine in the development of measures to provide more constant blood flow to the rectum of dogs. Such advances may prevent prolonged healing following subtotal colectomy.

Keywords: artery, blood, cranial, colectomy, internal, median, medicine, megacolon, pudendal, rectal, rectum, sacral, subtotal, veterinary.