ADVANCES IN VETERINARY MEDICINE RESEARCH WEEK
APRIL 11 – 14, 2011
ALL SEMINARS TAKE PLACE AT 12:00 PM IN THE
VETERINARY MEDICAL CENTER AUDITORIUM
Corner of Coffey Road and Tharp Street
Enter from Coffey Road and go up the stairs

MONDAY, APRIL 11TH
“Biomedical Imaging: Insight, Imagination and Innovation”
Dr. Michael Knopp
Professor of Radiology and Novartis Chair of Imaging Research

TUESDAY, APRIL 12TH
“Gut Reactions to Enteric Viruses and Vaccines”
Dr. Linda Saif
Distinguished University Professor
Departments of Veterinary Preventive Medicine and Food animal Health
Research Program

WEDNESDAY, APRIL 13TH
“Scientific Discovery in Infectious Diseases at the Intersect of Animal and Human Health; effective partnerships that Breed Success”
Dr. Larry Schlesinger
Samuel Saslaw Professor of Medicine
Director, Division of Infectious Diseases and the Center for Microbial Interface Biology

2011 SPONSORS:
Ohio State College of Veterinary Medicine Alumni Society
Center for Clinical and Translational Science
Pfizer Animal Health
Fisher Scientific
PROGRAM

April 14, 2011

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. Guy Palmer
Regents Professor and Creighton Endowed Chair, Pathology and Infectious Diseases
Director of the Paul G. Allen School for Global Animal Health
Washington State University

“The Role for Veterinary Medicine in Global Health”

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

PROGRAM CHAIR
Dr. Xin Li

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 14TH
12:15 – 2:00 pm

AWARDS PRESENTATION AND KEYNOTE ADDRESS

“The Role for Veterinary Medicine in Global Health”

Dr. Guy Palmer
Regents Professor and Creighton Endowed Chair
Pathology and Infectious Diseases
Director of the Paul G. Allen School for Global Animal Health
Washington 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 13th: 3:00-5:00 pm for Professional Students
April 14th: 8:00-10:30 am for Graduate Students

2011 SPONSORS:
Ohio State College of Veterinary Medicine Alumni Society
Center for Clinical and Translational Science
Pfizer Animal Health
Fisher Scientific
POSTER JUDGING SESSIONS

Wednesday, April 13, 2011
3:00 – 5:00 pm
Veterinary Student Poster Judging

Thursday, April 14, 2011
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 80 posters.

Melanie Abley  Chris Adin  Famke Aeffner
Matthew Allen  Raj Anupam  Jim Belknap
Andrew Bowman  Prosper Boyaka  Puja Buch
Andrew Dahlem  Josh Daniels  Rebecca Garabed
Wondwossen Gebreyes  Susan Giovengo  Pat Green
Kat Ham  Kate Hayes-Ozello  Kazu Ishihara
Eason Hildreth  Seth Jump  Bill Kerns
Bill Kisseberth  Krista La Perle  Xin Li
Sharell Mikesell  Mike Oglesbee  Judith Radin
Paivi Rajala-Schultz  Mike Rohovsky  John Sagartz
Karsten Schober  Judi Stella  Ramiro Toribio
Tom Wittum
The following posters are located in Sisson Hall

**Immunology and Infectious Diseases**

**IMID – 1** PROGRESSION FROM ACUTE LUNG INJURY TO ARDS IN MICE INFECTED WITH H1N1 INFLUENZA A VIRUS. F. Aeffner¹, Z. Traylor¹, A. Bratasz, E. Flaño; K.A. Powell, I.C. Davis¹. ¹Departments of Veterinary Biosciences, & ²Biomedical Informatics, The Ohio State University, Columbus, OH 43210, USA; ³The Research Institute at Nationwide Children’s Hospital, Columbus, OH 43205, USA

**IMID – 2** H-2 ALLELES CONTRIBUTE TO ANTIGEN 85-SPECIFIC INTERFERON-GAMMA RESPONSES DURING *MYCOBACTERIUM TUBERCULOSIS* INFECTION. G. Beamer¹,², J. Cyktor¹, B. Vesosky¹, J. Turner¹,³ ¹Center for Microbial Interface Biology, The Ohio State University, Columbus, OH 43210. ²Department of Veterinary Biosciences, The Ohio State University, Columbus, OH 43210. ³Division of Infectious Diseases, Department of Internal Medicine, The Ohio State University, Columbus, OH 43210

**IMID – 3** 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 – 4** INSIGHTS INTO THE REGULATORY MECHANISM CONTROLLING THE INHIBITION OF VACCINE-INDUCED SEROCONVERSION BY MATERNAL ANTIBODIES. D. Kim, D. Huey, M. Oglesbee and S. Niewiesk. Department of Veterinary Biosciences, Ohio State University, Columbus, Ohio, 43210.

**IMID – 5** HSP70 AND A NOVEL AXIS OF INNATE IMMUNITY IN THE VIRUS-INFECTED BRAIN Kim, M.Y.¹, Shu, Y¹, Carsillo, T.¹, Popovich, P.²,³, Niewiesk, S.¹, Oglesbee, M.¹,² The Ohio State University, Departments of Veterinary Biosciences¹, Molecular Virology, Immunology and Medical Genetics², and Neuroscience³, Columbus, Ohio, U.S.A.

**IMID – 6** IN UTERO AND COLOSTRAL TRANSMISSION OF *MYCOPLASMA HAEMOLAMAE* AS DETECTED BY PCR R. L. Pentecost, A. E. Marsh, J. Daleccio, J. Daniels, J. Lakritz, Departments of Veterinary Clinical Sciences and Preventive Medicine, Ohio State University
MCB – 1  **HUMAN T-LYMPHOTROPIC VIRUS TYPE 1 p30 INTERACTS WITH REgY AND ATM (ATAXIA TELANGIECTASIA MUTATED) TO PROMOTE CELL SURVIVAL.** R Anupam\(^1,2\), A Datta\(^1,2\), N Bowden\(^1,2\), N Shkriabai\(^1,3\), M Kvaratskhelia\(^1,3\), M Lairmore\(^1,2,4\)  
\(^1\) Center for Retrovirus Research, \(^2\) Department of Veterinary Biosciences, \(^3\) College of Pharmacy, and \(^4\) Comprehensive Cancer Center, Arthur G. James Cancer Hospital and Solove Research Institute, The Ohio State University, Columbus, Ohio 43210

MCB – 2  **DETERMINING THE IMPACT OF HIGHLY CONSERVED SERINE RESIDUES ON THE FUNCTION OF HTLV-1 p30** N Bowden, R Anupam, M Kesic, M Lairmore Department of Veterinary Biosciences

MCB – 3  **ANTITUMOR EFFECTS OF COMBINED CARBOPLATIN AND GEMCITABINE ON CANINE TRANSITIONAL CELL CARCINOMA CELLS.** JF de Brito Galvao\(^1\), S Murahari\(^2\), WC Kisseberth\(^1\), DJ Chew\(^1\), S Sutayatram\(^2\), N Inpanbutr\(^2\). The Ohio State University, Department of Veterinary Clinical Sciences\(^1\) and Veterinary Biosciences\(^2\).

MCB – 4  **IDENTIFICATION OF THE FUNCTIONAL DOMAINS AND CELLULAR BINDING PARTNERS OF HUMAN T-CELL LEUKEMIA VIRUS TYPE 2 p28 PROTEIN.** R Doueiri\(^1,2\), M Kesic\(^1,2\), S. O. John\(^5\), P. L. Green\(^1,2,3,4\)  
Center for Retrovirus Research\(^1\), Departments of Veterinary Biosciences\(^2\) and Molecular Virology, Immunology, and Medical Genetics\(^3\), Comprehensive Cancer Center and Solove Research Institute\(^4\), The Ohio State University, Columbus, OH 43210, USA.  
Department of Microbiology and Molecular Cell Biology, Cancer Biology and Infectious Disease Research Center, Eastern Virginia Medical School, Norfolk, Virginia 23508, USA\(^5\).

MCB – 5  **BREED-ASSOCIATED DIFFERENTIAL MICRORNA EXPRESSION IN CANINE OSTEOSARCOMA.** Fenger, J.M., Jalkanen, A.L., Ozer, H.G., Sarver, A.L., Subramanian, S., Breen, M., Modiano, J.F., London, C.A., Kisseberth, W.C. Departments of Veterinary Clinical Sciences and Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA

MCB – 6  **STRIKE-COUNTER STRIKE INTERFACE OF THE ANTIVIRAL RESPONSE: RETROVIRUS RNA SILENCING SUPPRESSOR**
AFFECTS CELLULAR MIRNAS WITH STRUCTURAL RESEMBLANCE TO VIRAL RNA. A. M. Hayes, K. Boris-Lawrie, Dept. of Veterinary Biosciences

MCB – 7 RNA HELICASE A IS NECESSARY FOR TRANSLATION OF SELECTED COMPLEX mRNAs: HETERODIMERIZATION MODULATES THE RNA BINDING DOMAIN W. Jing1,2, A. Ranji3,4, C. Bolinger2,3, J. M. Hernandez3,4 and K. Boris-Lawrie1,2,3,4,5
1Department of Veterinary Biosciences, 2Graduate Program in Molecular, Cellular and Developmental Biology, 3Center for Retrovirus Research, 4Center for RNA Biology, 5Comprehensive Cancer Center, The Ohio State University, Columbus, OH 43210, USA

MCB – 8 THE EXPRESSION OF CCAAT/ENHANCER BINDING PROTEINΔ (CEBPD) AND CEBPD TARGET GENES, IS REDUCED IN CHRONIC LPS TREATED RAW 264.7 MOUSE MACROPHAGES. Hicks, M, Couto K and J DeWille. Dept of Veterinary Biosciences, OSUCCC Molecular Biology/Cancer Genetics Program.

MCB – 9 IMMUNORESPONSE TO ALLOGENEIC SYNOVIAL OR XENOGENIC MESENCHYMAL STROMAL CELLS IN A CO-CULTURE MODEL. S.S. Jump, PhD2,3, D.S. Smith3, D.C. Flanigan, MD1,2, and A.L. Bertone, DVM, PhD1,2,3 Comparative Orthopedics Research Laboratory. 1Department of Orthopedics, The Ohio State University Medical Center. 2 Sports Medicine Center, The Ohio State University Medical Center. 3Department of Veterinary Clinical Sciences, The Ohio State University Veterinary Medical Center.

MCB – 10 DIRECT HUMAN ADENOVIRAL BMP-2 OR BMP-6 GENE THERAPY FOR BONE AND CARTILAGE REGENERATION IN A PONY OSTEOCHONDRAL MODEL. M. I. Menendez‡†, D.J. Clark‡, M. Carlton‡, D.C. Flanigan§, G. Jia‡, S. Sammet‡, S. E. Weisbrode¥, M. V. Knopp‡, A. L. Bertone†
† The Wright Center for Innovation in Biomedical Imaging, Department of Radiology, College of Medicine. † Comparative Orthopaedic Research Laboratory, Department of Clinical Sciences, College of Veterinary Medicine. ¥ Department of Biosciences, College of Veterinary Medicine. § Department of Orthopedics, The Ohio State University Medical Center, Columbus, OH, 43210, USA.
MCB – 11 GENETICALLY ENGINEERED JUVENILE HUMAN NEOCARTILAGE FORMATION IN VITRO FOR ARTICULAR CARTILAGE IMPLANTATION. VY Ng1, S Jump2,3, J Mayerson4, D Flanigan1,2, AL Bertone1,2,3 Comparative Orthopaedics Research Laboratory 1Department of Orthopaedics, The Ohio State University Medical Center 2Sports Medicine Center, The Ohio State University Medical Center 3Department of Veterinary Clinical Sciences, The Ohio State University Veterinary Medical Center

MCB – 12 THRIVING IN AN EVER-CHANGING WORLD: CASPASE 3-DEPENDENT PROTEOLYSIS DOWNREGULATES TRANSLATION OF SPECIFIC GENES J. Picking, A. Sharma, J. M. Hernandez, K. Boris-Lawrie, Dept. of Veterinary Biosciences

MCB – 13 IN VIVO REDUCTION OR BLOCKADE OF INTERLEUKIN-1B IN PRIMARY OSTEOARTHRITIS INFLUENCES EXPRESSION OF MEDIATORS IMPLICATED IN PATHOGENESIS. K. S. Santangelo, G. Nuovo, A.L. Bertone. Depts. of Veterinary Biosciences and Veterinary Clinical Sciences

MCB – 14 HUMAN T-CELL LEUKEMIA VIRUS TYPE 2 (HTLV-2) APH-2, AN ANTISENSE GENOME STRAND ENCODED PROTEIN, IS DISPENSABLE FOR VIRAL INFECTIVITY AND PERSISTENCE IN VIVO. H Yin1,3, N Dissinger1,3, R Haines1,3 and P. L. Green1,2,3,4 Departments of Veterinary Biosciences1 and Molecular Virology, Immunology, and Medical Genetics2, Center for Retrovirus Research3, and Comprehensive Cancer Center and Solove Research Institute4, The Ohio State University, Columbus, OH 43210.

MCB – 15 FLLL100, A HIGHLY SOLUBLE ANALOG OF FLLL32, INHIBITS PROLIFERATION OF OSTEOSARCOMA CELL LINES THROUGH DOWNREGULATION OF BOTH P-STAT3 AND TOTAL STAT3 Couto J., Bear M.D., Scwhartz E.B., Li P.K., Fuchs J.R., Kisseberth W.C., London C.A. Department of Veterinary Biosciences, Department of Veterinary Clinical Sciences, Division of Medicinal Chemistry, and the Comprehensive Cancer Center, The Ohio State University, Columbus, OH.

Structure/Function
SF – 1  EVALUATION OF LH RELEASE AFTER THE INTRAUTERINE ADMINISTRATION OF GnRH IN LACTATING DAIRY CATTLE. S. Bas, C.G. Pinto, M.L. Day, G.M. Schuenemann. Department of Veterinary Preventive Medicine, Department of Animal Sciences, The Ohio State University.

SF – 2  EFFECTS OF INTRAPERITONEAL ADMINISTRATION OF BILIRUBIN ON INFARCT AREA AND LEFT VENTRICULAR FUNCTION IN A RAT MODEL OF ACUTE CORONARY OCCLUSION. R. Ben-Amotz, C. Adin, J Bonagura, R. Hamiln. Depts. of Veterinary Biosciences and Veterinary Clinical.

SF – 3  THE NOVEL ENERGY RESTRICTION-MIMETIC AGENT OSU-CG5 SUPPRESSES THE EXPRESSION OF ENZYMES THAT PROMOTE THE WARBURG EFFECT IN VITRO. Berman-Booty, P.C. Chu, D. Wang, S. Kulp, and C.S. Chen. Department of Veterinary Biosciences, Division of Medicinal Chemistry.

SF – 4  MEASUREMENT OF THE FELINE HIPPOCAMPUS USING MAGNETIC RESONANCE IMAGING. Francis KA, Drost WT, Schmalbrock P, Burns P, Buffington CAT. The Ohio State University, Veterinary Clinical Sciences and Wright Center of Biomedical Innovation, Columbus, OH 43210.


SF – 6  REGULATION OF THE GLUCOSE TRANSPORT PATHWAY IN VISCERAL AND SUBCUTANEOUS ADIPOSE DEPOTS IN HORSES WITH INSULIN RESISTANCE. K. Kohler, A. Waller, T. Burns, M. Mudge, J. Belknap and Lacombe VA. College of Pharmacy; Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University.

SF – 7  DILTIAZEM TREATMENT ATTENUATES ARRYTHMOGENESIS DURING DIABETIC CARDIOMYOPATHY BY STABILIZING
RYR2-MEDIATED SR CA RELEASE. V.A. Lacombe, D. Terentyev, S. Viatchenko-Karpinski, R. Hamlin, S. Györke; C.A. Carnes. College of Pharmacy; Department of Veterinary Clinical Sciences; Department of Physiology and Cell Biology, College of Medicine, The Davis Heart and Lung Research Institute; The Ohio State University.

SF – 9 CALCIUM CHANNEL BLOCKER THERAPY SELECTIVELY ALTERS GLUCOSE TRANSPORTERS DURING DIABETIC CARDIOMYOPATHY. M. Parriman, A. Waller, and V. Lacombe. College of Pharmacy and Department of Veterinary Clinical Science, The Ohio State University.


SF – 11 OVEREXPRESSION OF SARCOPLASMIC RETICULUM CALCIUM ATPASE PUMP SELECTIVELY REGULATES GLUCOSE TRANSPORTERS IN THE HEART OF HEALTHY AND DIABETIC SUBJECTS. A.P. Waller¹, A. Kalyanasundaram², S. Hayes¹, M. Periasamy², V.A. Lacombe¹,³ ¹College of Pharmacy, ²Dept. of Physiology and Cell Biology, College of Medicine, ³Dept. of Veterinary Clinical Sciences, The Ohio State University

SF – 12 PROTECTIVE EFFECTS OF MAPK PHOSPHATASE 1 (MKP-1) DURING ACUTE ACETAMINOPHEN TOXICITY. L.M. Wancket, X. Meng, L. Rogers, and Y. Liu. Department of Veterinary Bioscience and Center for Perinatal Research (The Research Institute at Nationwide Children's Hospital)

SF – 13 MOLECULAR CHARACTERIZATION OF THE CANINE BETA-GLOBIN GENE CLUSTER. S Zaldívar-López¹,², J Rowell²,⁴, E Fiala², CG Couto¹, CE Alvarez¹,²,³ Departments of ¹Veterinary Clinical Sciences, ²Pediatrics, ³The Research Institute at Nationwide Children’s Hospital, ⁴College of Nursing

Clinical Research
CR-1 PHARMACOKINETICS OF INTRA-ARTICULAR BETAMETHASONE
SODIUM PHOSPHATE AND BETAMETHASONE ACETATE AND ENDOGENOUS HYDROCORTISONE SUPPRESSION IN EXERCISING HORSES. M. I. Menendez†*, M. A. Phelps‡ AND A. L. Bertone†

†Comparative Orthopaedic Research Laboratory, Department of Clinical Sciences, College of Veterinary Medicine; *Department of Radiology, College of Medicine; ‡Division of Pharmaceutics, Department of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio, 43210, USA.

CR – 2 THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM (RAAS) IN SEPTIC FOALS. K.A. Dembek1; K. Onasch1; R.J. Barsnick1; S.D. Hurcombe1; N.M. Slovis2; B. Barr3; R.E. Toribio1. 1The Ohio State University, College of Veterinary Medicine; Columbus, OH, USA; 2Hagyard Equine Medical Institute, Lexington, KY, USA; 3Rood and Riddle Equine Hospital, Lexington, KY, USA

CR – 3 ASSESSMENT OF MICROCIRCULATORY PERFUSION IN HEALTHY ANESTHETIZED CATS UNDERGOING OVARIOTOMY USING SIDESTREAM ARK FIELD MICROSCOPY. M. Goodnight, E. Cooper. Department of Veterinary Clinical Sciences

CR – 4 ENGINEERED CELL THERAPY FOR BONE AND JOINT REPAIR IN HORSES. I. Ishihara, E. Santschi, and A. Bertone. Depts. of Veterinary Clinical Sciences

CR – 5 ACCURACY OF COMPUTED TOMOGRAPHY IN DETERMINING LESION SIZE IN CANINE OSTEOSARCOMA OF THE APPENDICULAR SKELETON. KS Karnik, EM Green, VF Samii, SE Weisbrode, CA London. The Ohio State University, OH 43210. Veterinary Clinical Sciences

CR – 6 EFFECTS OF AQUALASE® CAPSULE WASHING ON LENS EPITHELIAL CELLS AND PCO FORMATION IN VITRO FOLLOWING PHACOEMULSIFICATION. EA Lutz, DA Wilkie, AJ Gemensky-Metzler, HL Chandler. The Ohio State University, College of Veterinary Medicine, Department of Veterinary Clinical Sciences, Comparative Ophthalmology, Columbus, OH.

Mosseri, S. Reed, VA Lacombe. College of Pharmacy; Department of Veterinary Clinical Sciences; The Ohio State University, Columbus, OH. Rood & Riddle Equine Hospital, Lexington, KY.

CR – 8  THE EFFECT OF MECHANICALARYTENOID CARTILAGE ABDUCTION BEFORE KNOT TYING ON FAILURE OF EQUINE LARYNGOPLASTY IN A CYCLICAL ADDUCTION MODEL. N.R. McClellan1, E.M. Santschi1, S.D.A. Hurcombe1, A.S. Litsky2. Department of Veterinary Clinical Sciences1, and Orthopedic BioMaterials Laboratory2.

CR – 9  CANAL FLARE INDEX IN GERMAN SHEPHERD DOGS, GOLDEN RETRIEVERS, AND LABRADOR RETRIEVERS PRESENTING FOR TOTAL HIP REPLACEMENT: IMPLICATIONS FOR FEMORAL IMPLANT SELECTION. Pugliese LC; Dyce J; Allen MJ. College of Veterinary Medicine, The Ohio State University, Columbus OH

CR – 10  CHONDROGENEIC POTENTIAL OF HUMAN SYNOVIAL-DERIVED MESENCHYMALSTROMAL CELLS AS VECTORS FOR KEY GROWTH FACTORS IN A CO-CULTURE MODEL. Seth S Jump, PhD2,3, Eric B Skinner, BSc3, Vincent Y Ng, MD1 David C Flanigan, MD1,2 & Alicia L Bertone, DVM, PhD1,2,3. 1Department of Orthopedics, The Ohio State University Medical Center 2 Sports Medicine Center, The Ohio State University Medical Center 3Department of Veterinary Clinical Sciences, The Ohio State University Veterinary Medical Center

Epidemiology and Applied Research

EAR – 1  ACTIVITY OF SANGROVIT® AGAINST LAWSONIA INTRACELLULARIS IN GROWER PIGS AND ITS IMPACT ON GUT PHYSIOLOGY AND HOST IMMUNITY. V. Artuso Ponte, M. Abley, B. Molla, G. Rajashekara, P. Boyaka, W. Gebreyes. 1Department of Veterinary Preventive Medicine, 2OARDC Food Animal Health, and 3Department of Veterinary Biosciences.

EAR – 2  SEROPREVALENCE OF NEOSPORA CANINUM AND TOXOPLASMA GONDII IN WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) FROM SIX REGIONS IN THE CLEVELAND METROPARKS. G. Ballash and P. Dennis. Dept. of Veterinary Preventive Medicine

EAR – 3  AN ORTHOTOPIC XENOGRAFT MODEL OF OSTEOSARCOMA WITH METASTASIS. B.K. Chaffee1, F. Xu1, T. J. Sharschmidt2, J.L. Mayerson2, M.J. Allen1, Departments of Veterinary Clinical
EAR – 4 PREVALENCE OF STAPHYLOCOCCUS AUREUS IN BULK TANK MILK AND MANAGEMENT PRACTICES IN OHIO L. da Costa and P. Rajala-Schultz. Department of Veterinary Preventive Medicine, College of Veterinary Preventive Medicine.

EAR – 5 PASSIVE SURVEILLANCE AND GENOTYPING OF STAPHYLOCOCCAL SPECIES ISOLATED FROM PATIENTS AT A VETERINARY TEACHING HOSPITAL. J. Mathews1,2, N. Tiao1, P. Patchanee1, W. Gebreyes1, and Members of the College Infectious Diseases Committee (CIDC)1,2,3. Departments of Veterinary Preventive Medicine1, Veterinary Biosciences2, and Veterinary Clinical Sciences3

EAR – 6 CHARACTERIZATION OF ESCHERICHIA COLI CARRYING blaCTX-M ISOLATED FROM COMMENSAL FLORA OF DAIRY CATTLE. D. Mollenkopf, M. Weeman, M. Abley, J. Daniels, W. Gebreyes, T. Wittum. Dept. of Veterinary Preventive Medicine and Veterinary Clinical Sciences

EAR – 7 IDENTIFICATION OF STAPHYLOCOCCUS AUREUS FROM BOVINE MILK USING BIOCHEMICAL TESTS AND PCR. H. Muftah, P. Rajala-Schultz, W. Gebreyes, F. DeGraves, J. Daniels, L. da Costa. Departments of Veterinary Preventive Medicine and Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA.


Core and Shared Resources
COR – 1 RESEARCH PATHOLOGY SUPPORT FOR ANIMAL MODELS PROVIDED BY THE COMPARATIVE PATHOLOGY & MOUSE PHENOTYPING SHARED RESOURCE.
K. La Perle, Dept. of Veterinary Biosciences

COR – 2 CLINICAL TRIALS OFFICE
N. Stingle, T. Mathie, R. Portela, H. Borghese, C.A. London, and WC Kisseberth, Departments of Veterinary Biosciences and Veterinary Clinical Sciences

COR – 3 VETERINARY BIOSPECIMEN REPOSITORY (TISSUE BANK)
Departments of Veterinary Biosciences and Veterinary Clinical Sciences

Educational
EDU – 1 DEVELOPMENT OF A VIRTUAL REALITY SIMULATOR FOR TEACHING CANINE ARTHROSCOPY. T. Motta, M. Shaw, D. Stredney, J. Au, M. Allen. Dept. Veterinary Clinical Sciences and Ohio Supercomputer Center

The following posters are on display in the Hummel Grand Lounge located in the Veterinary Medicine Academic Building.

VETERINARY STUDENT POSTERS

Clinical Research
VME – 1 PREGNANCY DIAGNOSIS BEFORE COLLECTION AND TRANSFER OF HORSE EMBRYOS. E. Coppelman and C.R.F. Pinto. Dept. of Veterinary Clinical Sciences

VME – 2 EFFECTS OF FLUPHENAZINE DECANOATE ON STRESS AND REPRODUCTIVE CYCLICITY IN BISON (BISON BISON) AM
Curtis1, MM Vick2, P Bapodra3, BA Wolfe1,3
1The Ohio State University College of Veterinary Medicine
2Cleveland Metroparks Zoo
3The Wilds, Cumberland, OH

VME – 3 THE IMPACT OF BEDDING TYPE ON CAGE CHANGEOUT FREQUENCY. R. Erickson, D. Domer, and V. Bergdall. University Laboratory Animal Resources

VME – 4 EFFECTS OF LACTOFERRIN ON POST-BREEDING UTERINE INFLAMMATION IN THE MARE. B. S. Forshey, C. A.
VME – 5 EVALUATION OF RODENT ANESTHESIA: DO STRAIN SPECIFIC RESPONSES EXIST?  C. Hilty, A. Dardenne DVM, and C. Freed MLAS, DVM, DACLAM. University Laboratory Animal Resources.

VME – 6 THE WHITE-COAT EFFECT ON BLOOD PRESSURE IN RETIRED RACING GREYHOUNDS. C.L. Marino, C.G. Couto, M.C. Iazbik. Department of Veterinary Clinical Sciences

VME - 7 HYPOTHALAMIC CONTROL OF PITUITARY AND ADRENAL FUNCTION IN CRITICALLY ILL FOALS. K. Onasch1; K.A. Dembek1; K. Hernon1; S.D. Hurcombe1; N.M. Slovis2; P. Morresey3; R.E. Toribio1. 1The Ohio State University, College of Veterinary Medicine; Columbus, OH, USA; 2Hagyard Equine Medical Institute, Lexington, KY, USA; 3Rood and Riddle Equine Hospital, Lexington, KY, USA

VME – 8 IN VITRO MEASUREMENT OF CIPROFLOXACIN MUTANT PREVENTION CONCENTRATION OF E. COLI ISOLATED FROM CANINE URINARY TRACT INFECTIONS AND ACHIEVABLE CIPROFLOXACIN CONCENTRATION IN THE URINE OF HEALTHY DOGS. G.M. Tracy, S.J. Irom, J.L. Mathews, W.A. Gebreyes, D.C. Chew, J.B. Daniels. Department: Veterinary Clinical Sciences

VME – 9 HEMOSTATIC ACTIVITY OF CANINE PLASMA STORED FOR TRANSFUSION. R. Urban, G. Couto, M.C. Iazbik, D. Hudson, A. DeFelice. Department of Veterinary Clinical Sciences

VME – 10 USE OF COTTON-SWABS FOR DETECTION OF STAPHYLOCOCCUS AUREUS IN BOVINE MILK. A. Wagner, P. Rajala-Schultz, L. da Costa. Department of Veterinary Preventive Medicine

Epidemiology and Applied Research
VME – 11 CENTRALIZED OPERATIONAL WEB-BASED-TECHNOLOGIES PROMOTING ANIMAL TRACEABILITY AND HEALTH (COWPATH). S. Baker¹, D. Roberts¹, K. Cantwell⁵, L. Sparks⁵, S. Huo⁵, R. Garabed¹, L. King¹ Department of Veterinary Preventive Medicine¹, Ted^Edge Summer at the Edge – Wright Brothers Institute²

VME – 12 OCCURRENCE AND MOLECULAR EPIDEMIOLOGY OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) ON FARM, AT SLAUGHTER AND PORK. M. Byrne, B.Z. Molla, M. Abley, W.A. Gebreyes. Dept. of Veterinary Preventative Medicine

VME – 13 IDENTIFICATION OF NEOSPORA CANINUM OOCYSTS FROM FECES OF OHIO COYOTES (CANIS LATRANS) USING QUANTITATIVE PCR. S Gupta, B Wolfe, P Rajala-Schultz. The Ohio State University College of Veterinary Medicine Department of Veterinary Preventive Medicine & The Wilds

VME – 14 THE IMPACT OF CEFTIOFUR REMOVAL ON RECOVERY OF SALMONELLA SPP. AND E. COLI RESISTANT TO THIRD GENERATION CEPHALOSPORINS. K. Kleinhenz¹, D. Mollenkopf¹, L. Heider¹, J. Funk², T. Wittum¹ ¹ Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio; ² Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan

VME – 15 THE USE OF HEAVY METALS IN SWINE FEED AND ITS ASSOCIATION WITH THE PRESENCE OF COPPER AND ZINC TOLERANT SALMONELLA. M. Nicol, B. Molla, and W. Gebreyes. Dept. of Veterinary Preventive Medicine

VME – 16 PREVALENCE OF MRSA IN INCOMING HORSES AT THE OSU VETERINARY MEDICAL CENTER. M. Piraino, J. Braman, J. van Balen, R. Nava-Hoet, C. Kohn, A.E. Hoet. Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University.

VME – 17 CENTRALIZED OPERATIONAL WEB-BASED-TECHNOLOGIES PROMOTING ANIMAL TRACEABILITY & HEALTH (COWPATH). S. Baker, D. Roberts. Dept. of Veterinary Preventive Medicine. Dr. L. King and Dr. R. Garabed
VME – 18  INFLUENCE OF ABIOTIC FACTORS ON THE PREVALENCE OF AVIAN ORIGIN TYPE A INFLUENZA VIRUSES IN THE ENVIRONMENT. C. Schwarten1, R. Slemons1, R. Gates2. 1The Ohio State University Department of Veterinary Preventive Medicine, 1920 Coffey Road, Columbus, Ohio 43210. 2The Ohio State University School of Environment and Natural Resources, 2021 Coffey Road, Columbus, Ohio 43210

VME – 19  SAMPLING TSETSE (GLOSSINA SPP) FLY POPULATIONS TO MODEL TRYPANOSOMIASIS RISK: A PILOT STUDY IN THE FAR NORTH REGION OF CAMEROON. S. Valerius, E. Walz, R. Garabed. Department of Veterinary Preventive Medicine, the Ohio State University

VME – 20  A CASE STUDY OF TWO VECTOR-BORNE DISEASES IN HUMANS AND ANIMALS OF THE FAR NORTH REGION OF CAMEROON: IMPLICATIONS FOR PREVENTATIVE MEASURES E. Walz, R. Garabed, D. Ewing, M. Moritz, Dept. of Veterinary Preventive Medicine and Dept. of Anthropology


VME – 23  PRACTICAL APPLICATION OF ONE HEALTH PRINCIPLES IN THE DEVELOPING WORLD: THE ROLE OF VETERINARY MEDICINE IN SIERRA LEONE. T. Strickler1, J. Zientek1, A. Sesay2, R. Suluku2 and F. Silveira1. 1 Department of Veterinary Preventive Medicine, The Ohio State University, USA. 2 Animal Science Department, Njala University, Sierra Leone.

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VME – 24  IHC AND ISH IDENTIFICATION OF REPLICATION CELL TYPES AND DISPERSION OF INFECTED CELLS IN TTV, AND TTV/PRRSV CO-INFECTED GNOTOBIOTIC PIGS. C. Cheney. Department of Veterinary Biosciences
VME – 25 MOLECULAR EVOLUTION OF PORCINE CIRCOVIRUS TYPE 2 (PCV2): ACQUISITION OF VIRULENCE BY MUTATIONAL EVENT(S) IN THE NUCLEOCAPSID PROTEIN. D. Corsmeier, S. Krakowka. Dept. of Veterinary Biosciences

VME – 26 DETECTION OF RABBIT FOXP3+CD4+CD25+ REGULATORY T CELLS IN THE GALT OF A HUMAN T-LYMPHOTROPIC VIRUS TYPE 1 (HTLV-1) RABBIT MODEL. M. O’Brien, R. Haines, P. Kannian, R. Urbiztondo, and M.D. Lairmore. 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 43210 USA

VME – 27 AEROSOLIZED NUCLEOTIDE SYNTHESIS INHIBITORS FOR TREATMENT OF INFLUENZA. Rivera, P. Davis, I. Department of Veterinary Biosciences

VME – 28 EFFECT OF H1N1 INFLUENZA VIRUS (A/PR/8) ON LUNG FUNCTION IN BALB/C MICE. Sherman, A. Aeffner, F. Traylor, Z. Davis, IC. Department Of Veterinary Biosciences

VME – 29 EFFECTS OF ESTRIOL (E3) ON REGULATORY DENDRITIC CELLS: A POTENTIAL THERAPEUTIC FOR AUTOIMMUNE DISEASE. A. Bedarf, D. Muth, T. Papenfuss, A. Singh, C. Taylor, A. White, Z. VanGundy. Department of Veterinary Biosciences, The Ohio State University, Columbus, OH 43210

VME – 30 SUPPRESSIVE EFFECTS OF THE TUMOR MICROENVIRONMENT ON CANINE MYELOID CELLS. J Wasserman, L Diese, Z VanGundy (Department of Veterinary Biosciences), A Singh, C London (Department of Veterinary Biosciences), T Papenfuss (Department of Veterinary Biosciences)

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VME – 31 ACUTE LPS TREATMENT INDUCES CCAAT/ENHANCER BINDING PROTEINδ (C/EBPδ) EXPRESSION AND NUCLEAR FACTOR-KAPPAB (KB-κB) DNA BINDING ACTIVITY IN THE RAW 264.7 MONOCYTE/MACROPHAGE CELL LINE. K. Couto, J. Dewille, and X. Yu. Department of Veterinary Biosciences

VME – 33  EVALUATION OF A PROTOTYPE NEEDLE TO CONCENTRATE AND ISOLATE STEM CELLS FROM BONE MARROW ASPIRATES. H. Helbig, B.S., A. Ishishara, DVM, PhD, R. Sanchez-Hodge, M. Wellman DVM, MS, PhD, DACVP, and A. L. Bertone, DVM, PhD, DACVS. Depts. Of Veterinary Clinical Sciences and Veterinary. Biosciences, College of Veterinary Medicine, The Ohio State University.

VME – 34  NEORICKETTSIA SURFACE ANTIGEN RECOGNITION BY HORSES WITH POTOMAC HORSE FEVER. S. Moesta, K. Gibson, Y. Rikihisa. Depts. of Veterinary Biosciences

VME – 35  ENHANCING ANTIPROLIFERATIVE EFFECTS OF CALCITRIOL ON CANINE TRANSITIONAL CELL CARCINOMA OF THE URINARY BLADDER BY CURCUMIN IN VITRO E. Shull, W. Smith, N. Inpanbutr, Department of Veterinary Biosciences

VME – 36  ENHANCING RADIOSENSITIVITY OF CANINE TRANSITIONAL CELL CARCINOMA OF URINARY BLADDER BY CURCUMIN IN VITRO. Smith, W., Shull, B., Green, E., Inpanbutr, N. The Ohio State University College of Veterinary Medicine, Department of Veterinary Biosciences

VME – 37  GENETIC CHARACTERIZATION AND CHEMOTHERAPEUTIC STUDIES ON FELINE ORAL SQUAMOUS CELL CARCINOMA. D. Yanik; S. P. S. Pillai; C. K. Martin; T. J. Rosol. Dept of Veterinary Biosciences.

Structure/Function

VME – 38  IN-VITRO EFFECTS OF BILIRUBIN ON PANCREATIC ISLET CELL VIABILITY. D. Carabetta, E. Zmuda, F. Xu, T. Hai, G. Hadley, C. Adin. Departments of Veterinary Clinical Sciences (D.C., F.X., C.A.), Surgery(G.H.) and Cellular/Molecular Biochemistry (E.Z., T.H.), The Ohio State University, Columbus, OH

VME – 39  LAMINAR INFLAMMATORY EVENTS AND EPITHELIAL STRESS AT OBEL GRADE 3 LAMENESS IN THE CARBOHYDRATE OVERLOAD MODEL OF EQUINE LAMINITIS. M. Hensel¹, L.A. Fugler², B. Leise¹, M. Watts¹, S. Eades², and J. Belknap¹. ¹. Dept. of Veterinary Clinical Sciences, The Ohio State University College of Veterinary Medicine, Columbus, OH. ². Louisiana State University School of Veterinary Medicine, Baton Rouge, LA.
P38 MAPK SIGNALING IN EQUINE LAMINITIS: THE EFFECT OF DIGITAL HYPOTHERMIA. C. Kelly, M. Watts, A. Van Eps, J. Belknap. Dept of Veterinary Clinical Sciences
IMID - 1

PROGRESSION FROM ACUTE LUNG INJURY TO ARDS IN MICE INFECTED WITH H1N1 INFLUENZA A VIRUS. F. Aeffner1, Z. Traylor1, A. Bratasz, E. Flaño; K.A. Powell, I.C. Davis1. 1Departments of Veterinary Biosciences, & 2Biomedical Informatics, The Ohio State University, Columbus, OH 43210, USA; 3The Research Institute at Nationwide Children’s Hospital, Columbus, OH 43205, USA

Rationale: Patients with severe influenza pneumonia exhibit many pathophysiologic features of ARDS: acute hypoxemia; decreased P_aO_2:F_iO_2 (P:F) ratios when mechanically ventilated; increased airway resistance and decreased lung compliance; and non-cardiogenic pulmonary edema. However, in mouse models, influenza disease severity is often based solely upon histopathologic criteria. The current study aimed to determine whether mice infected with influenza A exhibit pathophysiologic changes consistent with ARDS.

Methods: 8 week-old BALB/c mice were infected intranasally with mouse-adapted H1N1 influenza (A/WSN/33; 10,000 FFU/mouse), or mock-infected with virus diluent (PBS/0.1% FCS). Lung function was analyzed by the forced oscillation technique. Water signal in lungs was detected by magnetic resonance imaging (MRI). Carotid P_aO_2 was measured using an iSTAT blood gas analyzer.

Results: Influenza infection resulted in significantly increased basal airway resistance and a progressive decrease in lung compliance from day 2. In mice infected with influenza for 2 or 6 days, respectively, the P:F ratio was consistent with current definitions of ALI (P:F < 300) and ARDS (P:F < 200) following ventilation on 100% O_2 (F_iO_2 = 1) for 15 minutes. Uninfected mice maintained a normal P:F ratio (P:F > 600) under the same conditions. At day 6, bilateral hyperintense regions, indicative of severe pulmonary edema, were detectable by MRI in the lungs of influenza-infected mice. Influenza-infected mice exhibited normal heart weight:tibia length ratios, with no evidence of either cardiac pathology or cardiac infection with influenza.

Conclusions: Mice infected with a lethal dose of mouse-adapted influenza A meet current pathophysiologic definitions of ARDS. These findings further validate the mouse influenza model and suggest that analysis of treatment effects on these functional readouts may provide more relevant information than simple histopathology.

Keywords: Blood Gas Analysis; H1N1 Influenza A Virus; Magnetic Resonance Imaging; Mice; Acute Respiratory Distress Syndrome.
H-2 ALLELES CONTRIBUTE TO ANTIGEN 85-SPECIFIC INTERFERON-GAMMA RESPONSES DURING MYCOBACTERIUM TUBERCULOSIS INFECTION.

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The in vitro immune responses to mycobacterial antigens have been linked to the major histocompatibility (H-2) loci in mice. We evaluated in vitro and in vivo immune responses during early Mycobacterium tuberculosis (M.tb) pulmonary infection of C57BL/6 (H-2b), C57BL/6 (H-2k), CBA/J (H-2k), and C3H/HeJ (H-2k) mice to determine H-2k-dependent and -independent effects. H-2k-dependent effects included delayed and diminished Ag85-specific Th1 cell priming, a reduced frequency of Ag85-specific IFN-γ producing cells, reduced IFN-γ protein in vivo, and increased M.tb lung burden as demonstrated by C57BL/6 H-2k mice vs C57BL/6 mice. H-2k-independent factors controlled the amount of Ag85-specific IFN-γ produced by each cell, T cell numbers, granuloma size, and lymphocytic infiltrates in the lungs. Overall, these results suggest that an H-2k-dependent suboptimal generation of Ag85-specific cells impairs control of early M.tb growth in the lungs. H-2k-independent factors influence on the potency of IFN-γ producing cells and on immune cell trafficking during pulmonary M.tb infection. These results have implications for understanding the immune responses in humans with known major histocompatibility polymorphisms associated with increased risk of tuberculosis.

Key words: tuberculosis, major histocompatibility complex, immune response, interferon-gamma, antigen 85
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

HTLV-1 is the causative agent of a highly aggressive CD3+CD25+CD4+CD8- T-cell malignancy, adult T-cell leukemia/lymphoma (ATL). Vertical transmission of the virus from mother to child via breast milk is thought to be the primary route of exposure in endemic areas and has been specifically linked to the development of ATL. Our overall goal is to characterize the rabbit model by functionally analyzing distinct subpopulations of T-cells (CD4+, CD8+, T-regulatory cells) in normal rabbits and during mucosal transmission of the virus. I hypothesize T-cell subsets (T-regulatory and cytotoxic T-cells) in the GALT are key determinants of orally-acquired HTLV-1 infection. The objectives of this study were to develop and characterize an oral model of HTLV-1 infection in the rabbit model. In phase one, mononuclear cells were isolated from key GALT sites and analyzed using flow cytometry and immunohistochemistry to determine reference ranges of phenotype populations. In phase two, 12 week old female New Zealand White rabbits were orally inoculated with R-49 cells, then monitored with complete blood counts and differential white blood cell counts, serum antibody response to HTLV-1 antigens, detection of p19 antigen and pro-viral load in peripheral blood mononuclear cells (PBMCs), and evaluation at necropsy of key gut-associated lymphoid tissues. In phase one, our data indicate that rabbits, like humans, have a predominant CD4+ lymphocyte population throughout the GALT. In phase two, the rabbits that developed infection had a delayed and less intense humoral response, variable leukocytosis, and a delayed p19 antigen in PBMCs as compared to IV exposed rabbits. We conclude that this model mimics infant infection by repeated exposure to infected lymphocytes through breast milk. This model will provide fundamental information about the mucosal microenvironment during the early stages of orally-acquired HTLV-1, critical to understanding establishment of infection in breastfeeding children.

Keywords: HTLV-1, Mucosal Immunology, Oral transmission, Cytotoxic T-cells, T-regulatory cells
INSIGHTS INTO THE REGULATORY MECHANISM CONTROLLING THE INHIBITION OF VACCINE-INDUCED SEROCONVERSION BY MATERNAL ANTIBODIES. D. Kim, D. Huey, M. Oglesbee and S. Niewiesk. Department of Veterinary Biosciences, Ohio State University, Columbus, Ohio, 43210.

The inhibition of vaccination by maternal antibodies is a widely observed phenomenon in human and veterinary medicine. Maternal antibodies are known to suppress the B cell response. This is similar to antibody feedback mechanism studies where passively transferred antibody inhibits the B cell response against particulate antigens due to epitope masking. In the absence of experimental data addressing the mechanism underlying inhibition by maternal antibodies, it has been suggested that epitope masking explains the inhibition by maternal antibodies, too. Here we report that in the cotton rat model of measles virus (MV) vaccination passively transferred MV-specific IgG inhibit B cell responses through cross-linking of the B-cell receptor (BCR) with FcγRIIB. The extent of inhibition increases with the number of antibodies engaging FcγRIIB and depends on the Fc region of antibody and its isotype. This inhibition can be partially overcome by injection of MV-specific monoclonal IgM antibody. IgM stimulates the B-cell directly through cross-linking the BCR via complement protein C3d and antigen to the complement receptor 2 (CR2) signaling complex. These data demonstrate that maternal antibodies inhibit B cell responses by interaction with the inhibitory/regulatory FcγRIIB receptor and not through epitope masking.

Keywords: Maternal antibodies, Vaccination, Measles, Cotton rat
HSP70 AND A NOVEL AXIS OF INNATE IMMUNITY IN THE VIRUS-INFECTED BRAIN

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The major inducible 70 kDa heat shock protein (hsp70) promotes viral gene expression, but also stimulates innate immunity when released into the extracellular environment. It is unknown how these intracellular and extracellular functions of hsp70 combine to influence outcome of viral infection. We addressed this deficiency using the mouse model of measles virus (MeV) infection of neurons in brain. We generated transgenic (TG) mice that constitutively express hsp70 in neurons (i.e., a primate-pattern of hsp70 expression), and showed that these mice are completely protected from Edmonston MeV intracranial inoculation, in contrast to 35% mortality in non-transgenic (NT) infected controls. Complete protection required a viral transcriptional response to hsp70, reflected enhanced innate immunity, and was associated with elevated expression of Toll-like receptors (TLR) 2 and 4 and markers of brain macrophage (microglial) activation. In the current work, analysis of infected brain RNA showed that hsp70-dependent innate immunity was associated with a significant interferon beta (IFN-β) response that was not observed in NT mice. In addition, infected TG mice showed increased expression of genes associated with IFN signaling, macrophage activation and antigen presentation. In vitro data established a model for the hsp70-dependent innate immunity. We showed that MeV-infected mouse neuronal cell lines release hsp70 and that purified extracellular hsp70 stimulates production of IFN-β by a mouse microglial cell line. The result is consistent with extracellular hsp70 stimulation of TLR and represents a novel mechanism by which any virus causing hsp70 release may activate innate immune responses within uninfected cells.

Key words: extracellular hsp70, measles virus, IFN-β, innate immunity
IN UTERO AND COLOSTRAL TRANSMISSION OF MYCOPLASMA HAEMOLAMAE AS DETECTED BY PCR

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*Mycoplasma haemolamae*, a hemoparasite affecting camelids, causes variable degrees of parasitemia and anemia. Juvenile and immunocompromised individuals are more likely to become heavily parasitized with development of severe anemia leading to death. Treated individuals may recover but remain carriers. Understanding parasite biology, transmission, and host-parasite interactions allows for parasite control and prevention through alterations in husbandry practices. *M. hameolamae* mode of transmission is unknown. The study aim was to define whether *M. haemolamae* is transmitted *in utero* or through colostrum ingested by new-born cria. Blood was obtained from 56 pregnant female alpacas and their newborn cria as follows: A) mother’s blood (0 hour), B) pre-suckle cria blood (0 hour), C) mother’s colostrum (0 hour), D) post-colostral cria blood, (48-72 hours after colostrum ingestion). Whole blood DNA was extracted for PCR analysis using *M. haemolamae* specific primers. Plasma immunoglobulin (IgG) concentrations were determined in cria blood to compare and ensure uptake of maternal IgG from colostrum. *M. haemolamae* DNA was detected in 16/56 mother’s blood at parturition. *M. haemolamae* DNA was detected in 16/56 mother’s blood at parturition. The pre-colostrum plasma IgG concentration of this cria’s was > 400 mg/dL suggesting *in utero* response to an antigen; in contrast the remaining 53/54 crias were <100 mg/dl. Clinical disease was not present in this cria. All colostrum samples tested negative by PCR, indicating the organism is not shed in colostrum. After colostrum ingestion no crias were *M. haemolamae* PCR positive. Demonstration of colostral antibodies to *M. haemolamae* was possible using IFA slide preparation in 16/56 dam’s colostrum. This study has demonstrated: 1) rare *in utero* transmission of *M. haemolamae*, 2) *M. haemolamae* is not detected in colostrum, and 3) colostral antibodies exist. Additional research is required to evaluate blood sucking insects’ role as vectors in transmission.

Keywords: alpaca, PCR, *Mycoplasma haemolamae*
HUMAN T-LYMPHOTROPIC VIRUS TYPE 1 p30 INTERACTS WITH REGγ AND ATM (ATAAXIA TELANGIECTASIA MUTATED) TO PROMOTE CELL SURVIVAL

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Human T cell leukemia virus type 1 (HTLV-1), is complex deltaretrovirus and the first retrovirus to be associated with a human malignancy. HTLV-1 primarily infects CD4+ T-cells however it can also infect CD8+ T-cells, B-cells and macrophages. Approximately 15-25 million individuals worldwide are infected by HTLV-1 of which approximately 2-5% develop HTLV-1 associated disorders. HTLV-1, causes adult T-cell leukemia/lymphoma (ATL), an aggressive CD4+ T cell malignancy, and initiates a variety of immune-mediated disorders. HTLV-1 encodes typical structural and enzymatic genes gag, pol and env and various regulator and accessory genes. One such regulatory protein is p30, encoded in pX ORF II. Our lab has pioneered the study to understand the role of p30 in viral infection and transformation. p30 interacts with key cellular proteins such as CBP/p300 and Myc/TIP60 to differentially modulate host and viral gene expression. We hypothesize that interaction of p30 with host cellular proteins modulates cellular microenvironment in favor of viral spread and establishment of infection. In this study we used mass spectrometry and biochemical techniques to identify REGγ and ATM as two novel host proteins that interact with p30. ATM is a critical protein in DNA damage repair pathway and is activated by phosphorylation at ser-1981 (pATM) upon DNA damage. The expression of p30 reduced levels of phosphorylated ATM (pATM) and ATM and enhanced cell survival upon genotoxic stress. REGγ a nuclear proteasome activator binds to key cell cycle regulators and targets them to proteasome degradation. Surprisingly, the levels of p30 were in concert with the expression of REGγ indicating that p30 is stabilized by interacting with REGγ. Co-elution of p30, REGγ and ATM in size exclusion fractions suggest p30 being a part of a multiprotein complex. The current focus of our lab is to use deletion mutants of p30 and REGγ to map the regions which are involved in the interaction. Our data suggests that HTLV-1 p30 interacts with ATM and REGγ providing a novel explanation of how the viral protein may serve to increase viral spread by facilitating cell survival.

Keywords: DNA damage, Cell survival, Proteasome degradation
Human T-cell lymphotrophic virus type 1 (HTLV-1) is the causative agent of Adult T-cell leukemia/lymphoma (ATL). ATL is generally fatal within one year of diagnosis. During the long latency period between infection and disease the virus must balance the maintenance of its genome with avoiding elimination by the immune system. HTLV-1 p30 is a viral protein involved in maintaining this critical balance. p30 regulates viral and cellular gene expression, plays a role in DNA damage repair and cell cycle regulation. The specific residues associated with these functions are unknown.

We examined several p30 sequences and noted 11 highly conserved serines. Based on the level of conservation, ability of serines to be components of motifs and ability to be post translationally modified, we hypothesize that the multiple functions of p30 are related to the 11 highly conserved serines. The objectives of our study are to 1. Determine the phosphorylation status of the highly conserved serines 2. Determine which motifs and mini motifs present in p30 incorporate these highly conserved serines.

We used PhosPhoNet 2 to predict the probability of the eleven highly conserved serine residues being phosphorylated (phosphorylation scores ranging from 0.71-0.99). $^{32}$P orthophosphate labeling of 293T cells transfected with the s-tag p30 expression plasmid was used to test if p30 serines were phosphorylated.

Unexpectedly, in our orthophosphate labeled cells, there was no evidence that p30 is a phosphoprotein.

The evidence that p30 is not a phosphoprotein encourages a more intense focus on motifs and mini motifs which may reveal the biologic significance of the highly conserved serines present in p30. Ultimately, by determining the biologic significance of serines, we will better understand how p30 aids the virus in achieving balance between viral persistence and immune evasion. This increased understanding may reveal potential viral specific drug and or therapeutic targets.

Keywords: HTLV-1, p30, posttranslational modifications, phosphorylation
ANTITUMOR EFFECTS OF COMBINED CARBOPLATIN AND GEMCITABINE ON CANINE TRANSITIONAL CELL CARCINOMA CELLS. JF de Brito Galvao1, S Murahari2, WC Kisseberth1, DJ Chew1, S Sutayatram2, N Inpanbutr2. The Ohio State University, Department of Veterinary Clinical Sciences1 and Veterinary Biosciences2.

Carboplatin has shown little activity as a single agent for the treatment of canine transitional cell carcinoma (TCC). However, gemcitabine has shown synergism with carboplatin in human cell lines. The purpose of this study was to evaluate the activity of gemcitabine against canine TCC cell lines alone or in combination with carboplatin. We hypothesized that gemcitabine in combination with carboplatin would have synergistic effects in vitro. The results of this study could provide a rationale for treatment of canine TCC with the combination of these drugs.

TCC cell lines TCC-Kiss (KISS), K9TCC (JS), K9TCC-PU- AxA (AXA), K9TCC-PU-AxC (AXC), and K9TCC-PU-Sh (SH) were treated with gemcitabine, carboplatin, or the combination. Cell proliferation was assessed using CyQUANT assay, cell cycle was evaluated using propidium iodide staining, and apoptosis was assessed by measuring caspase-3/7 activation. Synergy was quantified by combination index analysis using Compusyn software.

Treatment of canine TCC cell lines with carboplatin or gemcitabine decreased cell proliferation, induced cell cycle arrest, and apoptosis. When TCC cell lines were treated with gemcitabine and carboplatin in combination at a therapeutically relevant concentration, a significant decrease in cell proliferation was observed compared to gemcitabine or carboplatin alone, and drug combination was synergistic in 3 of 5 cell lines, and additive in remaining 2 lines.

Gemcitabine exhibits biologic activity against canine TCC cell lines and carboplatin combined with gemcitabine exhibits synergistic activity at biologically relevant concentrations. Our results support further evaluation of these drugs in dogs with TCC to determine the clinical efficacy of this combination.

Keywords: bladder cancer, in vitro, cell culture, proliferation, combination index, caspase, cell cycle
IDENTIFICATION OF THE FUNCTIONAL DOMAINS AND CELLULAR BINDING PARTNERS OF HUMAN T-CELL LEUKEMIA VIRUS TYPE 2 p28 PROTEIN

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HTLV-1 and HTLV-2 are related but distinct complex retroviruses. HTLV-1 is associated with adult T-cell leukemia and a variety of neurological disorders. In contrast, HTLV-2 is less pathogenic, with few cases of leukemia and neurological disease. In addition to the structural and enzymatic proteins, HTLV encodes regulatory (Tax and Rex) and accessory proteins. Tax and Rex positively regulate virus production and are critical for viral replication and pathogenesis. We previously reported that the accessory gene product of the HTLV-1 and HTLV-2, p30xI and p28xII respectively, acts posttranscriptionally as a negative regulator of both Tax and Rex, by binding to and retaining their mRNA in the nucleus, leading to reduced protein expression and virion production. In cell culture p28 is dispensable, but is required for efficient viral persistence in infected animals. We seek to identify the functional domains of p28 responsible for its biochemical properties and posttranscriptional repression function. In addition we will determine the contribution of phosphorylation and acetylation to p28 function. Finally, using proteomics and molecular techniques we plan to determine the binding partners of p28 to better understand its mechanism of action. Thus far, we have generated and tested several p28 truncations in a reporter assay. Preliminary data shows that the last 60 amino acids of p28 are not required to repress Tax function, and the region 50-100 might be exercising an inhibitory role on p28 function. We also identified 6 serines and 1 tyrosine that are phosphorylated in cells. We are assessing their contribution to the overall function of p28. This study will allow us to better understand the interplay between the viral proteins and the host proteins that govern viral persistence, infectivity and pathogenesis and using comparative studies determine key factors responsible for the increased pathogenesis of HTLV-1 compared to HTLV-2.

Keywords: HTLV-1, HTLV-2, accessory protein p28, posttranscriptional, phosphorylation, acetylation, functional domains.
MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression primarily by targeting mRNAs according to the degree of complementarity with their 3’ untranslated regions. MiRNAs are often dysregulated in cancer, suggesting they play a role in tumorigenesis. Osteosarcoma (OSA) is the most common bone tumor in dogs, however, little is known regarding the mechanisms underlying malignant transformation in these tumors. Breeds such as Rottweilers and Greyhounds are at higher risk for developing OSA, suggesting that heritable factors play a role in this disease. We hypothesize that canine OSA is characterized by breed-associated patterns of miRNA expression. MiRNA profiling of primary canine OSA tumors was performed using the NanoString nCounter human microRNA Expression Assay, interrogating the expression profile of 752 human miRNAs; 168 of whose mature sequences are 100% conserved between human and dog. MiRNA profiling of a panel of normal canine tissues revealed tissue-specific miRNA expression signatures. Independent real time PCR validation of a subset of tissue-specific miRNAs validated the use of the NanoString nCounter Assay as a platform for evaluating miRNA expression in canine tissues. MiRNA expression was evaluated in 48 primary OSA tumors from Greyhound, Golden Retriever, Rottweiler, and mixed breed dogs. Hierarchical cluster analysis revealed distinct breed-associated miRNA expression signatures in canine OSA. 189 miRNAs were differentially expressed in Greyhound, Rottweiler, Golden Retriever, and Mixed Breed tumors (p<0.01). In an expanded cohort of Greyhound and Rottweiler tumors, real time PCR demonstrated that one of these, miR-494 is highly expressed in Rottweiler OSA as compared to Greyhound OSA or normal canine osteoblasts. Studies are currently underway to assess the biological consequences of miR-494 overexpression in normal canine osteoblasts. These data reveal significant correlations between breed and miRNA expression in canine OSA, suggesting breed-associated patterns of miRNA dysregulation may play a role in the pathogenesis of this disease.

Keywords: microRNA, osteosarcoma, canine
The primary line of protection against an infectious agent is the antiviral state. A major mechanism of the antiviral state is RNA silencing by small-RNA. This innate response is conserved across the animal and plant kingdoms. Viruses strike back using viral RNA silencing suppressors (RSS) that are RNAs or RNA binding proteins. A major scientific milestone is to understanding the interface of RSS with the RNA silencing pathway. Retroviruses that infect primates encode Tat RNA binding protein; Tat is an RSS of microRNA (miRNA). Herein, we hypothesized that Tat RSS decreases the abundance of a subset of miRNA. The targets of the differentially expressed cellular miRNA are postulated to be host genes that modulate HIV-1 biology. Herein genome-wide analysis screened the abundance of lymphocyte miRNAs. Microarray and bioinformatics measured change in lymphocyte miRNA abundance upon infection with HIV-1NL4-3 or HIV-1 deficient in Tat RSS (HIV-1(RSS)). Of ~350 host miRNA screened, most miRNA were similar between HIV-1 and HIV-1(RSS) infected CEMx174 cells. However, the RSS mutant HIV-1(RSS) increased the abundance of 24 miRNAs that are downregulated in HIV-1 infection and which bear a U-rich bulge that resembles the viral RNA at the Tat transactivation response (TAR) element in the 5’ untranslated region. Possible mechanisms are: 1) Tat selectively sequesters these miRNA in a complex inaccessible for RNA silencing; 2) pseudo-TAR-Tat interaction is a decoy substrate to TAR RNA binding protein and suppresses localized RNA silencing activity. This analysis requires definition of the targets of the differentially expressed cellular miRNAs and the cognate effect of Tat RSS activity. In sum, our results reveal a new interface of Tat RSS with a subset of miRNA target genes.

Keywords: microRNA (miRNA); RNA silencing suppression (RSS); HIV-1; Tat
RNA HELICASE A IS NECESSARY FOR TRANSLATION OF SELECTED COMPLEX mRNAs: HETERODIMERIZATION MODULATES THE RNA BINDING DOMAIN

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RNA helicase A (RHA) is necessary for translation of selected mRNAs of viral and cellular origin, which are recognized by structural features of their complex 5' untranslated region (UTR). Herein we investigated RHA residues necessary for selective interaction with target RNA. RHA is composed of domains that are interchangeable amongst RNA interactive proteins: C-terminal arginine and glycine-rich domain; central DEIH helicase domain; and N-terminal double stranded RNA binding domains (dsRBD) with conserved α-β-β-α topology; Mutations in the N-terminal domain of RHA (N-term) are associated with human breast neoplasia.

We isolated individual recombinant N-term, central helicase and C-term domains and applied in vitro biochemical measurements and fluorescent polarization assays, and mRNA translation assays in cells. The results indicate that the redundant double-stranded RNA binding domains within N-term directly engage structural features of the target RNA. Exogenously expressed N-term blocks RHA translation activity and substitution mutation of three lysine residues eliminate interaction with target RNA. A second activity of N-term was identified by immunoprecipitations (IPs) to identify protein cofactors. IP results with two epitope-tagged RHA molecules determined RHA heterodimerizes in cells. Moreover, the N-term and C-term domains are necessary and sufficient for the co-IP. Mutation of a lysine residue at the N-term weakens the domain interaction and the RNA binding activity, and abolishes the translational activity. The results indicate that N-term interaction with C-term residues facilitate translation of the target RNA. The outcome is modulation of RHA translation activity between active and inactive states: I) In the active state, N-term tethers ATP-dependent helicase activity to retrovirus and junD mRNA that is necessary for their translation; II) In the inactive state, N-term does not interact with C-terminal residues, and prevents translation of the target RNAs. Our findings are of broad importance to members of the dsRBD family, and post-transcriptional regulation of complex mRNAs.

Keywords: Retrovirus, Post-transcriptional control, RHA, Translation
THE EXPRESSION OF CCAAT/ENHANCER BINDING PROTEIN Δ (CEBPD) AND CEBPD TARGET GENES, IS REDUCED IN CHRONIC LPS TREATED RAW 264.7 MOUSE MACROPHAGES. Hicks, M, Couto K and J DeWille. Dept of Veterinary Biosciences, OSUCCC Molecular Biology/Cancer Genetics Program.

The objective of this study is to determine the influence of acute and chronic lipopolysaccharide (LPS) treatment on CEBPD and CEBPD target genes in the RAW 264.7, a mouse macrophage cell line. CEBPD is a member of the highly conserved CEBP family of DNA binding proteins. Previous work demonstrates that CEBPD functions as a tumor suppressor gene in mammary epithelial cells and induces the expression of downstream target genes that function in inflammation, growth control, differentiation, apoptosis, DNA damage and adhesion. Chronic inflammation has been linked to 15-20% of cancer deaths worldwide. Emerging evidence indicates that chronically activated immune cells secrete products into the tumor microenvironment that promote tumor development and progression. Studies focusing on inflammatory cells indicate that CEBPD is induced in response to acute LPS treatment, but the effects of chronic treatment on CEBPD expression and expression of its target genes have not been reported. We hypothesize that chronic inflammation by LPS in the microenvironment "reprograms" macrophages and alters the profile of secreted products from tumor inhibitory to tumor promoting. Selected CEBPD target genes analyzed in this study were previously identified in ChIP-Chip, ChIP-Seq and microarray studies performed in mammary epithelial cells.

These results demonstrate that acute LPS treatment induces CEBPD and its target genes, including the expression of pro-inflammatory mediators IL-6, IL-1β and CXCL2, demonstrating the key role of CEBPD in the acute innate immune response. CEBPD protein levels, along with expression of IL-1β were undetectable in a chronic LPS environment while IL-6, CXCL2, GP5 and PCDH9 were still induced. This suggests that chronic LPS treatment can "reprogram" CEBPD and its targets. Previous reports indicate that IL-1β induces growth arrest in breast cancer cells. The tumor suppressor function of CEBPD may be reprogrammed by chronic inflammation, resulting in the loss of expression of IL-1β.

Keywords: lipopolysaccharide, CEBPD, IL6, IL10, CXCL2, GP5, PCDH9, IL-1β, chronic inflammation, breast cancer, and macrophages.
IMMUNORESPONSE TO ALLOGENEIC SYNOVIAL OR XENOGENIC MESENCHYMAL Stromal CELLS IN A CO-CULTURE MODEL.

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Advancements in regenerative medicine may harness the potential of an engineered cell source for the repair and restoration of multiple human tissues. Allogeneic cells, from a different organism of the same species or xenogenic cells, from a different species, could provide a ready supply of therapeutic cells for use in tissue repair. Allogeneic mesenchymal stem cells in Phase III clinical trials have been used as a treatment for inflammatory conditions including Crohn’s disease, and autologus cell-based strategies, including autologous chondrocyte implantation and osteochondral grafts, are currently used in orthopedic clinical practice with some promising results. We propose to investigate recipient/host immunoreactions in an in vitro cell co-culture model. The immunoresponse to allogeneic human synovial-derived mesenchymal stromal cells alone or transduced with adenoviral green fluorescent protein (hSD-MSC or hSD-MSC/GFP) or the immunoresponse to xenogeneic equine mesenchymal stromal cells (eqMSC) or equine dermal fibroblasts (eqDFb), characterized by the proportion of CD3+, CD4+, and CD8+ human splenocytes (hSpl), was measured on Day 0 and Day 6 of co-culture by flow cytometry. In culture with hSD-MSC, hSD-MSC/GFP, eqDFb, or eqMSC, the proportion of CD3+ and CD8+ hSpl increased with time in culture but not with exposure to cell allo- or xeno-antigens. Both hSD-MSC and hSD-MSC/GFP increased in number during culture and were not affected in viability or proliferation by co-culture with allogeneic hSpl. In this primary exposure study, hSpl demonstrated a natural selection and adaptation to a short-term cell culture environment, and that neither allogeneic nor xenogeneic cell antigens incited a greater cellular immunoactivation than co-cultured hSpl alone. The clinical applicability of our work extends to potential cell-based strategies for the repair of human tissue. Allogeneic or xenogeneic cell sources could serve as a powerful clinical tool to enhance current medical practices that promote tissue healing.

Keywords: regenerative medicine, stem cell, cell vector, immunoactivation, tissue repair
DIRECT HUMAN ADENOVIRAL BMP-2 OR BMP-6 GENE THERAPY FOR BONE AND CARTILAGE REGENERATION IN A PONY OSTEOCHONDRAL MODEL

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Objective: To evaluate healing of surgically created large osteochondral defects in a weight-bearing femoral condyle in response to percutaneous direct injection of adenoviral (Ad) vectors containing coding regions for either human bone morphogenetic proteins 2 (BMP-2) or -6.

Methods: Four 13 mm diameter and 7 mm depth circular osteochondral defects were drilled, 1 per femoral condyle (n=20 defects). Direct injection of Ad-BMP-2, Ad-BMP-6, Ad-green fluorescence protein (GFP), or saline into the defect was performed 14 days after surgery. Quantitative magnetic resonance imaging (qMRI) and computed tomography (CT) were serially performed at 12, 24, and 52 weeks. At 52 weeks, histomorphometry and microtomographic analyses were performed to assess final subchondral bone and cartilage repair tissue quality.

Results: Direct delivery of Ad-BMP-6 into healing large femoral condyle lesions demonstrated dGEMRIC qMRI and histologic evidence of greater GAG-content in repair tissue at 12 weeks (p<0.05), while Ad-BMP-2 had greater nonmineral cartilage at the surface at 52 weeks. Ad-BMP-2 demonstrated greater CT subchondral bone mineral density (BMD) by 12 weeks and both Ad-BMP-2 and -6 had greater subchondral BMD at 52 wks (p<0.05). Despite these observations of earlier (Ad-BMP-6) and persistent (Ad-BMP-2) cartilage repair and greater subchondral bone regeneration (Ad-BMP-2 and -6), the tissue within the large weight-bearing defects at 52 weeks was suboptimal in all groups due to poor quality repair cartilage, central fibrocartilage retention, and central bone cavitation. Delivery of either BMP by this method had greater frequencies of subchondral bone cystic formation (p<0.05).

Conclusions: Delivery of Ad-BMP-2 or Ad-BMP-6 to large weight-bearing osteochondral defects via direct injection provided evidence of support to cartilage and subchondral bone regeneration but was insufficient to provide long-term quality osteochondral repair in this femoral condyle pony model.

Keywords: bone morphogenetic protein, cartilage and bone regeneration, knee, pony model
Articular cartilage defects have limited ability to self-heal, resulting in osteoarthritis. Current treatments result in suboptimal fibrocartilage. To create a superior chondral implant, we combined 3 cutting edge techniques: use of juvenile human chondrocytes (jCh), genetic engineering with BMP2 adenoviral (Ad) vectors (jCh-BMP2), and de-novo scaffold-free neocartilage (NC) formation using a chondrogenic differentiation media with TGF\(\beta\) (CDM). Our goal was to optimally produce a disc of human NC for potential implantation into chondral defects. Our hypotheses were that CDM would be superior to DMEM/FBS in NC formation, and further, jCh-BMP2 would enhance this effect.

NC was grown for 2 wks in DMEM or CDM. Ad vectors were used to transduce cells with hBMP-2. Analysis included physical parameters, RT-PCR, ELISA, and histology. Differences between treatments (DMEM vs CDM; jCh vs jCh-BMP2) were compared with ANOVA (p<0.05 significant).

JCh-BMP2 in CDM formed significantly larger and equivalent weight discs to CDM alone, greater than DMEM. Until day 7, jCh-BMP2 discs were larger regardless of media, but jCh-BMP2-DMEM trailed off after day 7. DMEM alone produced the smallest discs. By day 14, CDM alone produced discs with the best chondrogenic index followed by jCh-BMP2. JCh-BMP2, regardless of media, had reduced cell number and viability (70-85%) by day 14. JCh discs produced a mean of 0.8ng/ml BMP2. JCh-BMP2 discs in DMEM produced less BMP2 (4.0ng/ml) than jCh-BMP2 discs in CDM (180.8ng/ml). NC grown in CDM, regardless of presence of BMP2 had greater ratios of collagen type II:type I. Only jCh-BMP2 discs in CDM expressed high levels of type X collagen.

Our outcomes suggested in vitro chondrogenesis and BMP2 production were superior in NC grown in CDM, with selected parameters further enhanced by BMP2 transduction. BMP2 transduction increased BMP2 production in de-novo scaffold-free NC confirming the constructs could serve to deliver BMP2 to adjacent chondrocytes.

Keywords: cartilage, neocartilage, adenovirus, bone morphogenetic protein 2, genetic engineering
THRIVING IN AN EVER-CHANGING WORLD: CASPASE 3-DEPENDENT PROTEOLYSIS DOWNREGULATES TRANSLATION OF SPECIFIC GENES

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Over the course of their life, cells must successfully navigate a variety of stresses. Downregulation of protein synthesis is one important cellular response to unfavorable conditions. We hypothesize that RNA helicase A (RHA) is a regulatory node for this response because of its role in the translation of specific mRNAs. RHA is necessary for translation of selected mRNAs; these particular mRNAs are distinguished by their complex 5' untranslated region (UTR), which is a barrier to the scanning ribosome. We have demonstrated the molecular basis of RHA translational control is specific and selective interaction with structural features of the 5' UTR and its ATP-dependent helicase activity, which facilitates ribosome scanning. The outcome is efficient translation of genes including junD proto-oncogene, bovine and human T-cell leukemia virus type I, avian spleen necrosis virus, feline leukemia virus, and retroviruses of simian origin.

Herein, we evaluated RHA activity under two conditions of stress: apoptosis-inducing drug and serum deprivation. We determined that activation of caspase 3 by stress triggers cleavage of RHA. Biochemical experiments identified D96 as the major site of this cleavage. Amino acids 1-96 of RHA contain a double-stranded RNA binding domain (dsRBD) necessary for recognition and binding of target mRNAs. Therefore, we expect loss of this domain to modulate RHA translational activity. Indeed, we demonstrate that RHA cleavage by caspase 3 results in a specific decrease in junD translational efficiency. We have generated a caspase cleavage-resistant RHA mutation (D96A) and expect this mutation to rescue the stress-dependent loss of RHA translational activity. Mounting evidence suggests that control of gene expression by RHA is critical to normal function of cells. We speculate that perturbation of RHA’s regulatory role may result in increased susceptibility to environmental stress.

Keywords: RNA helicase A, 5' untranslated region, retrovirus, stress response, gene expression, caspase 3
IN VIVO REDUCTION OR BLOCKADE OF INTERLEUKIN-1B IN PRIMARY OSTEOARTHRITIS INFLUENCES EXPRESSION OF MEDIATORS IMPLICATED IN PATHOGENESIS. K. S. Santangelo, G. Nuovo, A.L. Bertone. Depts. of Veterinary Biosciences and Veterinary Clinical Sciences

Osteoarthritis (OA) is the leading cause of physical disability in developed nations, resulting in large burdens on society and the health care industry. Interleukin-1β (IL-1β) is cited as a major cytokine involved in OA-related pathology but in vivo characterization of its function in primary disease remains unclear. We recently described the temporal expression and tissue distribution of IL-1β through progression of spontaneous OA in two strains of guinea pigs with varying propensity for spontaneous disease. Persistent IL-1β expression was found in pertinent articular tissues in OA-prone animals at 120 and 180 days, while OA-resistant animals demonstrated a significant reduction in immunostaining, a finding that may correlate to early incidence of OA in the former strain. Here, we proceeded with in vivo studies to reduce or block IL-1β-mediated pathways such that evidence of its mechanistic contribution to OA could be determined. Male OA-prone guinea-pigs were obtained for data collection at 120 and 180 days of age following injection with viral vectors at 60 days of age. Successful reduction of IL-1β-mediate signaling was achieved: (1) via RNA interference (RNAi) techniques using a novel adeno-associated viral vector (AAV) vector containing an IL-1β targeting knockdown sequence; or (2) administration of an adenovirus (Ad) vector encoding interleukin-1 receptor antagonist protein (IRAP). Importantly, manipulation of IL-1β was demonstrated over a 120-day period with no statistical difference in reporter gene expression from either viral vector. This work confirmed that a diminution in IL-1β signaling by RNAi or IRAP via AAV and Ad vectors, respectively, was accomplished, resulting in decreased expression of three inflammatory mediators and one catabolic agent, and increased expression of a key anabolic molecule. Thus, we provided biological evidence that IL-1β serves an important role in primary OA in vivo and that these translational tools may provide long-term beneficial disease modification.

Keywords: osteoarthritis; interleukin-1β; adeno-associated viral vectors; adenoviral vectors; RNA interference; interleukin-1 receptor antagonist protein
HUMAN T-CELL LEUKEMIA VIRUS TYPE 2 (HTLV-2) APH-2, AN ANTISENSE GENOME STRAND ENCODED PROTEIN, IS DISPENSABLE FOR VIRAL INFECTIVITY AND PERSISTENCE IN VIVO

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Human T-cell leukemia virus type 1 (HTLV-1) and type 2 (HTLV-2) are human tumorigenic retroviruses that encode Tax viral oncogene. Tax is critical for viral transcription and replication and T-cell transformation. Transcription from the antisense strand of the HTLV-1 genome has been described and the encoded protein termed HBZ has been shown to reduce Tax-mediated viral transcription, promote the proliferation of HTLV-1 infected cells, and be required for viral persistence in vivo. Recently, an anti-sense HTLV-2 protein (APH-2) with limited homology to HBZ has been identified. However, the functional role of APH-2 in the context of a replicating virus has yet to be tested. We seek to test the hypothesis that APH-2 contributes to the balance of HTLV-2 gene transcription potentially disrupting viral replication and cell transformation in vitro and the ability of the virus to persist in vivo. We first compared the capacity of inhibiting Tax-mediated viral transcription between HBZ and APH-2. We found that APH-2 is much less efficient in repressing Tax-mediated viral transcription compared to HBZ. We also investigated the functional significance of APH-2 in HTLV-2-mediated immortalization of primary T-lymphocytes in cell culture and viral survival in the rabbit animal model. The immortalization capacity of APH-2 mutant viruses was indistinguishable from the wild type virus, which is consistent with what we observed on HBZ. However, we found that rabbits infected with APH-2 mutant viruses displayed an increased antibody response to viral gene products and a higher proviral load in PBMCs compared to wild type HTLV-2 infected rabbits. This suggests APH-2 mutant viruses undergo a higher viral gene expression and replication due to a loss of negative control on viral transcription. Our findings indicate that, unlike HBZ which is important for viral persistence in vivo, APH-2 is dispensable for enhancing viral replication and persistent infection in vivo.

Keywords: retrovirus, antisense, immortalization, T-lymphocyte
FLLL100, A HIGHLY SOLUBLE ANALOG OF FLLL32, INHIBITS PROLIFERATION OF OSTEOSARCOMA CELL LINES THROUGH DOWNREGULATION OF BOTH P-STAT3 AND TOTAL STAT3
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Background: Signal transducer and activator of transcription 3 (STAT3) plays an important role in cancer cell proliferation, survival and metastasis. STAT3 phosphorylation has been linked with prognosis in several cancers, and it has proven to be an effective target for therapeutic intervention in a number of tumors. We have previously shown that STAT3 is dysregulated in canine and human osteosarcoma (OSA) and have evaluated several potential small molecule inhibitors of STAT3 in OSA cell lines. One of these, FLLL32, an analog of curcumin, exhibited significant activity against OSA cell lines in vitro, inducing downregulation of P-STAT3 and total STAT3, caspase activation and cell death. However, FLLL32 is highly insoluble significantly limiting its use in vivo. The Medicinal Chemistry group at OSU has developed a highly soluble analog of FLLL32, termed FLLL100. The purpose of this study was to assess whether this FLLL100 was capable of modulating STAT3 and inducing biologic effects in OSA tumor cells similar to those observed with FLLL32.

Methods and Materials: The canine OSA line OSA8 and human line SJSA were treated with DMSO, FLLL32 or FLLL100 for 72 hours and effects on cell proliferation were assessed. OSA8 and SJSA cells were treated with DMSO, FLLL32 or FLLL100 for 24 hours and caspase-3,7 activity was assessed. OSA8 and SJSA cells were treated for 24 hours with DMSO, FLLL32 or FLLL100 Western Blotting for P-STAT3, total STAT3, and b-actin was performed.

Results: FLLL100 inhibited proliferation of OSA cell lines in a dose dependent manner with a calculated IC50 of 3.27 mM for OSA8 and 3.42 mM for SJSA. This compares to those obtained for FLLL32 (1.62 mM for OSA8 and 1.09 mM for SJSA) which were on average 2-3 times lower. FLLL100 also induced caspase 3,7 activation in treated OSA cell lines, and downregulation of P-STAT3 and total STAT3 within 24 hours of drug exposure, although this occurred at concentrations higher than those required for FLLL32.

Conclusions: FLLL100 is a highly soluble analog of FLLL32 that inhibits the proliferation of OSA cell lines, induces apoptosis of OSA cell lines, and downregulates both P-STAT3 and total STAT3. Although the biologic effects of FLLL100 require concentrations of drug approximately 2-3 times higher than those necessary for FLLL32, its increased solubility should permit the evaluation of drug activity in vivo.
EVALUATION OF LH RELEASE AFTER THE INTRAUTERINE ADMINISTRATION OF GnRH IN LACTATING DAIRY CATTLE. S. Bas¹, C.G. Pinto¹, M.L. Day², G.M. Schuenemann¹ Department of Veterinary Preventive Medicine¹, Department of Animal Sciences², The Ohio State University

Our objectives were to determine the preovulatory release of LH and ovulatory response after the intrauterine (i.u.) administration of GnRH in dairy cows. Lactating cows (n=23) were synchronized with a Presynch-Ovsynch protocol. Ovarian structures were recorded and a blood sample collected at the time of first GnRH and PGF of Ovsynch. Only cows presenting a CL ≥ 15 mm and at least one follicle ≥ 10 mm in diameter remained in the study. At the time of the second GnRH of Ovsynch (h 0), cows were blocked by parity and randomly assigned to 1 of 3 groups: control group (CON; n=7) received 2 mL, i.m., of sterile water; intramuscular group (IM; n=8) received 100 µg, i.m., of GnRH; and intrauterine group (IU; n=8) received 100 µg, i.u., of GnRH. Blood samples for determination of LH concentrations were collected at h 0, 0.5, 1, 1.5, 2, 3 and 4. Ultrasonography was performed twice daily for determination of ovulation. Progesterone concentrations at h 0 did not differ (P >0.05) between groups. Concentrations of LH were greater (P<0.05) in the IM than IU and CON groups at h 0, 0.5, 1, 1.5, 2, and 4 h but not at h 3 between the IM and IU group. IU cows started increasing LH concentrations at 1 h reaching maximum levels at 2-3 h post GnRH. The proportion of cows that ovulated by h 60 was greater (P < 0.05) for the IM (8/8) and IU (7/8) as compared to CON (2/7). Intrauterine GnRH resulted in lower serum concentrations of LH than in the IM group, but the proportion of cows that ovulated by h 60 did not differ between treatments. These findings provide evidence that i.u. administration may be an alternative route of delivery of GnRH to synchronize ovulation in synchronization programs.

Keywords: Intrauterine, GnRH, LH, ovulation.
EFFECTS OF INTRAPERITONEAL ADMINISTRATION OF BILIRUBIN ON INFARCT AREA AND LEFT VENTRICULAR FUNCTION IN A RAT MODEL OF ACUTE CORONARY OCCLUSION. R. Ben-Amotz, C. Adin, J bonagura, r. hamiln. Depts. of Veterinary Biosciences and Veterinary Clinical

Bilirubin was considered to be a toxin that accumulates after catabolism of heme by the enzyme heme oxygenase. However, a mounting body of evidence suggests that bilirubin, at a physiological (non-toxic) doses, is a powerful antioxidant and anti-atherosclerotic agent. Interestingly, recent clinical studies have shown that human beings with genetically induced hyperbilirubinemia (Gilbert Syndrome) are protected against coronary heart disease. The purpose of this study was to investigate whether administration of exogenous bilirubin to normal rats would convey similar protective effects in an experimental model of coronary ischemia. Our hypothesis was that bilirubin administration (20uM/kg, IP, 1 hour before injury) would decrease infarct area and preserve left ventricular function when compared to non-treated rats. Coronary ischemia was induced by temporary (30 min) ligation of the left anterior descending coronary artery in control rats (n=5), bilirubin treated rats (n=5), followed by a 1 hour period of reperfusion. Left ventricular function was estimated non-invasively using echocardiographic measurements of fractional shortening % and area shortening %. Effects of anesthesia on cardiac function were controlled for by using a sham group (n=5). There was a significant reduction of infarct size in the bilirubin treated group compared to the non-treated group (13.34% vs 25.5%, p<0.0067). Left ventricular function decreased in both experimental groups after ischemia and reperfusion, although bilirubin provided a protective effect on fractional shortening during the period of ischemia (18.8 vs 25.8%, P= 0.034). Based on the results of this study, bilirubin supplementation appears to provide significant decrease in infarct size although protective effects on left ventricular function were noted only during the period of ischemia.

Keywords: bilirubin, occlusion, ischemia-reperfusion injury
THE NOVEL ENERGY RESTRICTION-MIMETIC AGENT OSU-CG5 SUPPRESSES
THE EXPRESSION OF ENZYMES THAT PROMOTE THE WARBURG EFFECT IN VITRO. Berman-Booty¹,², P.C. Chu², D. Wang², S. Kulp², and C.S. Chen²

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Cancer cells gain growth advantages by shifting cellular energy metabolism to aerobic glycolysis, the so-called Warburg effect. The in vivo efficacy of dietary energy restriction, 2-deoxyglucose, and resveratrol in suppressing carcinogenesis in various animal models provides a proof-of-concept for targeting energy metabolism for cancer chemoprevention. We have developed a novel class of energy restriction-mimetic agents (ERMAs) based on our finding that thiazolidinediones share the abilities of glucose deprivation, 2-deoxyglucose, and resveratrol to elicit starvation-associated cellular responses in prostate cancer cells. Our novel ERMA, OSU-CG5, exhibits higher antitumor potency than resveratrol and 2-deoxyglucose without cytotoxicity to nonmalignant prostatic epithelial cells. In androgen-dependent LNCaP prostate cancer cells and the castration-resistant subline LNCaP-C4-2 (C4-2), 72-hour treatment with OSU-CG5 results in increased expression of β-TrCP and subsequent β-TrCP-mediated proteasomal degradation of β-catenin, cyclin D1, and Sp1, as well as downregulation of the androgen receptor. Additionally, OSU-CG5 causes AMP-activated protein kinase activation, consistent with a “starvation-response,” and increases expression of GRP78 and GADD153, biomarkers associated with endoplasmic reticulum stress. Treatment of LNCaP cells with OSU-CG5 for either 24 or 48 hours downregulates the expression of metabolic enzymes that promote the Warburg effect, including pyruvate dehydrogenase kinase isoenzyme 3, the M2 isoform of pyruvate kinase, fatty acid synthase, fatty acid desaturase 2, acyl-CoA synthase long-chain family member 3, ATP citrate lyase, and lactate dehydrogenase A. Based on these results, we will evaluate OSU-CG5’s tumor-suppressive effects in the C4-2 xenograft tumor model, and its chemopreventive activity in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model.

Keywords: Warburg effect, energy-restriction, energy-restriction mimetic agents, prostate cancer
MEASUREMENT OF THE FELINE HIPPOCAMPUS USING MAGNETIC RESONANCE IMAGING. Francis KA, Drost WT, Schmalbrock P, Burns P, Buffington CAT. The Ohio State University, Veterinary Clinical Sciences and Wright Center of Biomedical Innovation, Columbus, OH 43210.

Introduction: The human hippocampus has several unique attributes and is one of the more structurally discrete structures of the limbic system. Humans exposed to chronic stress or pain, most notably patients with posttraumatic stress disorder and chronic pelvic pain syndromes, have detectably smaller hippocampal structures.

Interstitial cystitis (IC) in humans is a recognized syndrome of chronic pelvic pain. Feline interstitial cystitis (FIC) is a spontaneous animal model for IC. Therefore, documenting volume changes in the hippocampus in cats may lead to further understanding of chronic pain in all species.

The aim of this study is to evaluate the feasibility of measuring the volume of the feline hippocampus using MRI.

Materials and Methods: MR imaging of the brains of 3 cats (2 nonsymptomatic cats from an FIC colony and 1 normal cat from an unrelated study) was performed using 3 Tesla (T) and 7T MR systems. T2w images were made with quadrature extremity coils designed specifically for each magnet, and a smaller surface coil in the 7T magnet. Transverse, sagittal, and dorsal plane images were acquired in each imaging session.

Cats were imaged in vivo, post perfusion fixation, and at various durations (4-181 days) of immersion fixation of the brain in 10% formalin using both magnets.

DICOM images were imported into ImageJ and each hippocampus was manually outlined on all images. The areas were then multiplied by the image thickness (including spacing) and the area from each slice was summed to obtain a volume.

Results: Measured volumes range from 216-375 mm$^3$ in transverse images in vivo with a trend of smaller volumes when measured on sagittal and dorsal images.

Discussion: At this stage, measurement of hippocampus volume using the current technology is difficult.

Keywords: MRI, cat, feline, morphometry
The N-terminus of PThrP promotes bone and cartilage formation from MSC, while concurrently inhibiting fat and muscle formation. However, the role of the other regions of PThrP is unknown. MSC from mice lacking the mid-region, NLS, and C-terminus of PThrP (Pthrp\(^{-/-}\)) and wild-type (WT) littermates were seeded in monolayer (osteogenesis, adipogenesis, and myogenesis) or pellet cultures (chondrogenesis). MSC were exposed to differentiation media for 24 days. Cell proliferation, histochemical indices, secreted biomarkers, and gene expression were compared. Unstimulated Pthrp\(^{-/-}\) MSC proliferated faster than WT MSC (\(P < 0.01\)) and had greater alkaline phosphatase (ALP) activity under osteogenic conditions (\(P < 0.0001\)). However, Pthrp\(^{-/-}\) MSC had reduced osteoblast differentiation, demonstrated by decreased mineralization and osteocalcin secretion (\(P = 0.016\) and 0.029, respectively). Similarly, Pthrp\(^{-/-}\) MSC-derived cartilage pellets were smaller (\(P < 0.0001\)) and expressed less Sox9 (\(P = 0.042\)), however they expressed greater Ihh (\(P = 0.035\)). Despite Pthrp\(^{-/-}\) MSC producing a greater number of adipocytes (\(P = 0.023\)) and a trend towards increased Ppar\(\gamma\) expression (\(P = 0.087\)), both genotypes had similar adipogenic gene expression profiles. Interestingly, Pthrp\(^{-/-}\) MSC produced a larger number of myocytes (\(P < 0.0001\)) and expressed more desmin and myogenin (\(P = 0.002\) and 0.031, respectively). In conclusion, regions distinct from the N-terminus influence MSC commitment. Increased proliferation, ALP, and Ihh by Pthrp\(^{-/-}\) MSC, but less bone and cartilage formation, indicate that the mid-region, NLS, and C-terminus inhibit proliferation, but promote terminal differentiation of osteoblasts and chondrocytes. This is the first time that the mid-region, NLS, and C-terminus have been implicated in regulating adipocyte and myocyte formation, and they complement the inhibitory function of the N-terminus. Similarities in adipogenic gene expression profiles, despite a greater number of Pthrp\(^{-/-}\) MSC-derived adipocytes, suggest dysregulated energy metabolism, which may contribute to the perinatal lethality observed in Pthrp\(^{-/-}\) mice.

Keywords: PThrP, osteogenesis, adipogenesis, chondrogenesis, myogenesis
REGULATION OF THE GLUCOSE TRANSPORT PATHWAY IN VISCERAL AND SUBCUTANEOUS ADIPOSE DEPOTS IN HORSES WITH INSULIN RESISTANCE. K. Kohler, A. Waller, T. Burns, M. Mudge, J. Belknap and Lacombe VA. College of Pharmacy; Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University.

Although the importance of adipose tissue (AT) glucose transport in regulating whole-body insulin sensitivity is becoming increasingly evident and insulin resistance (IR) has been widely recognized, the underlying mechanisms of altered glucose transport and its contributions to IR are still not well understood. The purpose of the present study was to determine the early pathological changes in glucose transport by characterizing alterations in glucose transporters (GLUTs) in multiple visceral and subcutaneous adipose depots in a large animal model of naturally occurring compensated IR. AT biopsies were collected from horses, which were classified as insulin-sensitive (IS) or compensated IR based on the results of an insulin-modified frequently sampled intravenous glucose tolerance test. Protein expression of GLUT4 (major isoform) and GLUT12 (one of the most recently discovered isoforms) were measured by Western blotting in multiple AT depots, as well as AS160 (a potential key player in GLUT trafficking pathway). Using a biotinylated bis-mannose photolabeled technique, active cell surface GLUT4 content was also quantified. Omental AT had the highest total GLUT-4 and-12 content compared to other sites during the IS state. IR was associated with a significantly reduced total GLUT4, but not GLUT12, content in omental AT, without a change in content in other visceral or subcutaneous adipose sites. In addition, active cell surface GLUT4 was lower in subcutaneous and visceral AT of IR compared to IS horses. Impairment in GLUT4 trafficking occurred independently of any changes in AS160 phosphorylation between groups. Our data suggest that GLUT4, but not GLUT12, is a pathogenic factor in AT during naturally occurring compensated IR, despite normal AS160 activation.

Keywords: Omental; Biotinylated photo-affinity label; GLUT (4 and 12) trafficking; AS160.
DILTIAZEM TREATMENT ATTENUATES ARRYTHMOGENESIS DURING DIABETIC CARDIOMYOPATHY BY STABILIZING RYR2-MEDIATED SR CA RELEASE. V.A. Lacombe, D. Terentyev, S. Viatchenko-Karpinski, R. Hamlin, S. Györke; C.A. Carnes. College of Pharmacy; Department of Veterinary Clinical Sciences; Department of Physiology and Cell Biology, College of Medicine, The Davis Heart and Lung Research Institute; The Ohio State University.

Diabetes increases the risk of ventricular arrhythmias, for which the underlying mechanisms are not well known, and specific therapies for this patient population are not well defined. The potential beneficial effect of diltiazem (DILT), a widely prescribed calcium (Ca) channel blocker, to treat arrhythmia has not been investigated in diabetes. We hypothesized that impaired Ca handling may contribute to arrythmogenesis, which will be restored by long-term treatment with DILT in a model of diabetic cardiomyopathy.

Rats with streptozotocin-induced diabetes were randomized to treatment with DILT or placebo for 8 weeks, and compared to controls. Electrocardiograms were measured at baseline and at 8 weeks. Ventricular myocytes were isolated for electrophysiological measurements and confocal Ca imaging.

Diabetes significantly increased QRS amplitude, QTc interval and beat-to-beat QT variability. Action potential durations (APD) were prolonged and afterdepolarizations occurred in diabetic myocytes. DILT treatment normalized QT (interval and variability), APD (50 and 90), and reduced the occurrence of afterdepolarizations in DILT-treated compared to untreated diabetic rats. Ca transient amplitude and sarcoplasmic reticulum (SR) Ca load (estimated by measuring caffeine-evoked \( \int_{\text{NCX}} \) and Ca transient amplitudes) were reduced in diabetic compared to control myocytes (P<0.05). DILT therapy resulted in partial repletion of SR Ca content, thus increasing peak amplitude and partially normalizing Ca transient in diabetic myocytes. In permeabilized myocytes, Ca spark frequency was decreased in treated and untreated diabetic compared to control rats (P<0.05).

The decreased Ca spark frequency, in the face of increased SR Ca load and Ca transient peak amplitude in treated diabetic myocytes, suggests that DILT therapy improves Ca homeostasis by blocking diastolic SR Ca leak, which subsequently attenuated arrythmogenesis observed in vivo and in vitro during diabetes. In addition, reducing Ca influx may improve diabetic cardiomyopathy by altering Ca-dependent signaling pathways with subsequent posttranslational modifications and stabilization of ryanodine receptors.

Keywords: Diabetes, Arrhythmias, Calcium channel blockers, Electrophysiology, Excitation-contraction coupling, Patch clamping.
CALCIUM CHANNEL BLOCKER THERAPY SELECTIVELY ALTERS GLUCOSE TRANSPORTERS DURING DIABETIC CARDIOMYOPATHY. M. Parriman, A. Waller, and V. Lacombe. College of Pharmacy and Department of Veterinary Clinical Science, The Ohio State University.

Diabetes results in hyperglycemia due to decreased glucose uptake into insulin-sensitive tissues, leading to multi-organ dysfunction, including heart diseases. Glucose transport into cells, the rate-limiting step in whole body glucose utilization, occurs via a family of membrane proteins called the glucose transporters (GLUTs), and is regulated by both insulin- and Ca\textsuperscript{2+}/contraction-mediated pathways. Although calcium channel blockers (CCB) are widely prescribed for cardiovascular diseases, including in diabetic patients, its effects on glucose transport in the heart are unknown. Therefore, our aim was to determine the effect of long-term treatment with a CCB, diltiazem, on glucose transport in the diabetic myocardium. Diabetes was induced in male Wistar rats by injection of a low dose of streptozotocin in two groups, while the other two groups received a placebo injection. One group of diabetic (Dx-CCB) rats and one group of healthy rats (Co-CCB) randomly received diltiazem (25 mg/kg/day orally) for 8 weeks. Echocardiographic examinations were performed at baseline and at 8 weeks. GLUT4 and GLUT 12, a recent discovered isoform, were measured from a myocardial membrane-enriched preparation by Western Blot. As expected, STZ-treated rats displayed hyperglycemia within 72 hours of injection, which persisted up to 8 weeks, while there was no change in blood [glucose] of either control group. Diltiazem therapy did not improve the observed hyperglycemia. Diabetic rats displayed systolic dysfunction characteristic of diabetic cardiomyopathy, which was not significantly improved in the diltiazem-treated group. Cardiac GLUT4 protein content was significantly reduced in untreated diabetic compared to healthy control rats, with the most pronounced effect in the DX-CCB Group. There was no difference in cardiac GLUT12 protein content. In conclusion, our data suggest that administering diltiazem to diabetic patients does not improve hyperglycemia or systolic function, and in fact decreases cardiac glucose transport, and thus may be contraindicated as a treatment for diabetic cardiomyopathy.

Keywords: Diabetes, Diltiazem, Heart, GLUT-4, GLUT-12
EPITHELIAL MIGRATION ON THE CANINE TYMPANIC MEMBRANE.


Epithelial migration (EM) is a process that serves as a self-cleaning and repair mechanism for the ear canal and tympanic membrane (TM) and has been evaluated in humans and several other species, but not dogs. The objective of this study was to determine the rate and pattern of EM on the TM in clinically normal laboratory dogs. Eighteen dogs (eight beagles, ten fox hounds) were anesthetized and three drops of waterproof drawing ink were placed on the TM, two (PT1, PT2) on the caudal half of the pars tensa and one (PF) on the pars flaccida (PF). Images were recorded with a video otoscope and digital capture system. Each dog was evaluated and images recorded every six to eight days for four evaluations. Migration pattern analysis and EM rate calculation were performed with image processing software. Descriptive statistics for EM rate (mean, standard deviation, 95% confidence interval) were calculated for all ink drop locations on the TM (PT1, PT2, PF) at each time point. No significant differences in the mean EM rates were identified between ears (eight fox hounds), breeds, or locations PT1 and PT2. The mean overall EM rates (+standard deviation) were 96.4 (±43.1) and 225.4 (±128.1) micrometers per day for the pars tensa and pars flaccida, respectively. All ink drops moved outward, the majority in a radial direction, from the original location to the TM’s periphery. The ink drop placement method evaluated in this study can be used to determine the EM rate of a normal canine TM.

Keywords: tympanic membrane, epithelial migration, canine
OVEREXPRESSION OF SARCOPLASMIC RETICULUM CALCIUM ATPASE PUMP SELECTIVELY REGULATES GLUCOSE TRANSPORTERS IN THE HEART OF HEALTHY AND DIABETIC SUBJECTS. A.P. Waller¹, A. Kalyanasundaram², S. Hayes¹, M. Periasamy², V.A. Lacombe¹,³

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Diabetes results in hyperglycemia due to decreased glucose uptake into insulin-sensitive tissues. The translocation of glucose transporters (GLUTs) to the cell surface is the limiting step in cardiac glucose uptake, and is regulated by insulin and calcium dependent processes, although the latter has received scarce attention. Since the sarcoplasmic reticulum Ca²⁺ ATPase (SERCA) pump tightly regulates the cytosolic [Ca²⁺] and plays a major role in the pathogenesis of diabetic cardiomyopathy, we hypothesized that increased SERCA activity will not only rescue cardiac function but also glucose transport in the diabetic myocardium. Using a transgenic (TG) mouse model overexpressing SERCA1a pump in the heart, streptozotocin (STZ)-induced diabetic TG and wild type (WT) mice were compared with age-matched non-diabetic controls. Active cell surface GLUT-4 and -12 was quantified using a novel cell-surface biotinylation photolabeled technique in intact perfused hearts. Cardiac function was assessed by echocardiography and electrocardiography.

In healthy mice, overexpression of SERCA1a pump in the heart resulted in a 2 fold increase in cell-surface GLUT4 content compared to WT mice (P=0.07). STZ-treated mice displayed hyperglycemia (which persisted up to 8 weeks in the WT mice) and diabetic cardiomyopathy, as evidenced by the bradycardia. Overexpression of SERCA partially rescued the hyperglycemia starting from 2 weeks post injection and enhanced cardiac glucose transport during diabetes, as total myocardial GLUT4 content was significantly decreased in diabetic WT mice compared to healthy WT, but GLUT4 content was not different in diabetic TG vs healthy WT. In addition, there was a 1.5 fold increase in cell surface GLUT4 in the diabetic TG mice compared to diabetic WT. There were no differences in the cell surface content of the recently discovered GLUT12 however. We concluded that SR Ca²⁺ cycling regulates cardiac GLUT translocation, and that overexpression of SERCA in the diabetic heart rescues cardiac function and glucose transport.

Keywords: biotinylated photo-affinity label, GLUT (4 and 12) trafficking, cardiomyopathy
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MAPK phosphatase (MKP)-1 is a critical negative regulator of p38 and JNK MAP kinases. Bacteria-infected Mkp-1-/- mice have prolonged p38 and JNK activation, multi-organ failure, and high mortality rates. While Mkp-1 is clearly protective against microbial insults, little is known about the role of Mkp-1 in other conditions where JNK activation enhances clinical disease. JNK activation promotes hepatocyte death in acetaminophen overdose, one of the most common causes of drug-induced liver failure in the United States. In this study, we evaluated the role of Mkp-1 in the host response to acute acetaminophen toxicity. Fasted Mkp-1+/+ and Mkp-1-/- mice received an intraperitoneal injection of PBS or 300 mg/kg acetaminophen. Mice were euthanized 1-6 hours post dosing and tissues collected to assess glutathione levels, organ damage, and MAPK activation. Separately, fasted Mkp-1+/+ and Mkp-1-/- mice received an intraperitoneal injection of 400 mg/kg acetaminophen, were re-fed after 6 hours, and survival was monitored through 120 hours post treatment. Serum ALT, a marker of hepatocyte injury, was significantly higher in Mkp-1-/- compared to Mkp-1+/+ mice at 4 hours post dosing. Histologically, Mkp-1-/- mice had more rapid and extensive hepatocyte degeneration and necrosis than did Mkp-1+/+ mice at 2-6 hours post dosing. Although hepatic glutathione levels were higher in PBS-treated Mkp-1-/- mice, acetaminophen treatment depleted glutathione to comparable levels in both mouse strains, indicating that higher initial glutathione stores did not protect Mkp-1-/- mice. Mkp-1-/- livers had more sustained JNK activation through 6 hours post treatment compared to Mkp-1+/+ livers. Finally, survival was lower in Mkp-1-/- compared to Mkp-1+/+ mice, including overall survival (32% vs. 43%) and median survival time (23.5 vs. 71 hours). Together, these data support the model that Mkp-1 plays an important protective role in mediating host survival and tissue damage during acute acetaminophen toxicity, likely through regulation of JNK.

Keywords: Mouse, acetaminophen, JNK, Mkp-1
MOLECULAR CHARACTERIZATION OF THE CANINE BETA-GLOBIN GENE CLUSTER. S Zaldívar-López1,2, J Rowell2,4, E Fiala2, CG Couto1, CE Alvarez1,2,3
Departments of 1Veterinary Clinical Sciences, 2Pediatrics, 3The Research Institute at Nationwide Children’s Hospital, 4College of Nursing

We reported that hemoglobin in retired racing Greyhounds (RRG) has higher oxygen carrying properties and affinity than other breeds. The latter was an unexpected finding (counterintuitive based on previous reports in exercise physiology). Due to the high prevalence of hemoglobinopathies (i.e. thalassemias), globin genes in other species are well characterized. Surprisingly, very little is known about canine hemoglobin genetics. The purpose of this study was to characterize genetics of canine beta globins. Using computational BLAST analysis of the dog genome, we identified five beta globin genes in a single locus: two human $HBE$-like followed by three $HBB$-like genes. We isolated DNA and RNA from blood of RRGs, AKC registered Greyhounds (AKCG), and German Shepherd Dog (GSD). All beta globin exons and splice sites were sequenced, and the beta globin locus was examined by array comparative genomic hybridization (custom 1M Agilent array; whole genome, tiling design). Additionally, we determined the number of common haplotypes that span this locus in RRGs and AKCGs using high density SNP array (180k Illumina HD). Expression and sequence analysis of cDNA showed all five beta globin genes are actively expressed in adults. CanHBB1 and 2 were created by relatively recent segmental duplication and have identical protein sequence. CanHBB1/2 are abundantly expressed in adults; CanHBB3 is expressed at greatly reduced levels. Sequencing results revealed one rare non-synonymous single nucleotide polymorphism (SNP) in HBE1 of RRGs, but no variation that could explain their abnormal hemoglobin. We did not detect structural variation overlapping or near the beta globin locus. Notably, RRG and AKCG do not share haplotypes spanning the beta globin locus. This is the first characterization of canine hemoglobin genetics, and the first report of canine embryonic hemoglobins and their expression in adults.

Keywords: hemoglobin, beta-globin, canine, Greyhound, DNA sequencing, hybridization
PHARMACOKINETICS OF INTRA-ARTICULAR BETAMETHASONE SODIUM PHOSPHATE AND BETAMETHASONE ACETATE AND ENDOGENOUS HYDROCORTISONE SUPPRESSION IN EXERCISING HORSES
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Reasons for performing the study: No reports exist of the pharmacokinetics (PK) of betamethasone (BTM) sodium phosphate and betamethasone acetate administered intra-articular (IA) into multiple joints in exercising sport horses. Endogenous hydrocortisone (HYD) suppression may serve as a biomarker of BTM administration.

Objectives: To determine the PK of BTM and HYD concentrations in plasma and urine after IA administration of a total of 30 mg BTM.

Methods: Eight 4.5-year old Thoroughbred mares were exercised on a treadmill and BTM was administered IA. Plasma and urine BTM and HYD were determined via high performance liquid chromatography spectrometry for 6 weeks. Concentration-time profiles of BTM and HYD in plasma and urine were used to generate PK estimates for non-compartmental analyses and comparisons among times and HYD concentrations.

Results: BTM in plasma had greater Tmax (Tmax 0.8 hours) vs. urine (Tmax 7.1 hours; P<0.05). Urine BTM concentration (ng/mL) and amount (AUClast, hr*ng/mL) were greater than plasma. HYD was suppressed for at least 3 days (<1 ng/mL) for all horses (P<0.05). The time of last quantifiable concentration of BTM (Tlast) was not significantly different in plasma and urine.

Conclusions: A faster detection of BTM occurred in plasma. The time of last quantifiable concentration of BTM was similar in plasma and urine. Use of highly sensitive HPLC-MS/MS assays enabled early detection (0.8 hours) and prolonged and consistent determination of BTM in plasma and urine. Plasma HYD suppression was a reliable indicator of exogenous glucocorticoid (GC) administration.

Potential Relevance: Use of more sensitive assays, exercising horses, and a multiple joint injection protocol of clinically used total dosages of BTM may serve to establish new detection parameters that may harmonize withdrawal recommendations in typical sport horses in plasma and urine. The plasma HYD suppression may serve as a screening tool for detection of exogenous GC.

Keywords: Horse; Betamethasone Sodium Phosphate; Betamethasone Acetate; Hydrocortisone; Intra-articular; Pharmacokinetics.
THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM (RAAS) IN SEPTIC FOALS. K.A. Dembek; K. Onasch; R.J. Barsnick; S.D. Hurcombe; N.M. Slovis; B. Barr; R.E. Toribio. 1The Ohio State University, College of Veterinary Medicine; Columbus, OH, USA; 2Hagyard Equine Medical Institute, Lexington, KY, USA; 3Rood and Riddle Equine Hospital, Lexington, KY, USA

Sepsis is the number one cause of mortality in neonatal foals. Septic foals are often hypovolemic from systemic inflammation, electrolyte imbalances, decreased milk intake, and fluid loss. The stress of sepsis activates the hypothalamic-pituitary-adrenal axis (HPAA) and the renin-angiotensin-aldosterone system (RAAS) to regulate metabolic functions, blood pressure, tissue perfusion, and inflammation. The role of the RAAS is well documented in critically ill human neonates, but limited information exists in foals. The goal of this study was to evaluate the RAAS response in sick and healthy foals. We hypothesized that in septic foals the RAAS will be activated and the degree of activation will be associated with severity of sepsis and mortality. Blood samples were collected on admission from 60 septic (sepsis score >12), 102 sick non-septic (SNS), and 18 healthy foals of <7 days of age. Measured RAAS factors included renin, angiotensin II (ANG-II), and aldosterone. Since the HPAA and RAAS interact, adrenocorticotropin (ACTH) and cortisol were measured. 

ANG-II, aldosterone, ACTH, and cortisol concentrations were higher in septic and SNS foals compared to healthy foals (P<0.05). Septic foals had a higher ACTH:aldosterone ratio than healthy foals (P<0.001). Septic non-survivor foals had higher aldosterone, cortisol, and ACTH, and lower ANG-II concentrations than survivors. Renin activity and ANG-II concentrations were not different among groups.

This study shows that in response to sepsis there is RAAS and HPAA activation in critically ill foals. Result of this study will enhance our understanding of foal sepsis, improving future treatments and outcome.

Keywords: corticotropin-releasing-hormone, adrenocorticotropin, aldosterone, cortisol, angiotensin II, renin, sepsis, foals
ASSESSMENT OF MICROCIRCULATORY PERFUSION IN HEALTHY ANESTHETIZED CATS UNDERGOING OVARIOHYSTERECTOMY USING SIDESTREAM ARK FIELD MICROSCOPY. M. Goodnight, E. Cooper.
Department of Veterinary Clinical Sciences

Introduction: Specialized imaging techniques have been developed to directly assess the microcirculation but have not been previously used in cats. We sought to establish normal values for microcirculatory parameters in healthy, anesthetized cats using sidestream dark field microscopy (SDM) and to determine if surgical manipulation alters these values during ovariohysterectomy.

Methods: Eighteen healthy cats presenting for elective ovariohysterectomy were anesthetized using a standardized protocol. Three 20-second microcirculatory videos were obtained from the sublingual mucosa at three intervention points. Videos were assessed for quality; only those deemed acceptable were included. Qualifying videos were analyzed by a single observer blinded to intervention point. Total vessel density (TVD), proportion of perfused vessels (PPV), perfused vessel density (PVD) and microvascular flow index (MFI) were determined using vascular analysis software. Microvascular parameters were analyzed for significant changes between intervention points and correlation to macrovascular parameters.

Results: Twelve cats were included in the final video analysis, six were removed owing to poor video quality. TVD, PPV and PVD did not vary with surgical manipulation. MFI had independent positive correlation with surgical tissue manipulation, mean arterial pressure, heart rate, respiratory rate and systolic blood pressure and had negative correlation with temperature [p < 0.05].

Conclusion: Surgical tissue manipulation did not significantly alter microvascular perfusion parameters. Changes in macrovascular parameters do not correlate well to changes in TVD, PPV or PVD, but an association was found with MFI. SDM allowed for real-time imaging of the sublingual microvasculature in cats. This technology has potential as a tool in experimental and clinical monitoring of microcirculatory changes.

Keywords: microcirculation, feline, surgery, anesthesia, microscan, OPS
Regenerative strategies for inferior bone and cartilage repair are a growing need in equine industry. Live cell transplantation for cartilage or bone damage have limited proof of efficacy and are burdened by donor morbidity, cell amplification costs, dual surgeries, cost of case-by-case inefficient cell processing, and with allografts, extensive, regulatory paperwork for accountability, disease screening, and tracking of human tissue. Our laboratory is one of the first to publish this effectiveness in a large animal model, using cell numbers, bone voids, and injection volumes. We have applied bone-marrow derived stem cells and dermal fibroblasts for bone regeneration in equine clinical patients. Eight horses enrolled had failure of bone fusion, bone healing or with bone cysts that met the inclusion criteria for our clinical trial. These horses had $5 \times 10^7$ autologous dermal fibroblasts, engineered to express bone morphogenetic protein-2 (BMP2), injected from an extraarticular approach, into bone voids. In some cases, cells were secured in place with thrombin and Platelet Rich Plasma. Horses were reevaluated at 2 and 6 months for soundness and radiographic appearance of the bone. Clinical improvement in lameness occurred in all cases within 2 months. Radiographic evidence of increased bone density at the bone void was evident in 5 of 7 horses within 60 days. Three horses had complete soundness within 60 days. Dermal biopsies were shipped from practices on ice for 4 of these cases demonstrating proof of feasibility for practical application. No complications such as joint swelling or lameness immediately after injection were identified. Cells are easy to grow and high cell yield occurred in every case. In Summary, cell BMP2 therapy effectively accelerates bone regeneration in equine bone, both cancellous and cortical bone and in clinical cases of failed bone repair.

Keywords: Horses, Cell therapy, Bone repair, Joint repair, Bone morphogenetic protein
ACCURACY OF COMPUTED TOMOGRAPHY IN DETERMINING LESION SIZE IN CANINE OSTEOSARCOMA OF THE APPENDICULAR SKELETON.
KS Karnik, EM Green, VF Samii, SE Weisbrode, CA London. The Ohio State University, OH 43210. Veterinary Clinical Sciences

Introduction/Purpose
Osteosarcoma (OSA) is the most common bone neoplasm in dogs and is associated with rapid progression of metastasis and high rate of mortality. To our knowledge, use of multi-detector CT technology performed before and after intravenous contrast medium administration to identify the extent of appendicular OSA has not been performed in the dog. The goal of this study is to assess the extent of appendicular OSA with multi-detector contrast enhanced CT and compare that with the gold standard of histopathology. Accurately determining the extent of diseased bone is of importance for surgical margins in limb-spare procedures and also in serial monitoring of response of diseased bone to chemotherapy or radiotherapy when amputation is not performed.

Materials and Methods
Ten dogs with confirmed appendicular OSA underwent CT imaging. A series of 0.625 mm transverse contiguous images of the affected and contralateral limb were acquired before and after intravenous contrast medium administration prior to amputation. The extent of intramedullary/endosteal, periosteal, and contrast enhancement of lesions on CT images were measured. The extent of disease was confirmed with histopathology following amputation. Linear regression analysis was used to correlate the extent of the lesions measured with CT to the gold standard of histopathology.

Results
The intramedullary/endosteal extent of neoplasia on CT correlated best with histopathology using a statistical model ($r^2 = 0.85$, slope = 0.98). There was poor correlation using the extent of periosteal disease ($r^2 = 0.27$, slope = 0.67) and contrast enhancement ($r^2 = 0.42$, slope = 0.59).

Discussion/Conclusions
The intramedullary/endosteal extent of neoplasia on CT correlates well with the gold standard of histopathology. This may be helpful for surgical planning for limb spare surgery and in assessing progression/regression of response to chemotherapy or radiotherapy.

Keywords
CT, computed tomography, OSA, osteosarcoma, multidetector, measure, limb spare, accuracy
EFFECTS OF AQUALASE® CAPSULE WASHING ON LENS EPITHELIAL CELLS AND PCO FORMATION IN VITRO FOLLOWING PHACOEMULSIFICATION. EA Lutz, DA Wilkie, AJ Gemensky-Metzler, HL Chandler. The Ohio State University, College of Veterinary Medicine, Department of Veterinary Clinical Sciences, Comparative Ophthalmology, Columbus, OH.

Purpose: To evaluate the in vitro effects of the Alcon Infiniti Vision System AquaLase Liquefaction Device® on lens epithelial cell (LEC) removal and posterior capsule opacification (PCO) formation following phacoemulsification.

Methods: 24 canine cadaver eyes were randomly assigned to one of three in vitro treatment groups. 6 eyes received phacoemulsification only, 9 eyes received capsule washing with Alcon recommended settings (vacuum limit=40mmHg, pulses per second (PPS)=50, 30 seconds of washing per capsule hemisphere), and 9 eyes received aggressive capsule washing (limit=60mmHg, PPS=50, 60 seconds of washing per capsule hemisphere). Lens capsules were monitored daily for 24 days following treatment, and migration and proliferation of LEC onto the posterior lens capsule (PCO) were evaluated.

Results: Lens capsules that received only phacoemulsification had marked, diffuse LEC remaining immediately following treatment, and complete PCO formation (confluent LEC on the posterior capsule) within 4±2 days (range 2-8 days). Capsules that received recommended AquaLase capsule washing had focal, equatorial LEC clusters remaining immediately following treatment, and complete PCO formation within 9±2 days (range 5-11 days). Capsules that received aggressive AquaLase capsule washing had no LEC grossly observed immediately following treatment; 5 capsules had complete PCO formation within 13±2 days (range 9-14 days), and 4 capsules had no PCO formation or LEC proliferation by 24 days post-treatment.

Conclusions: Alcon AquaLase® capsule washing is capable of removing LEC and delaying PCO formation following phacoemulsification in vitro. Use of more aggressive capsule washing settings resulted in more effective LEC removal and a greater delay in PCO formation.

Keywords: posterior capsule opacification, lens epithelial cell, phacoemulsification, Alcon AquaLase® capsule washing, canine
CLASSIFICATION OF EPILEPTIC SEIZURES IN HORSES: AN EPIDEMIOLOGIC STUDY OF 104 CASES (1988-2009). ME Mayes, S Mosseri, S Reed, VA Lacombe. College of Pharmacy; Department of Veterinary Clinical Sciences; The Ohio State University, Columbus, OH. Rood & Riddle Equine Hospital, Lexington, KY.

Although many studies have been performed to classify epileptic seizures (ES) in humans and small animals, no similar epidemiologic study is available in horses. Therefore, the objectives of this study was to classify ES in horses (all ages except neonates) presented for seizure disorders at The OSU Equine Veterinary Teaching Hospital between 1988 and 2009, following small animal classification and the current guidelines of the International League Against Epilepsy.

ES were classified by type and etiology, based on history, clinical observations, diagnostic investigations (e.g., electroencephalograms, CSF and computed tomography imaging of the head) and postmortem examinations (when available) in 104 horses.

Regarding their type, ES were defined as primary generalized in 16% of horses, secondary generalized in 21% of cases, simple and complex partial in 54% of cases, and unclassified in 9% of cases. Regarding their etiology, symptomatic ES, with underlying structural brain lesions (including brain tumor, abscess, traumatic or infectious meningoencephalitis), were identified in 41 horses and suspected in 48 horses. In contrast, reactive ES were only reported in 2 cases. Epilepsy (i.e., recurrence of ≥ 2 ES) was identified in 73 cases, and further classified as symptomatic (26 cases), cryptogenic (i.e., probably symptomatic, 40 cases), idiopathic (i.e., suspected genetic predisposition, 2 foal) or unknown in 5 cases.

This study is the first attempt to classify ES and epilepsy in a referral equine population. As in humans, such classifications are dynamic concepts but could help clinicians in the establishment of a diagnosis, a therapeutic plan or prognosis.

Keywords: Epilepsy, electroencephalogram, idiopathic, cryptogenic, symptomatic
THE EFFECT OF MECHANICAL ARYTENOID CARTILAGE ABDUCTION BEFORE KNOT TYING ON FAILURE OF EQUINE LARYNGOPLASTY IN A CYCLICAL ADDUCTION MODEL. N.R. McClellan1, E.M. Santschi1, S.D.A. Hurcombe1, A.S. Litsky2. Department of Veterinary Clinical Sciences1, and Orthopedic BioMaterials Laboratory2.

Objectives – To (1) develop a model that applies cyclical adductory forces on the arytenoid cartilage similar to swallowing or coughing. To (2) determine if a clamp that abducts the arytenoid cartilage during knot tying will improve the maintenance of arytenoid cartilage abduction following laryngoplasty.

Study Design – Experimental

Sample Population – Equine Larynges (n=14)

Methods – Larynges (n = 14) were collected from horses aged 2 - 4 years (median 3.5). Left arytenoid laryngoplasty was performed using a single suture of #5 Ethibond with (n=7) and without (n=7) abducting the left arytenoid with a clamp before the suture was tied. Each laryngoplasty was tested under cyclic loading of 2 to 26 N at 1 Hz for 5000 cycles. Data were assessed between groups using a Mann-Whitney U test and a Friedman statistic for repeated measures; significance was set at P < 0.05.

Results – Left arytenoid cartilages were abducted a median of 18.5 mm (range 11-24 mm). The median loss of abduction in the non-clamped group was 5.74 mm (31%) and in the clamped group was 7.52 mm (41%). This model of cyclical adduction of left arytenoid resulted in displacements similar to those seen clinically in the first week after surgery.

Conclusions – Abducting the left arytenoid cartilage with a clamp before knot tying does not improve laryngoplasty resistance to cyclical adductory forces.

Clinical Relevance – Cyclical adductory forces in the form of coughing and swallowing may be responsible for early postoperative loss of laryngoplasty abduction. This model will be useful in testing novel techniques for laryngoplasty.

Keywords: Horse; laryngoplasty; laryngeal clamp; cyclical adduction; arytenoid displacement
CANAL FLARE INDEX IN GERMAN SHEPHERD DOGS, GOLDEN RETRIEVERS, AND LABRADOR RETRIEVERS PRESENTING FOR TOTAL HIP REPLACEMENT: IMPLICATIONS FOR FEMORAL IMPLANT SELECTION

Pugliese LC; Dyce J; Allen MJ. College of Veterinary Medicine, The Ohio State University, Columbus OH

Introduction: Canal flare index (CFI), the ratio of the intracortical diameter at the lesser trochanter divided by that at the mid-diaphysis, has been used to assess proximal femoral geometry in total hip replacement (THR) patients. A CFI of ≤1.8 is classified as stovepipe morphology, while 1.8-2.5 is considered normal. The aim of this study was to compare CFI in three dog breeds commonly referred for THR. We hypothesized that German Shepherd Dogs (GSD) would have lower CFI than Golden Retrievers (GR) or Labrador Retrievers (LR).

Materials and methods: Thirty-two dogs of each breed were identified from a THR database. CFI values were calculated from ventrodorsal pelvic radiographs. One-way analysis of variance (ANOVA) was used to compare CFI, body weight and age in the three groups. Changes in CFI with age were assessed using linear regression. Implant use (CFX versus BFX stem) was compared using Chi-squared test.

Results: Mean CFI values were lowest in GSD (1.57 versus 1.78 in GR and 1.84 for LR; p=0.001). Age distributions were similar in the 3 groups but differences in body weight existed (GSD>GR; p=0.007). The relationship between age and CFI was weak and not significant (p>0.05 for all breeds). The use of BFX and CFX implants differed in the three breeds (p=0.00014) and dogs receiving BFX had higher CFI than those receiving CFX stems (p=0.003).

Discussion: Breed-specific variations in CFI have not been reported in dogs. GSD have a stovepipe femoral morphology and CFX implants are used more frequently in this breed.

Keywords: Dog, total hip replacement, implant selection
CHONDROGENIC POTENTIAL OF HUMAN SYNOVIAL-DERIVED MESENCHYMALSTROMAL CELLS AS VECTORS FOR KEY GROWTH FACTORS IN A CO-CULTURE MODEL. Seth S Jump, PhD\textsuperscript{2,3}, Eric B Skinner, BSc\textsuperscript{3}, Vincent Y Ng, MD\textsuperscript{1} David C Flanigan, MD\textsuperscript{1,2} & Alicia L Bertone, DVM, PhD\textsuperscript{1,2,3}

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Exposure of chondrocytes to synovial-derived mesenchymal stromal cells carrying chondrogenic growth factors increases proliferation and growth of the extracellular matrix. Human synovial-derived mesenchymal stromal cells (SDMSC) were co-cultured with autologous human chondrocytes (CH) for 14 days. The purpose of this cell culture experiment was to determine the effectiveness of SDMSCs to deliver selected genes via the shared cell culture media to promote the chondrogenesis of CH. In this experiment, the hypothesis was that SDMSC transduced with bone-morphogenetic protein 2 (AdBMP2), bone-morphogenetic protein 14 (AdBMP14), or fibroblast growth factor 18 (AdFGF18) would promote chondrogenesis compared to SDMSC transduced with Ad containing green fluorescent protein (AdGFP) or non-transduced SDMSC. SDMSCs were grown alone or in cell culture inserts and shared media with CH, which were seeded on the cell culture plate in monolayer. Media were harvested at 7 and 14 days, and cells were harvested at day 14. Outcome measurements included cell number and cell viability assessment (CyQUANT assay and hemacytometer count), determination of protein concentration for BMP2, pro-matrix metalloproteinase 1 (pro-MMP1), interleukin-1 beta (IL-1\textbeta), and hyaluronic acid (HA), and mRNA for aggrecan and type I, II, and X collagen.

Under all conditions, after 14 days there was an increase in SDMSC and CH cell number. The mRNA for aggrecan was upregulated in CH exposed to human synovial-derived mesenchymal stromal cells (SDMSC) transduced with AdBMP2, AdBMP14, or AdFGF18. Furthermore, there was an increase in the mRNA for collagen type I alpha in CH exposed to SDMSC transduced with AdGFP, AdBMP2, AdBMP14, or AdFGF18. In SDMSC alone, transduction with AdGFP, AdBMP2 and AdFGF18 increased the mRNA for aggrecan, while AdBMP14 decreased aggrecan mRNA. Exposure of CH to SDMSC carrying growth factors increases proliferation and growth of the extracellular matrix. Our data supports the potential clinical use of the cell for cartilage restoration.

Keywords: cartilage restoration, adenovirus, cell vector, growth factors
ACTIVITY OF SANGROVIT® AGAINST LAWSONIA INTRACELLULARIS IN GROWER PIGS AND ITS IMPACT ON GUT PHYSIOLOGY AND HOST IMMUNITY. V. Artuso Ponte,1 M. Abley,1 B. Molla,1 G. Rajashekara,2 P. Boyaka,3 W. Gebreyes1.

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Sanguinarine, a quaternary benzophenanthridine alkaloid plant extract of Macleaya cordata, has demonstrated to have anti-inflammatory, antimicrobial and immunomodulatory effect. It increases the availability of aromatic amino acids and decreases the levels of toxic biogenic amines. This study was aimed to evaluate the effect of Sangrovit® supplementation as compared to tylosin on growth performance, feed efficiency and Lawsonia intracellularis shedding in pigs, and to determine the effect of Sangrovit® on the immune system. A total of 24 pigs, four weeks-old challenged with Lawsonia intracellularis were randomly allocated to a treatment group (control non-supplemented, 40 g Sangrovit®/mton, 75 g Sangrovit®/mton, and 22g /kg tylosin). Pigs were weighed weekly and average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F) were calculated. Fecal samples were collected weekly for isolation and quantification of Lawsonia intracellularis using qPCR as well as blood samples for determination of IgA and IgG levels. After 21 days (acute phase), three pigs from each treatment group were euthanized for recording of lesions of the acute stage of the disease; the remaining 12 pigs were euthanized 90 days after challenge (chronic phase). Results showed that ADG and ADFI was higher for pigs receiving tylosin as compared to the other groups (p>0.05). Pigs receiving 75 gr. Sangrovit®/mton showed a higher G:F ratio as compared to the other groups (p>0.05). None of the treatment groups showed significant differences in Lawsonia shedding level based on quantitative PCR. Only control group presented characteristic lesions of Lawsonia infection at the acute stage of the disease (21 days). At the chronic stage, the highest ileum thickness score was observed in pigs receiving tylosin. Findings suggest that Sangrovit® supplementation is effective for improving growth performance and reducing pathognomonic lesions. Further studies are needed to determine the effect of Sangrovit® on the immune system.

Keywords: Sangrovit®, Lawsonia intracellularis, growth performance, immunity.
SEROPREVALENCE OF \textit{NEOSPORA CANINUM} AND \textit{TOXOPLASMA GONDII} IN WHITE-TAILED DEER (\textit{ODOCOILEUS VIRGINIANUS}) FROM SIX REGIONS IN THE CLEVELAND METROPARKS. G. Ballash and P. Dennis. Dept. of Veterinary Preventive Medicine

Sera from 444 white-tailed deer (\textit{Odocoileus virginianus}) from six distinct Cleveland Metropark reservations in Cuyahoga County, Ohio were tested for antibodies to \textit{Toxoplasma gondii} and \textit{Neospora caninum} by the Modified Agglutination Test and NcGRA6 ELISA, respectively. Overall, 261 (58.8\%) deer tested seropositive to \textit{T. gondii} and 105 (23.6\%) tested positive for \textit{N. caninum}. There was a significant increase in \textit{T. gondii} seropositivity from fawns to yearlings (P<0.001) and fawns to adults (P=0.001), however there was no significant increase from yearling to adults (P=0.326). A significant increase in \textit{T. gondii} seroprevalence was observed among the deer in the western reservations compared to the eastern reservations (P<0.001). Results for \textit{N. caninum} will be discussed. Any consumer of venison from the white-tailed deer population should practice safe food handling and cooking techniques to prevent potential \textit{T. gondii} infection.

Keywords: \textit{Toxoplasma gondii}, \textit{Neospora caninum}, seroprevalence, white-tailed deer
AN ORTHOTOPIC XENOGRAFT MODEL OF OSTEOSARCOMA WITH METASTASIS
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Background: Osteosarcoma (OSA) is the most common primary malignancy of bone in humans and is the second most frequent cause of cancer related deaths in children. In addition, OSA is the most common primary bone tumor in dogs. Despite improvements in the management of the primary bone disease, many human and veterinary patients will eventually succumb to distant metastasis involving the lungs. Although a number of OSA cell lines have been characterized in vitro, a widely used orthotopic mouse model of OSA with reliable metastasis has not been established. The objective of this study was to develop a relevant and predictable mouse model of osteosarcoma that recapitulates the clinical disease process with particular emphasis on the development of lung metastasis.

Methodology: Homozygous nude mice were injected subcutaneously with canine osteosarcoma cells. The subcutaneous tumors were allowed to grow for 4-6 weeks. The tumors were harvested and 6 additional nude mice underwent intra-tibial implantation of the solid tumor tissue. Mice were monitored weekly by digital radiography. At 5 weeks post implantation, the mice underwent hind limb amputations to 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.

Results and conclusions: During the intra-tibial implantation procedure, one of the tibias fractured and the mouse was euthanized. The remaining 5 mice developed lytic and proliferative radiographic lesions in the proximal tibia. This was further demonstrated by the post-amputation micro-CT imaging. Histology sections confirmed the presence of osteosarcoma in the proximal tibia of all mice. Two mice had gross evidence of lung metastasis and an additional two mice had microscopic lung metastases. In conclusion, this model successfully fulfills our goal of developing an orthotopic mouse model of osteosarcoma with lung metastasis.

Keywords: osteosarcoma, metastasis, mouse model
PREVALENCE OF STAPHYLOCOCCUS AUREUS IN BULK TANK MILK AND MANAGEMENT PRACTICES IN OHIO

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

Mastitis, continues to be the most common dairy cow disease worldwide. *Staphylococcus aureus* (SA) is a major contagious mastitis pathogen with a great economic impact and thus implementation of successful prevention/control program for it in dairy herds is important. The objectives of this study were to estimate the prevalence of SA in bulk tank milk (BTM) and to describe adoption of recommended mastitis control practices in Ohio dairy farms. A survey containing 44 questions on herd characteristics, milking procedures, heifer raising, mastitis control and biosecurity practices, was mailed to 780 Ohio producers, and 386 surveys were returned (response rate of 49.5%). Overall, 60% of the responders had 50-199 cows and 28% less than 50 cows. Twenty-nine percent (29%) of the herds with less than 50 cows were positive for SA compared to 53% when herd size increase to 50-199 cows. Of the responders, 36.6% have an open herd and of those only 22.7% quarantined purchased animals and 31.2% tested animals for any diseases before introducing them to the herd. Sixty-seven percent (67%) of these open herds were positives for SA. Forty-two percent (42.6%) of the herds reported no practice of pre-strip, 65% were positive for SA. Eighty percent (80.3%) utilize pre and post-dip and single-use towels. In 32.5% of the herds, known infected cows were milked last or separately. Forty-seven percent (47%) of the BTM tested positive for SA. A more comprehensive understanding of the management practices should be accompanied by research efforts to promote animal health and welfare, improve milk quality and increase the profitability of the dairy herds.

Keywords: management practices, bulk tank milk, *Staphylococcus aureus*, dairy cows
PASSIVE SURVEILLANCE AND GENOTYPING OF STAPHYLOCOCCAL SPECIES ISOLATED FROM PATIENTS AT A VETERINARY TEACHING HOSPITAL

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Antimicrobial resistance is a persistent concern in human and veterinary medicine. Increasing prevalence of nosocomial pathogens, particularly methicillin-resistant \textit{Staphylococcus aureus} (MRSA), highlights the importance of pathogen monitoring in healthcare facilities. While sophisticated surveillance programs are universally implemented in human medicine, long-term epidemiologic studies for tracking and monitoring pathogen transmission and antimicrobial resistance patterns in veterinary hospitals lag behind their human counterparts. The Ohio State University Veterinary Medical Center (OSU-VMC) in conjunction with the Infectious Diseases Molecular Epidemiology Laboratory (IDMEL) established a passive surveillance system whose objectives include monitoring the prevalence of bacterial pathogens, phenotypic and genotypic characterization of methicillin-resistant Staphylococcal species, and determining the frequency of antimicrobial resistant strains and predominant resistance patterns. From 2007 to 2010, \textit{Staphylococcus aureus} (n = 139) and \textit{Staphylococcus pseudintermedius} (n = 827) isolates cultured from canine, equine, and feline patients were analyzed with PCR to determine the presence of the \textit{mecA} gene and characterize SCC\textit{mec} types. Sixty-nine (50.7\%) out of 136 \textit{S. aureus} isolates tested were phenotypically resistant to oxacillin, while sixty-seven (51.5\%) out of 130 isolates were considered MRSA based on carriage of the \textit{mecA} gene. Of the \textit{mecA} positive isolates, 62 out of 67 (92.5\%) isolates expressed phenotypic oxacillin resistance. Approximately 54\% of isolates contained SCC\textit{mec} type II; the remaining isolates were SCC\textit{mec} type III, V/VII, IV, or remained unclassified. Among \textit{S. pseudintermedius} isolates, 319 out of 812 (39.3\%) isolates tested were phenotypically resistant to oxacillin, while 363 out of 740 (49.1\%) isolates tested were considered MRSP based on presence of the \textit{mecA} gene. Of the \textit{mecA} positive isolates, 273 out of 363 (75.2\%) isolates tested expressed phenotypic oxacillin resistance. Approximately 38\% of isolates had a SCC\textit{mec} type that currently remains unclassified, while 35.6\% of isolates were SCC\textit{mec} type V or VII, followed by SCC\textit{mec} types III and IV.

Keywords: MRSA, MRSP, \textit{mecA}, oxacillin, and methicillin
CHARACTERIZATION OF *ESCHERICHIA COLI* CARRYING $bla_{\text{CTX-M}}$ ISOLATED FROM COMMENSAL FLORA OF DAIRY CATTLE. D. Mollenkopf, M. Weeman, M. Abley, J. Daniels, W. Gebreyes, T. Wittum. Dept. of Veterinary Preventive Medicine and Veterinary Clinical Sciences

First reported in fecal *E coli* of US livestock in 2010, CTX-M extended spectrum β-lactamase genes encode for production of enzymes capable of inhibiting the antimicrobial effects of important cephalosporin drugs. This plasmid-borne gene conveys resistance to penicillins and 1\textsuperscript{st}, 3\textsuperscript{rd}, and 4\textsuperscript{th} generation cephalosporins, but not to cephamycins or β-lactamase inhibitors.

A collection of 70 PCR confirmed fecal *E coli* isolates carrying $bla_{\text{CTX-M}}$ recovered from 5 Ohio dairy herds were characterized at the level of the bacterium, plasmid, and gene. Isolates were evaluated by PFGE and minimum inhibitory concentration (MIC) to compare and contrast bacterial relatedness and antimicrobial resistance phenotype. Plasmids were conjugated to a K12 MG1655 recipient, extracted and analyzed by restriction fragment analysis using *Acc I*. Plasmid replicon types were determined using multiplex PCR. Gene amplicons were bi-directionally sequenced and analyzed using BLAST.

Multiple CTX-M genes were recovered including CTX-M-1, -15, and -14, with specific genes clustering within herds. PFGE of bacterial backbones found similar within herd clustering of isolates with the exception of one herd which contained at least 6 different bacterial fingerprints. Isolates were resistant to ampicillin, cefazolin, cephalothin, cefotaxime, cefpodoxime, ceftriaxone, ceftiofur, and cefepime, and had ceftazidime MICs which ranged from 2 to 8 μg/ml. These isolates were susceptible to cefoxitin and to beta-lactam drug combinations that included clavulanic acid or tazobactam. An exception was isolates from one herd which were found to harbor an AmpC β-lactamase gene in addition to the CTX-M.

Key words: β-lactamase, CTX-M, antimicrobial resistance, dairy cattle
IDENTIFICATION OF STAPHYLOCOCCUS AUREUS FROM BOVINE MILK USING BIOCHEMICAL TESTS AND PCR. H. Muftah, P. Rajala-Schultz, W. Gebreyes, F. DeGraves, J. Daniels, L. da Costa. Departments of Veterinary Preventive Medicine and Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA.

*Staphylococcus aureus* (SA) is a major cause of mastitis, causing huge economic losses to dairy industry. Positive result on coagulase test is considered indicative of presence of SA in routine mastitis diagnosis, however, other coagulase positive staphylococcus (CPS) species also exist. The objectives of this study are to determine if presence of CPS in bovine milk is indicative of presence of SA and t determine the level of antimicrobial resistance among these isolates. So far, 89 CPS strains isolated from milk of subclinically infected udder of individual cattle and from hundreds of bulk tank milk of Ohio dairy herds have been included in this study. Bacterial colony morphology, hemolysis, mannitol fermentation, growth on CHROMagar™ Staph aureus, and reaction on coagulase tube and latex agglutination (PASTORE™ STAPH-PLUS) tests are examined for all isolates. Amplification of the *S. aureus* nuc gene is considered a final confirmation of the identity of SA. Antimicrobial susceptibility testing of the isolates against ampicillin, ceftiofur, cephalothin, erythromycin oxacillin, penicillin, streptomycin, and tetracycline is performed using Kirby-Bauer disc diffusion method. So far, we have examined 49 out of 89 of our isolates, and PCR and susceptibility testing for the remaining isolates is on-going. All the tested isolates so far were confirmed SA using PCR. Phenotypically, the majority of the isolates were pansusceptible (38), only eleven (22.4%) were resistant to one or more antibiotics. Interestingly, four isolates carried the mecA gene which encodes for the Penicillin binding protein 2A that indicates methicillin resistance. The preliminary results suggest that CPS other than SA are rare in bovine milk and coagulase tube test can be considered sufficient for the identification of SA in milk samples. More investigations about genes that might be responsible for methicillin resistance are recommended.

Keywords: mastitis, coagulase positive staphylococcus, *S. aureus*

Since cattle are a major source of food and the multi-faceted cattle industry engages people from farms to slaughter houses and meat markets, it is conceivable that Campylobacter contaminated beef products would pose a significant public health impact. However, the occurrence of Campylobacter in cattle and its potential impact on human health in the US require further analysis. In this study, we determined the prevalence, genotypic, and phenotypic properties of C. jejuni and C. coli that were isolated from cattle presented to slaughter across the USA. Campylobacter were detected in 181 (19.2%) out of 944 fecal samples. Specifically, 71 C. jejuni, 132 C. coli, and 10 other Campylobacter were identified. Our results showed that the prevalence of Campylobacter in cattle feces was regionally different. Specifically, Campylobacter were retrieved from 32.8% of the fecal samples collected from the South, which was significantly ($P<0.05$) higher than those from the North (14.8%), Midwest (15.83%), and East (12%). Further, PFGE analysis suggested that the C. jejuni and C. coli were genotypically diverse and geographically constrained. In addition, 11 new C. jejuni STs, including 3 novel alleles, and one C. coli ST were detected by MLST analysis. The most frequently observed clonal complexes were ST-21, ST-42, and ST-61, which are common in humans. Interestingly, the bovine Campylobacter isolates showed high resistance to several antimicrobials including ciprofloxacin, erythromycin, and gentamicin. In conclusion, our results highlight the importance of cattle as a potential reservoir for clinically important Campylobacter.

Keywords: cattle, Campylobacter, USA, Public Health

Salmonella is a zoonotic foodborne pathogen with several common serotypes which can be ingested through contaminated meat. In a previous study conducted by our team, feed, environmental, and fecal samples were all found to contain Salmonella. The purpose of this new study was to identify and characterize the possible role of Salmonella in the feed, and its phenotypic and genotypic relationship with fecal and environmental samples. A total of 280 isolates were tested and categorized based on serogrouping, genotyping, antimicrobial susceptibility testing, and PFGE DNA fingerprinting. The results demonstrated five genotypic clusters of highly similar isolates. Four of these clusters showed a genotypic relationship between the isolates of feed origin and those of fecal origin. The four clusters identified with feed and fecal isolate relationships were serogroup B with AmStTeKm resistance, serogroup C with SuTe resistance, serogroup B with Te resistance, and serogroup E with Te resistance. The study also identified a high proportion of multi-drug resistant isolates. The most common multi-drug resistant patterns were SuTe (20.4%), Te (28.2%), and AmStTeKm (12.9%). Serogroups C (26.8%), E (13.6%), and B (42.5%) were also found to be the most common. These patterns were found in the feed and fecal samples from the same barn at the same collection time. The significance of finding multidrug resistant, epidemiological connections between the isolates indicates feed as an important source of contamination. Therefore targeted intervention measures may be designed to reduce transmission and contamination of Salmonella, particularly multi-drug resistant strains, into the food supply.

Keywords: Salmonella, Swine, PFGE
RESEARCH PATHOLOGY SUPPORT FOR ANIMAL MODELS PROVIDED BY THE COMPARATIVE PATHOLOGY & MOUSE PHENOTYPING SHARED RESOURCE.
K. La Perle. Dept. of Veterinary Biosciences

The Comparative Pathology & Mouse Phenotyping Shared Resource (CPMPSR) provides expert, readily available and affordable experimental pathology support to investigators utilizing animal models to study human disease. Comparative pathologists affiliated with the CPMPSR are familiar with normal anatomy and physiology, as well as background age- and strain-related lesions of various animal models. Recognition of lesions and their interpretation in the context of individual investigations provides a critical component to research incorporating animal models. Services include comprehensive macroscopic and microscopic examinations of various species of laboratory animals with an emphasis on the phenotypic characterization of newly produced lines of genetically engineered mice. Additional services include hematology, clinical chemistry, radiography, routine frozen and paraffin slide preparation as well as tissue microarray preparation and special histochemical and immunohistochemical staining. In addition, the CPMPSR provides a referral service to experienced scientists within the OSU research community providing expertise in animal model development, experimental design, optimal sample collection, and data interpretation.

Keywords: Comparative pathology, animal models, genetically engineered mice, hematology, clinical chemistry, histology, immunohistochemistry
Clinical trials are investigations that test the safety and/or effectiveness of drugs, devices, treatments, or preventive measures in both humans and animals. Most clinical trials are conducted based on preliminary data from laboratory studies that have shown how a new treatment may be effective. Carefully conducted clinical trials are the safest and fastest way to evaluate newly discovered methods and treatment options. In many cases clinical trials are done to try and find a better treatment when the current therapies are not very effective.

The Clinical Trials Office 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 office 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).

As clinical trials continue to lead a role in the advancement of both veterinary and human medicine, the efforts of the CTO serve to enhance the ability of all at the College of Veterinary Medicine to successfully undertake clinical investigations in the veterinary patient population. 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
Recent advances in genetics and molecular biology have allowed researchers to identify genes and molecules associated with cancer in people. Understanding the genetics and behavior of genes and proteins in cancer cells provides information for prevention and early detection, and enables researchers to identify targets for new drug therapies.

Millions of dogs and cats are diagnosed with cancer each year. We have only begun to investigate the genetics and molecular biology of cancer in dogs and cats, but based on advances in human medicine, it is likely that similar progress can be made in the early detection, treatment, and prevention of cancer in veterinary medicine. The Ohio State University College of Veterinary Medicine established the Biospecimen Repository or “Tissue Bank” to collect tissue samples from dogs and cats with cancer so that the genetics and molecular biology can be more closely studied.

The Tissue Bank collects samples of tumors and normal tissue from dogs and cats, and stores these tissues under controlled conditions for future use by multiple investigators. Tissues are collected and archived only after receiving informed consent from the owners. The types of biological materials that are processed include a piece of the tumor, normal tissue adjacent to the tumor, whole blood, plasma, serum, and urine. Samples are preserved in liquid nitrogen, formalin and Optimum Cutting Temperature (OCT) medium. This tissue bank will serve as a tremendous resource with the ultimate goal of developing new prevention and treatment strategies for companion animals with a variety of illnesses.

Because the biology and behavior of many types of cancer in dogs and cats are similar to cancer in humans, knowledge gained from research on companion animal cancer patients benefits both people and pets.

Keywords: biospecimen, cancer, genetics, molecular biology, tumor
DEVELOPMENT OF A VIRTUAL REALITY SIMULATOR FOR TEACHING CANINE ARTHROSCOPY. T. Motta, M. Shaw, D. Stredney, J. Au, M. Allen. Dept. Veterinary Clinical Sciences and Ohio Supercomputer Center

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

To reconstruct the regional anatomy, we have acquired high-resolution images from a canine stifle joint using computed tomography scans to accurately model the bone. In addition, we have acquired magnetic resonance imaging data to display the soft tissue structures.

Currently our simulator provides visualization of the canine stifle joint and allows for interactive manipulation using a haptic device that simulates surgical tools with force reflection. We are able to view anatomical structures on the bone and highlight the important structures vital for proper orientation to successfully execute this surgical procedure. The simulator allows the surgeon in training to arbitrarily section through the hard and soft tissues providing an additional method for learning the spatial configurations of the regional anatomy.

Our ongoing development includes additional tools to physically investigate the canine stifle joint. During this stage, experts will assist in validating the simulator for its realism as well as help provide metrics for which trainees can be evaluated, thus providing an avenue to introduce automated assessment. Through these steps, we will move towards our objective of employing simulation technologies to replace the use of cadaver materials in the formative development of surgical technique for canine arthroscopy.

Keywords: simulation, surgery, arthroscopy, teaching, orthopedics
In commercial embryo transfer programs, horse embryos are generally collected between days 6-8 after ovulation without knowing pregnancy status. It would be desirable to attempt to diagnose pregnancy before embryo collection and transfer. In a previous study, embryos measuring < 10 mm in diameter were detected by transrectal ultrasonography with a 5 MHz transducer before embryo recovery and transfer (Kivett and Pinto, 2005). In that study, the authors reported a pregnancy rate of 33.3%. Furthermore, relatively greater pregnancy rates were obtained for embryos measuring 3-5 mm in diameter than pregnancy rates for embryos measuring 5-9 mm.

The aims of the present study were: 1) to determine whether embryos ≤ 3 mm diameter could be diagnosed by advanced transrectal ultrasonography; 2) to compare the pregnancy rate of conventional embryo transfer with that of transfer of large embryos. We hypothesized that the transfer of 2-3 mm horse embryos would result in pregnancy rates similar to that following conventional embryo transfer.

Preliminary work: four reproductively mature mares ranging from 6-15 years of age were utilized in this study. Mares found in estrus and with follicles ≥ 33 mm were artificially inseminated with fresh semen and treated with 2000 IU of human chorionic gonadotropin (hCG) to induce ovulation. Beginning 9 days post hCG injection, each mare was examined by transrectal ultrasonography (Voluson i GE Healthcare system ultrasound equipped with a 7.5 MHz transducer) every six hours until an embryo was detected and recovered by uterine lavage. Two of the four mares were diagnosed pregnant at 10.5 and 11 days following hCG administration, with embryos measuring 2 and 3 mm, respectively. These preliminary results indicate that embryos measuring ≤ 3 mm can be successfully detected by advanced ultrasonography. We expect that the transfer of embryos of this size would result in acceptable pregnancy rates.

Keywords: embryo, equine, ultrasonography
EFFECTS OF FLUPHENAZINE DECANOATE ON STRESS AND REPRODUCTIVE CYClicity IN BISON (BISON BISON) AM Curtis¹, MM Vick², P Bapodra³, BA Wolfe¹,³
¹The Ohio State University College of Veterinary Medicine
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Stress has recently come to the forefront of medicine as an important factor in health and reproduction. One significant detrimental effect of acute and chronic stress is reduced reproductive potential. This is critical in the management of non-domestic animals, many of which are considered endangered due to low population numbers. When captive populations are small, genetic management for increased diversity could most practically and safely be accomplished by applying assisted reproductive techniques. These techniques have historically met with poor results in nondomestic animals potentially due to intensive handling situations leading to higher levels of stress compromising reproductive success.

A promising method to mitigate the effects of stress is through the use of long acting neuroleptics which reduce the physiologic responses to stressful stimuli. However, the mechanism of action of neuroleptics could have a negative effect on reproduction by increasing prolactin levels, potentially leading to a delayed or absent LH surge thus impacting ovulation.

This study examined the effect treatment with fluphenazine decanoate had on cortisol levels, gonadotropin release, and ovulation with respect to pregnancy rates following synchronization and timed AI in bison. The animals were randomly divided into two groups with one group receiving fluphenazine. Both groups were synchronized and underwent timed AI with frozen semen. Fecal levels of progesterone and cortisol were monitored throughout. One control animal and no treatment animals were diagnosed as pregnant. Progesterone levels rose more slowly in treatment animals and all animals experienced early corpus luteum regression. Fecal cortisol levels were higher in treated animals and these animals were determined to be more resistant to handling. These results were unexpected and point to the need for more studies to fully ascertain how neuroleptic therapy might be utilized in managing and breeding nondomestic hoofstock on a species by species basis.

Keywords: Bison, stress, neuroleptic, estrus synchronization, AI
THE IMPACT OF BEDDING TYPE ON CAGE CHANGEOUT FREQUENCY. R. Erickson, D. Domer, and V. Bergdall. University Laboratory Animal Resources

Cage changeout frequency in laboratory mice can have impacts on animal health and stress levels, human health, and operational costs of husbandry. Cage changing frequency is dictated by intracage ammonia levels. Ammonia gas is released from urea by urease, which is produced by fecal bacteria and bacteria present in bedding before use. PureLite is a proprietary method of processing corncob bedding, and it has been shown to have significantly less bacteria than standard corncob bedding. The use of PureLite bedding could lead to a decrease in intracage ammonia levels and thus decrease the frequency of cage changing and sanitation without adverse effects to the animals. For this study, mice were placed 5 per cage with non-sterilized standard corncob, non-sterilized PureLite, sterilized standard corncob, or sterilized PureLite bedding. Intracage ammonia levels were measured daily using an ammonia tube test device, and the number of days to reach 25 ppm ammonia was documented. The study was performed in both static and ventilated cages. In static cages, sterile corncob bedding took significantly fewer days to reach 25 ppm ammonia than both non-sterile corncob and sterile PureLite; no significant difference was found between non-sterile corncob and non-sterile PureLite. In ventilated cages, sterile corncob took significantly longer to reach 25 ppm than sterile PureLite or non-sterile corncob; non-sterile PureLite lasted significantly longer than non-sterile corncob; and non-sterile PureLite lasted longer than sterile PureLite. Further experiments will include quantitative and qualitative analysis of bacterial growth in the above conditions.

Keywords: rodents, husbandry, ammonia, bedding, corncob, PureLite
EFFECTS OF LACTOFERRIN ON POST-BREEDING UTERINE INFLAMMATION IN THE MARE.  B. S. Forshey, C. A. Messerschmidt, C. R.F. Pinto and M. A. Coutinho da Silva.  Department of Veterinary Clinical Sciences, The Ohio state University, Columbus, OH 43210

Our objective was to determine the effects of lactoferrin on the post-breeding inflammatory process of the endometrium. Our hypothesis was that lactoferrin would modulate the inflammatory process post-breeding by altering expression of pro-inflammatory cytokines in the endometrium. Six cycling mares received either the control treatment (semen only) or lactoferrin (semen + 1 g lactoferrin) in a cross-over design. Estrous mares were inseminated with $1 \times 10^9$ dead sperm diluted in 50 mL of skim milk based extender with or without 1 g of lactoferrin, and received 2500 IU of human chorionic gonadotropin (hCG) to induce ovulation. The time of ovulation and the amount of intrauterine fluid (0 = none; 4 = large) was determined daily by ultrasonography. Endometrial culture, cytology, and biopsy were collected approximately at 24 h post-insemination. Endometrial swab was submitted for aerobic culture and the amount of bacterial growth was determined (0 = no growth; 4 = heavy growth). Endometrial cytology was stained with Diff-Quick® and evaluated to determine the percentage of white blood cells (WBC) in the smear. Endometrial biopsies were immediately frozen and then evaluated by RT-PCR to determine expression of the following genes: IL-1β, IL-6, IL-8, IL-10, and TNF-α. Data were analyzed by Wilcoxon Rank Sum test and significance was set at $P<0.05$. Ovulation was detected in all mares within 48 h of hCG administration. Twenty four hours after insemination, there were no significant differences between control and lactoferrin groups for intrauterine fluid (2.2 vs. 1.7), bacterial growth (1.2 vs. 0.8), and percentage of WBCs (37.3 vs. 21%). However, there was a decrease in the expression of the pro-inflammatory cytokines IL-1 ($P<0.05$) and IL-8 ($P<0.07$). In conclusion, lactoferrin decreased expression of pro-inflammatory cytokines and could potentially be used to prevent persistent post-breeding endometritis in mares.

Keywords: Lactoferrin, Endometritis, Inflammation, Equine, Mare.

Acknowledgements: Merial Summer Research Program and Laura L. Pierson, DMV International, Delhi, NY.
EVALUATION OF RODENT ANESTHESIA: DO STRAIN SPECIFIC RESPONSES EXIST? C Hilty, A Dardenne DVM, and C Freed MLAS, DVM, DACLAM. University Laboratory Animal Resources.

The mouse is the most frequently used animal model for biomedical research. To meet research needs, surgical anesthesia is commonly induced, yet information is limited regarding strain specific variability in physiologic responses. This study investigated isoflurane, a commonly used inhalant anesthetic and a parenterally administered cocktail consisting of a dissociative agent (ketamine), sedative (acepromazine) and an alpha-2 agonist (xylazine) in the C57BL/6 and BALB/c inbred mouse strains. For both groups, a surgical plane of anesthesia was maintained for 20 minutes. For the ketamine-xylazine-acepromazine (KXA) group, a range of drug doses was required as individual responses to the initial dose were not consistent. The alpha-2 antagonist, yohimbine, was administered during the recovery period. The induction rate, duration and depth of anesthetic plane, and recovery time were noted for each strain and each anesthetic protocol. Physiologic parameters were monitored during anesthetic events and electrocardiograms (ECG) were evaluated on a subset of animals. In addition, blood samples were collected at baseline and following anesthetic events for analysis. No statistically significant differences between strains were observed based on the evaluated parameters in the two strains that were selected. Not surprisingly, significant differences were noted between the anesthetic protocols for both the C57BL/6 and BALB/c strains. Specifically, the induction period and recovery time were significantly shorter for the isoflurane group when compared with the KXA group, which was consistent across both inbred strains. Overall, based on our study, isoflurane is still the recommended option for achieving a surgical plane of anesthesia. However, if injectable protocols are needed based on research needs, a dose of 150/20/3 mg/kg KXA effectively produces a surgical plane of anesthesia in both the C57BL/6 and BALB/c strains with minimal impact on the parameters measured.

Keywords: anesthesia, rodents, isoflurane, ketamine, xylazine, acepromazine
THE WHITE-COAT EFFECT ON BLOOD PRESSURE IN RETIRED RACING GREYHOUNDS. C.L. Marino, C.G. Couto, M.C. Iazbik. Department of Veterinary Clinical Sciences

The white-coat effect (WCE) is a phenomenon attributed to an autonomic response that raises blood pressure in a hospital setting when compared to normal, at home, ambulatory blood pressure. This has been well documented in humans and cats, with the degree of hypertension correlating to an increased risk of kidney disease; however, studies have failed to reliably show a WCE in dogs. Greyhounds are well known for their cardiovascular peculiarities, including a higher resting blood pressure than non-Greyhounds. In this study, the systolic, diastolic, and mean arterial pressure (SAP, DAP, MAP) and heart rate (HR) of 22 retired racing Greyhounds enrolled in the OSU Animal Blood Bank donor program were measured in three environments: the hospital blood bank (Hosp), at home by a student (H/S), and at home by the owner (H/O). Oscillometric measurements were taken from the left antebrachial and left tibial sites using a 5cm cuff with the Greyhounds in right lateral recumbency. The highest and lowest values were eliminated from a series of five measurements and the remaining three were averaged. An ANOVA for repeated measures revealed significant differences between the Hosp and H/S measurements (P< 0.001) and between the Hosp and H/O measurements (P<0.001) for SAP, MAP, and HR; interestingly, the DAP (H/O) was only significantly lower than the DAP (Hosp). No significant differences were found between the H/S and H/O measurements. The SAP was significantly higher in the rear leg in all settings (P<0.05). The results show a WCE on blood pressure and heart rate that appears to be due to the environment rather than people. The increased musculature and oxygen demand when racing may explain the higher hind limb SAP. It is clear that although Greyhounds are “hypertensive” in a hospital setting, the blood pressure is not sustained in their every day environment.

Keywords: white-coat effect, blood pressure, hypertension, Greyhounds
Sepsis, a condition where bacteria multiply in the blood and spread throughout the body, is the leading cause of death among newborn foals. Sepsis is a systemic inflammatory state, often leading to hypoperfusion, acid-base and electrolyte abnormalities, organ failure and death. The hypothalamic-adrenal-pituitary axis is central in the response to stress by regulating cardiovascular functions, immune response, and metabolic functions.

The hypothalamus controls the secretion of adrenocorticotropic hormone-(ACTH) from the pituitary gland by releasing corticotropin releasing hormone (CRH) into the pituitary portal system. In horses, arginine vasopressin (AVP) is also an ACTH releasing factor. ACTH stimulates the adrenal gland to produce cortisol and aldosterone. Cortisol has multiple metabolic functions, while aldosterone, acts in the kidney to retain sodium and eliminate potassium.

The goal of this project was to determine the blood concentrations of CRH, AVP as well as their association with ACTH, cortisol and aldosterone concentrations in septic, sick non-septic and healthy foals. We hypothesized that CRH, AVP, ACTH, cortisol and aldosterone concentrations will be elevated in septic foals and associated with mortality.

The study population included septic (n=37), sick non-septic (n=127) and healthy (n=20) foals of < 7 days of age. Foals with a sepsis score >11 were considered septic, while foals with a sepsis score <10 were considered non-septic. Blood samples were collected in plain and EDTA/ aprotinin tubes. Hormones were measured with validated radioimmunoassays.

Of the sick foals, 30% (27/127) were septic, with a mortality rate of 48% (17% for sick non-septic). AVP, ACTH, cortisol, and aldosterone concentrations were higher in septic foals compared to other groups (P<0.05). An unexpected but interesting finding was that CRH concentrations were lower in septic foals. We found no differences in CRH, ACTH, aldosterone, and cortisol concentrations between septic survivors and non-survivors foals.

These findings suggest that a coordinate response at the hypothalamic, pituitary and adrenal gland occur in septic foals and that unlike other species during sepsis, AVP (not CRH) is the main ACTH releasing hormone in critically ill foals.

Keywords: foal, sepsis, adrenocorticotropic hormone, corticotrophin releasing hormone, arginine vasopressin, cortisol, hypothalamus, pituitary gland, adrenal gland
Antimicrobial resistance in pathogenic bacteria is a serious concern in human and veterinary medicine. Fluoroquinolone antimicrobials are used to treat a variety of bacterial infections, including urinary tract infections in dogs. Enrofloxacin, a veterinary-licensed fluoroquinolone, is metabolized to ciprofloxacin, which also has antibacterial activity. Spontaneous mutations in bacterial DNA gyrase genes confer resistance to these compounds in *E. coli* and other uropathogenic bacteria. Using drug dosages that achieve urine fluoroquinolone concentrations exceeding the threshold that permit such mutant formation would be desirable in patients. This threshold value for a bacterial isolate is known as the Mutant Prevention Concentration (MPC). Drug concentrations below the MPC will enhance the selection of fluoroquinolone-resistant bacteria.

Urine enrofloxacin and ciprofloxacin concentrations in healthy dogs (n=6) were measured using high-performance liquid chromatography (Mark Papich, NCSU) following the highest labeled dose of oral enrofloxacin (20 mg/kg). Mean maximum urine concentrations of enrofloxacin and ciprofloxacin were 139 µg/mL (range 73 µg/mL–226 µg/mL) and 371 µg/mL (range 200 µg/mL–639 µg/mL) respectively. We also measured MPCs among clinical canine urinary isolates of *E. coli* (n=27), which were sourced from different patients and determined to be genetically distinct by Pulsed Field Gel Electrophoresis. Using standard clinical interpretive criteria, these isolates were all susceptible to ciprofloxacin, with minimum inhibitory concentrations that ranged 0.016 µg/mL to 0.031 µg/mL. The MPCs of the *E. coli* ranged from 0.125 µg/mL to 1.0 µg/mL. These results indicate 1) that ciprofloxacin is the major form of the drug in canine urine and 2) suggest that high dose enrofloxacin therapy may slow the emergence of fluoroquinolone resistance among isolates of *E. coli* that cause urinary tract infections in dogs.

Keywords: MPC, enrofloxacin, ciprofloxacin, fluoroquinolone resistance, *E. coli*, UTI, MIC
HEMOSTATIC ACTIVITY OF CANINE PLASMA STORED FOR TRANSFUSION. R. Urban, G. Couto, M.C. Iazbik, D. Hudson, A. DeFelice. Department of Veterinary Clinical Sciences

In humans, fresh frozen plasma (FFP) loses factor V and VIII activities (labile factors) after a year, and becomes frozen plasma (FP). Therefore, it is theoretically unsuitable for use in patients with coagulopathies. We hypothesized that FP is hemostatically active after 5 years of storage.

We evaluated fresh plasma (n=15) from blood donor Greyhounds within 2 hours of collection using thrombelastography (TEG), OSPT, APTT, fibrinogen (FIB), and antithrombin (AT); samples were evaluated again 42 days after either being refrigerated or frozen. Five-year old FP (n=10) was thawed and divided into aliquots. Aliquots were evaluated as above at days 0, 2, and 7 (stored in refrigerator), and at days 7 and 28 (stored in regular freezer). We also evaluated 8 samples for clotting factors V, VIII, IX, and X activities.

The OSPT and APTT were significantly longer and the FIB and AT significantly lower in the FP (p<0.05); however, most values in FP were within the reference range for dogs. There was a significant decrease in the R and increase in the angle of FP when compared to fresh plasma. Interestingly, the strength of the clot (MA) was not different between groups. FP stored at -30ºC for 42 days had significantly shorter R and higher angle (p=0.002) than FP immediately after thawing. The shortening of the R and increase in the angle in both groups suggest activation of hemostasis by refreezing, likely by platelet microparticles. As expected, activity of clotting factor VIII (but not of factor V) was low; however, the plasma appeared to be hemostatically active when evaluated by TEG.

These results support the fact that 5-year-old canine FP stored for transfusion at -30ºC is hemostatically active and should be clinically evaluated in patients with coagulopathies. If active, the monetary savings of using “old” plasma will be remarkable.

Keywords: canine, dog, hemostasis, plasma, FP, frozen plasma, TEG, thrombelastograph, APTT, OSPT, antithrombin, fibrinogen, Greyhound, transfusion
USE OF COTTON-SWABS FOR DETECTION OF \textit{STAPHYLOCOCCUS AUREUS} IN BOVINE MILK A. Wagner, P. Rajala-Schultz, L. da Costa. Department of Veterinary Preventive Medicine

\textit{Staphylococcus aureus} (SA) is a common cause of mastitis in dairy cows. Detection of SA from milk samples is important for effective control of the organism and a practical and sensitive method is necessary for clinical use. The purpose of this study was to compare the efficacy of detecting SA from bovine milk samples using sterile cotton-tipped applicators (cotton-swabs) compared to 10 $\mu$l calibrated loops and plating 100 $\mu$l, using micropipettors. Quarter milk samples ($n=156$) were examined from 40 cows on three Ohio Department of Rehabilitation and Correction dairies. Samples were plated on trypticase soy agar with 5\% sheep blood and growth was examined at 24 and 48 h. Using any method, SA was found in 24 quarters of 11 cows. Using cotton-swabs, SA was found in 23 quarters with a sensitivity of 95.8\% (95\% confidence interval [CI]: 78.9-99.9\%) and a specificity of 100\% (95\% CI: 97.2-100\%). With both the 10 and 100 $\mu$l volumes, SA was found in 21 quarters with a sensitivity of 87.5\% (95\% CI: 67.6-97.3\%) and a specificity of 100\%. The methods provided almost perfect agreement (Kappa > 0.88). In most cases, number of CFU/ml was >10 in the positive samples. Our results suggest that swabs provide a practical and accurate alternative for detecting SA in bovine milk.

Keywords: Bovine mastitis; detection; \textit{Staphylococcus aureus}
Animal traceability and disease monitoring are major concerns for veterinary preventive medicine and public health. To improve traceability in Ohio, added to the AgTraq® animal tracing system already in place, and used the data in a Food and Mouth Disease (FMD) outbreak simulation for the Ohio Department of Agriculture (ODA). Working with both ODA and Wright Patterson Air Force Base, we used geospatial engineering to create several maps of Ohio’s animal agriculture. We also made maps that included Ohio Veterinary Emergency Responders (OVER) data locations and area coverage, diagnostic laboratory locations, and roadways to facilitate different aspects of disease identification and control. Further, Android® cellular phone applications were developed for real-time disease monitoring to be used by the Veterinary Medical Officers (VMO) employed by ODA. The Android® application can be used by VMO’s to take pictures of diseased livestock and type up diagnoses to be submitted to ODA in real-time, while the VMOs simultaneously are sending the samples to diagnostic laboratories for confirmation. We tested the Android® application in our simulated FMD outbreak. Our system allows government officials responsible for the health and safety of humans as well as Ohio’s agriculture the ability to halt disease spread more rapidly before substantial damage can occur. The summation of our mapped agriculture data and FMD simulation provides a novel, real-time animal traceability and disease monitoring system for the state of Ohio.

Keywords: Foot and Mouth Disease (FMD), animal traceability, agriculture, geospatial engineering, Android® technologies
OCCURRENCE AND MOLECULAR EPIDEMIOLOGY OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) ON FARM, AT SLAUGHTER AND PORK.

M. Byrne, B.Z. Molla, M. Abley, W.A. Gebreyes. Dept. of Veterinary Preventative Medicine

Background: Specific strains of methicillin resistant *Staphylococcus aureus* (MRSA) associated with pigs (ST 398) have been reported in pigs and those in contact with pigs from a number of countries. MRSA in slaughtered pigs and pork products has not been addressed and therefore the potential food safety implications in the U.S. are not known.

Purpose: A serial cross sectional study targeting ten cohorts of commercial swine farms was conducted to determine the occurrence and prevalence of MRSA in market age pigs and assess the likelihood of carriage or cross contamination with MRSA at slaughter to determine the potential food safety implications.

Methods: Paired nasal and peri-anal swab samples (n=24/farm) were collected from pigs on-farm and the same cohort of pigs were followed and sampled at the lairage before slaughter and carcass swabs at the post evisceration stage. Pork samples from the same group of pigs were collected at retail market. *Staphylococcus aureus* isolates were recovered following conventional cultural methods using oxacillin resistance screening agar. Biochemically screened isolates were tested for the presence of a species-specific gene (*nuc*) and methicillin resistance marker gene (*mecA*).

Results: MRSA was detected in 40% (4/10) of the herds included in the study. The prevalence of MRSA in pigs was higher at lairage and ranged from 0 to 54.2% per farm compared to that same batch of pigs on-farm (0 to 12.5%). The proportion of MRSA positive isolates was relatively higher in nasal compared to peri-anal samples. We detected MRSA in 1.6% (4/240) of the carcass swab and 3.7% (5/135) of the retail pork samples. MRSA isolates showing phenotypic and genotypic similarity were recovered from batch of pigs before slaughter at the lairage, carcass swabs and retail meat.

Summary: The preliminary results show that MRSA is prevalent in market age pigs on-farm and at the lairage, on carcass and subsequently on retail pork. Persistence of MRSA in the food chain implies potential food safety significance.

Keywords: MRSA, swine, occurrence, molecular epidemiology, food safety
IDENTIFICATION OF *NEOSPORA CANINUM* OOCYSTS FROM FECES OF OHIO COYOTES (*CANIS LATRANS*) USING QUANTITATIVE PCR.
S Gupta, B Wolfe, P Rajala-Schultz. The Ohio State University College of Veterinary Medicine Department of Veterinary Preventative Medicine & The Wilds

Coyotes (*Canis latrans*) and domestic dogs are the definitive hosts for *Neospora caninum*, a coccidian parasite known to cause abortion in cattle and other ungulates, as well as fatal neurologic disease in dogs. *Neospora* oocyst shedding is not well characterized in coyotes, but is believed to occur in low numbers. *Neospora* oocysts are morphologically indistinguishable from the oocysts of *Hammondia hammondi*, a common coccidian of dogs, and *Toxoplasma gondii*, acquired incidentally through consumption of cat feces. This study surveyed the prevalence of *Neospora* oocyst shedding in coyote feces on the *Wilds*, a 10,000-acre conservation and research facility in southeastern Ohio housing 18 species of endangered ungulates. Herds tested at the *Wilds* demonstrate high levels of serpositivity to *Neospora caninum*, indicating exposure to the parasite potentially from horizontal transmission by ingestion of coyote feces or vertical transmission from dam to fetus. Coyote droppings were collected from May to September, 2010 at the *Wilds* for parasitic evaluation by fecal flotation. Parasites were identified in 75.9% (22/29) of samples, of which 68.2% (15/22) demonstrated *Neospora*-like oocysts. Quantitative PCR (qPCR) was used to identify the repetitive Nc5 region of the *Neospora* genome in sporulated oocysts and for quantification of DNA copy number. This ongoing study will result in a year-long seasonal profile of *Neospora caninum* oocyst shedding in southeastern Ohio coyotes, providing a framework for identification of at-risk populations of domestic and nondomestic ungulates in the area, for which coyotes may be an important vector of neosporosis.

Keywords: *Neospora caninum*, QPCR, Ohio, *Canis latrans*, Coyote, Neosporosis
THE IMPACT OF CEFTIOFUR REMOVAL ON RECOVERY OF *SALMONELLA* SPP. AND *E. COLI* RESISTANT TO THIRD GENERATION CEPHALOSPORINS

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**Introduction:** The use of the third generation cephalosporin, ceftiofur, is common in animal agriculture for the treatment of a wide range of production-limiting disease conditions. The veterinary use of ceftiofur in livestock is capable of selecting for the \textit{bla}\textsubscript{CMY-2} resistance gene in the intestinal flora. **Objective:** The purpose of this study was to determine the effect of ceftiofur use restriction on the recovery of \textit{bla}\textsubscript{CMY-2} within both the intestinal flora of livestock populations and their meat products. **Methods:** Two farms, each with established beef and dairy cow herds, were monitored over 15 months. At each farm, 50 fecal samples were collected monthly from both beef and dairy populations. Weekly ground meat samples were collected from the processing plant responsible for harvesting animals within these farm systems. The samples were screened for *Salmonella* spp. and *E. coli* resistant to third generation cephalosporins. **Results:** Overall, *E. coli* harboring \textit{bla}\textsubscript{CMY-2} was recovered from 44.1% of 2273 total fecal samples, and *Salmonella* spp. was isolated from 12.8% of samples. Of these, 47.4% of 860 dairy samples and 52.1% of 750 beef samples produced resistant *E. coli* isolates. As for *Salmonella* spp., 14% were cultured from dairy cattle and 4.8% from beef cattle. Variation in the presence of \textit{bla}\textsubscript{CMY-2} between farms was observed. From the fresh meat samples, only 1 resistant *E. coli* and 10 Salmonella spp. isolates were recovered during the course of the study. **Summary:** The removal of ceftiofur did not impact our ability to recover enteric bacteria resistant third generation cephalosporins, and we found little evidence that resistant organisms from animals were regularly transferred into the food chain.

Keywords: antimicrobial resistance, food safety, *Salmonella, E. coli*
THE USE OF HEAVY METALS IN SWINE FEED AND ITS ASSOCIATION WITH THE PRESENCE OF COPPER AND ZINC TOLERANT SALMONELLA. M. Nicol, B. Molla, and W. Gebreyes. Dept. of Veterinary Preventive Medicine

Non-typhoidal Salmonella serovars are among the most important foodborne bacterial pathogens. In swine production systems heavy metals in feed are used to assist with normal growth of pigs and to provide cytotoxic effects on bacteria. Many strains of commonly occurring serovars have been shown to exhibit multi-drug resistance (MDR) but selective pressure for emergence and persistence of MDR is poorly understood. The presence and significance of tolerance factors to copper and zinc and its potential association with antimicrobial resistance among food borne pathogens in the United States has not been investigated. This study looks to characterize the role of chemical interventions in swine production systems on the emergence of heavy metal tolerant Salmonella and its association with antimicrobial resistance. A total of 353 Salmonella isolates with different antimicrobial resistance profiles were randomly selected from feed (n=30), fecal (n=226), and environment (n=97) samples. Minimum inhibitory concentrations (MIC) were determined on Mueller-Hinton-II agar plates containing differing dilutions of copper sulfate (1-32mM) and zinc chloride (0.25-16mM). Zinc susceptibility was recorded at 4mM (42%) and 8mM (58%) and copper susceptibility at 16mM (15%), 20mM (36%), and 24mM (49%). The most common MDR patterns among the more heavy metal tolerant isolates were AmClStSuTe (n=81) and AmStTeKm (n=58), which are common multi-drug resistance patterns found in swine production systems. There was significant association between heavy metal tolerance and distinct multi-drug resistance types: the odds of finding high Zinc MIC were 15 times higher for the AmClStSuTe R-type than AmStTeKm (Chi-square= 47.2; p<0.05). On the other hand, the odds ratio value for association between copper tolerance and MDR AmStTeKm was 4.6 (Chi-square=17.9; P<0.05). Preliminary findings in this study strongly suggest that the use of heavy metals in swine feed could contribute to the persistence of unique MDR Salmonella strains that could be of major public health significance.

Keywords: Salmonella, Heavy Metal Use, Copper Tolerance, Zinc Tolerance, Antibiotic Resistance
PREVALENCE OF MRSA IN INCOMING HORSES AT THE OSU VETERINARY MEDICAL CENTER. M. Piraino, J. Braman, J. van Balen, R. Nava-Hoet, C. Kohn, A.E. Hoet. Department of Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio State University.

Horses have been reported as the source of Methicillin-resistant Staphylococcus aureus (MRSA) in multiple zoonotic and nosocomial outbreaks primarily in veterinary hospitals. However, the real prevalence of MRSA in the horse population arriving at the OSU-VMC, as well as important risk factors associated with this pathogen, is largely unknown. Therefore, we established an active surveillance system to monitor the prevalence of MRSA positive horses coming into the equine hospital. Samples were collected by independently swabbing sterile cotton-tip culture swabs along the anterior nares, the perianal area, and the axillary area. MRSA was isolated and identified using standard culture and molecular methods. Epidemiologic surveys were collected on each horse sampled in order to identify potential risk factors associated with MRSA colonization. Phenotyping of the MRSA isolates was performed using the Kirby-Bauer Disc Diffusion Method. Genotyping results of the isolates are pending. To date, 120 horses have been sampled with seven testing positive for MRSA, giving a prevalence of 5.8 percent. Five of the seven MRSA isolates (71.4 percent) were cultured from nasal swabs while two of the seven isolates were from axillary swabs. In addition to resistance to Beta-Lactam drugs and Polymyxin B, all 7 isolates were resistant to Gentamicin, 5 to Tetracycline and Sulfamethoxazole, and 3 to Erythromycin. All isolates were susceptible to Glycopeptides (i.e. Vancomycin), Phenicols, and Quinolones. Two isolates had intermediate susceptibility and one had inducible resistance to Lincosamide. No risk factors for positive horses have been identified yet. This study clearly shows that the Veterinary Medical Center is receiving MRSA positive horses on regular bases, which could serve as potential occupational hazards and a source for nosocomial infections. Further statistical analysis of the data will be performed at the conclusion of the MRSA Surveillance Program to look for risk factors and possible trends.

Keywords: MRSA, Horses, Prevalence, Veterinary Hospital
The COWPATH team represents collaboration between the OSU College of Veterinary Medicine, the Ohio Department of Agriculture (ODA), and the US Air Force Research Laboratory (AFRL). The collaboration represents the realization that the technologies being developed at the AFRL are valuable and relevant to OSU’s and ODA’s “One Health” goals. The COWPATH team was based at The Wright Brothers Institute as a part of the Tech Edge summer program. Our diverse team included undergraduate and professional students with varying expertise. Our mission was to utilize layered sensing techniques developed at the AFRL to create a program that offers real-time situational awareness and traceability for Ohio’s livestock populations. In the midst of a serious foreign animal disease outbreak there are going to be massive amounts of data and information presented to officials such as the state veterinarian and/or representatives from the Department of Homeland Security. In order for these decision makers to act most efficiently, these data need to be accessible and organized. Our team created a program to allow many layers of data to be viewed alone or in conjunction with other relevant layers. For example, a digital map of Ohio can be created that displays all the known farm locations by species, infectivity status, soil types, watershed information, major roadways, nearest airport, nearest Ohio Veterinary Emergency Responders, and projected spread of the disease. To test our data management system, we made a simulation of a foot and mouth disease outbreak in Ohio’s livestock populations. Our proof of concept demonstration of this system and simulation to the state veterinarian showed that all the information needed for disease tracking, depopulation, and the burn/bury of euthanized animals could be displayed in our real-time viewing system. A system modeled after our simulation has potential for improving livestock traceability and real-time situational awareness of disease spread here in Ohio and elsewhere.

Keywords: Foot and Mouth Disease, livestock traceability, agriculture, geospatial engineering, Android technologies, disease outbreak control
INFLUENCE OF ABIOTIC FACTORS ON THE PREVALENCE OF AVIAN ORIGIN TYPE A INFLUENZA VIRUSES IN THE ENVIRONMENT. C. Schwarten\(^1\), R. Slemons\(^1\), R. Gates\(^2\)

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Evidence from three decades of exploratory research supports the increasingly popular belief that the environment may be an important source of avian-origin type A influenza viruses (AIVs) infecting wild birds and poultry. Early observations led to the hypothesis that virus-contaminated water could serve as an indirect source of AIVs infecting poultry. Later, experiments supported this hypothesis by demonstrating the ability of several AIV strains to persist in water for extended periods of time under different abiotic laboratory conditions. More current research has now shown that selected AIV strains can persist equally well in humic soil as in water, and that biotic factors, including microorganisms and bivalves, also affect AIV persistence. These findings led us to hypothesize that marshes serve as an important temporary source of environmentally tolerant AIVs left behind by departing birds which indirectly infect subsequently arriving avian populations. We further hypothesized that multiple abiotic factors in these marshes influence the persistence, and therefore, the subsequent probability of indirect transmission of AIVs to new hosts.

The objective of this project was to develop and implement a field sampling protocol to create abiotic profiles for multiple marshes located in two different coastal wetland complexes located along the southwestern basin of Lake Erie. Sampling sites in deep water, shallow water, and estuary marshes were established in open water and near emergent vegetation. From July – September 2010, data were periodically collected for temperature, pH, conductivity, salinity, oxidation-reduction potential (ORP), dissolved oxygen (DO), water depth, and presence of emergent vegetation. Our results showed statistically significant differences exist between the abiotic profiles as well as between the marsh complexes studied. These results will be used in the future to determine which profiles are more favorable for virus survival.

Keywords: avian influenza, wildlife diseases, environmental persistence, environmental transmission, environmental science, virology
Trypanosomiasis is a deadly parasitic disease transmitted to humans and animals by tsetse flies across most of Africa. We conducted a pilot study early in the rainy season to identify what species of tsetse flies and trypanosomes were present in the Far North region of Cameroon and where and when they have been most prevalent. Two species of tsetse flies, *Glossina tachinoides* and *Glossina morsitans submorsitans*, were caught by using bi-conical fly traps. Tsetse flies were caught in locations near water, near village wells, and in open plains, but none were caught in cattle markets. DNA samples were collected from the trapped flies as well as cattle in the region and submitted for trypanosome testing. Semi-structured interviews were used to identify where and when local herders encountered the greatest numbers of flies. Herders identified some specific locations as high risk areas for biting flies and identified the cool dry season as the season with the most biting flies in general. This is the first report of tsetse flies in the Far North Region of Cameroon, however whether they were simply not reported or whether they have migrated north from known tsetse endemic areas is unknown. This information along with the seasonal distribution of biting flies reported by the herders will be useful to plan future trapping sites. Based on pending DNA results, future studies may need to expand to look at other biting flies as the main vector for trypanosomiasis in the area.

Keywords: Trypanosomiasis, Cameroon, tsetse fly
A CASE STUDY OF TWO VECTOR-BORNE DISEASES IN HUMANS AND ANIMALS OF THE FAR NORTH REGION OF CAMEROON: IMPLICATIONS FOR PREVENTATIVE MEASURES E. Walz, R. Garabed, D. Ewing, M. Moritz, Dept. of Veterinary Preventive Medicine and Dept. of Anthropology

Vector-borne diseases, such as trypanosomiasis and malaria, are causes of significant economic losses to livestock production and human productivity in the developing world. We conducted 35 semi-structured interviews with FulBe mobile pastoralists of the Far North region of Cameroon. After comparing perceptions on disease prevention, we found that pastoralists are more likely to take an active role in preventing a vector-borne disease in cattle rather than in human populations. This suggests that when working in an international public health setting, it is important to recognize how economic and cultural significance can affect the utilization of preventative medicine resources.

Keywords: international public health, vector-borne diseases, trypanosomiasis, malaria
CEFTIOFUR USE AND THE RECOVERY OF EXTENDED-SPECTRUM CEPHALOSPORIN RESISTANT FECAL FLORA FROM DAIRY COWS
M. Weeman, D. Mollenkopf, T. Wittum. Veterinary Preventative Medicine

Extended-spectrum cephalosporin resistance in enteric bacteria is an important concern for both veterinary medicine and public health. We investigated the relationship between ceftiofur use and the dissemination of extended-spectrum cephalosporin resistance in dairy cattle. Fecal samples from twenty Ohio dairy herds were screened for the presence of the \textit{bla}_{\text{CTX-M}} and \textit{bla}_{\text{CMY}} \beta\text{-lactamase genes. Information regarding herd size, types of ceftiofur use, frequency of ceftiofur use and dry cow therapy was also collected.} For the culture of extended-spectrum cephalosporin resistant \textit{E. coli}, a 4 g aliquot of each sample was incubated overnight in nutrient broth containing cefotaxime at 2 ug/ml. The following day, each aliquot was aseptically swabbed and streaked onto MacConkey agar containing 4 ug/ml cefepime for the identification of \textit{E. coli} with \textit{bla}_{\text{CTX-M}} and MacConkey agar containing 4 ug/ml cefoxitin for the identification of \textit{E. coli} with \textit{bla}_{\text{CMY}}. \textit{Salmonella} were recovered using standard procedures. \textit{bla}_{\text{CMY}} and \textit{bla}_{\text{CTX-M}} presence in isolates were confirmed by PCR. Extended-spectrum cephalosporin resistant phenotypes are normally only detectable in the presence of selection pressure. In this study, \textit{bla}_{\text{CMY}} and \textit{Bla}_{\text{CTX-M}} resistance genes were present in 100% and 40% of herds, respectively. In total, 94% and 13% of fecal samples were positive for \textit{bla}_{\text{CMY}} and \textit{Bla}_{\text{CTX-M}} respectively. Salmonella was recovered from 80% of herds and 43% of individual samples although no Salmonella contained \textit{bla}_{\text{CMY}} or \textit{bla}_{\text{CTX-M}}.

Keywords: Dairy Cattle, Antibiotic resistance, Escherichia coli, Salmonella spp.
MICROBIOLOGICAL EVALUATION OF COW’S MILK IN RURAL SIERRA LEONE:
INVESTIGATING PRESENCE, GENOTYPE, AND ANTIBIOTIC RESISTANCE OF
SALMONELLA, STAPHYLOCOCCUS, AND MYCOPLASMA SPP. IN A
REPRESENTATIVE HERD FROM THE SENEHUN REGION  J. Zientek, T. Strickler, F.
Silveira, and W. Gebreyes, Dept. of Veterinary Preventive Medicine

Small dual-purpose cattle herds in rural Sierra Leone account for a major portion of the
local protein supply. Thus, the disease-causing potential of agents such as Salmonella,
Staphylococcus, and Mycoplasma spp., and the relative lack of food safety and animal
health infrastructure make understanding their role in this area crucially important.
Twelve milk samples were collected from the primary herd in the Senehun region of
southern Sierra Leone, representing approx. 40% of the region’s small population.
Samples were stored and later transported to the Ohio State University Dept. of
Veterinary Preventive Medicine for analysis. Protocols for isolation of Salmonella,
Staphylococcus, and Mycoplasma spp. were carried out. Further characterization of
Staphylococcus isolates including speciation, genotyping, and presence of antibiotic
resistance was performed using Kirby-Bauer Susceptibility Testing and PCR.
Salmonella spp. were not found to be present in any of the samples. Ten
Staphylococcus isolates from eight individuals were analyzed using PCR for presence
of nuc, mecA, and blaZ genes. Ten of ten were positive for nuc, all ten were negative
for mecA, and one isolate was shown to carry the blaZ gene – this isolate also showed
resistance to penicillin on Kirby-Bauer testing. Another isolate exhibited Streptomycin
resistance on Kirby-Bauer testing. No other antibiotic resistance was evident with the
Kirby-Bauer assay. No Mycoplasma isolates were identified. The lack of Salmonella
and Mycoplasma is promising, although the presence of Staphylococcus indicates the
need for education in proper milking techniques and further attention to prevention of
mastitis. Additionally, the presence of β-lactam resistance genes in an area with very
little antibiotic use necessitates further investigation into the selection environment for
maintenance of resistance genes.

Keywords:  Salmonella spp., Staphylococcus spp., Staphylococcus aureus,
Mycoplasma, Antibiotic resistance, Ndama cattle, Agriculture in the developing world,
Food safety
Sierra Leone embodies a critical need for implementation of the One Health concept. Factors such as the exceptional thinness of the human-animal interface and the integral importance of animal agriculture necessitate continued attention to the intersections of human and animal health. Livestock and other domestic animals in Sierra Leone represent incredible potential as a much-needed source of protein, and yet zoonotic diseases that may originate from livestock comprise major public health risks. In addition, animal diseases that affect productivity are major detriments to the livelihood of humans in the region.

Our research team collaborated on several projects with Njala University Animal Sciences Department, aiming to improve livestock production and reduce animal-related public health risks. Goats are very important livestock as sources of meat and milk in arid and semi-arid regions of the world. As part of the Dual-Purpose Goat Development Project, we performed a timed artificial insemination protocol on a cohort of 17 West African Dwarf goats with frozen-thawed dairy goat semen, specifically Toggenburg and Nubian, which was imported from the United States.

In collaboration with Njala University Animal Health Club, we also endeavored on an outreach campaign to provide clinical veterinary services, disease surveillance, evaluation of animal husbandry methods, and education.

Keywords: artificial insemination, West African dwarf goat, outreach in veterinary medicine, capacity building, agricultural development, One Health, zoonotic disease
IHC AND ISH IDENTIFICATION OF REPLICATION CELL TYPES AND DISPERSION OF INFECTED CELLS IN TTV, AND TTV/PRRSV CO-INFECTED GNOTOBIOTIC PIGS.  C. Cheney, Department of Veterinary Biosciences

An immunohistochemical staining method using a monoclonal antibody specific for double-stranded DNAs was recently shown to be effective in identifying cellular sites of replication for porcine circovirus 2. Both PCV2 and the TTVs are members of the Circoviridae family and are characterized by single-stranded circularized DNA genomes. Both viruses replicate their DNAs by creation of a double-stranded DNA intermediate isoforms. These can be detected in cell nuclei and cytoplasm of ethanol-fixed tissue sections from infected gnotobiotic by IHC with this monoclonal. The use of single-stranded DNA anti-body was also shown to expose the existence of static virus within cells, and ISH using TTV-specific primers should allow visualization of this static virus as further confirmation.

It was determined that TTV involved in replication were located in tissue macrophages in bone marrow, spleen, and lung in those samples infected with TTV alone. For those samples co-infected with PRRSV, it was found that in low PRRSV load concentrations, ileum and lymph nodes became sights of replication, and for samples with a high load of PRRSV, the predominant sights of replication were lung and bone marrow, with the overwhelming percentage of positive sights located in the lung, also the major sight of infection for the PPRS virus itself. Static torque teno viral particles were determined to be in all tissues excluding kidney and liver via ss-DNA IHC and ISH, however, these two assays did not correlate to each other, leaving doubt as to their efficacy. The amount of TTV in these tissues was determined by PCR and no correlation with the IHC data, suggesting this method is not viable for use in TTV. It is of note, however, that all positive cells were determined to be of the histocytic/macrophage type.

Keywords: Gnotobiotic pigs, TTV, PRRSV, Co-infection amplification
MOLECULAR EVOLUTION OF PORCINE CIRCOVIRUS TYPE 2 (PCV2): ACQUISITION OF VIRULENCE BY MUTATIONAL EVENT(S) IN THE NUCLEOCAPSID PROTEIN.  D. Corsmeier, S. Krakowka. Dept. of Veterinary Biosciences

Subclinical PCV2 infections are common in current hog populations; moreover, widespread prevalence of PCV2 is documented long before the first reported outbreaks of the porcine circovirus-associated diseases, including postweaning multisystemic wasting syndrome (PMWS). PCV2 DNAs were recovered from archived porcine tissue samples dating back to nearly 40 years ago. Upon reconstruction and sequencing of the archival PCV2 genome, a consistent nine-nucleotide difference was found in the second open reading frame (ORF2), which codes for the viral nucleocapsid protein. This change in nucleotide sequence translates to a three amino acid change at positions 133-135 of the nucleocapsid protein. In the archival PCV2 genome, the residue configuration is threonine-glycine-asparagine, a hydrophilic motif. In contemporary PCV2 genomes, these three residues are configured as alanine-threonine-alanine, a substantially more hydrophobic motif. This variation is reflected on a hydrophobicity plot as a critical change in the middle of the second immunogenic epitope of the capsid protein. Further, with our developed predictive models, we demonstrate here that the alteration in primary amino acid sequence corresponds to a considerable morphological difference in tertiary protein structure in the second immunogenic epitope of the molecule. With the models, the nucleocapsid of archival PCV2 is predicted to project outward in the second epitope region in a locally convex conformation, whereas contemporary PCV2 genomes project inward forming a hydrophobic “pocket” or concave configuration. Repeated attempts to reproduce PMWS \textit{in vivo} with archival PCV2 have shown that it is nonpathogenic. Collectively, these three-dimensional model predictions and the \textit{in vivo} data support the hypothesis that specific mutational events in the archival PCV2 conferred upon contemporary PCV2 a critical change in the three-dimensional structure and this change is directly associated with acquired pathogenicity of the new contemporary PCV2. Once widely distributed in swine populations as a subclinical infection, both infectious and noninfectious cofactors promoted contemporary PCV2 burden in these pigs resulting in PMWS.

Keywords: porcine circovirus pcv pcv2 PMWS postweaning multisystemic wasting protein structure prediction hydrophobicity hydrophobic pocket
DETECTION OF RABBIT FOXP3+CD4+CD25+ REGULATORY T CELLS IN THE GALT OF A HUMAN T-LYMPHOTROPIC VIRUS TYPE 1 (HTLV-1) RABBIT MODEL

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Human T-lymphotropic virus-1 (HTLV-1) is a retrovirus associated with development of lymphoproliferative diseases. The rabbit animal model of HTLV-1 provides invaluable insight into virus immunopathogenesis, but limited rabbit-specific diagnostic reagents pose a challenge in immunophenotyping rabbit lymphoid cells. The hypothesis is that rabbit T-regulatory cells with a CD4+CD25+Foxp3+ phenotype can be characterized using commercially available reagents. The primary focus of this project is to develop a staining protocol to identify rabbit T-regulatory cells using flow cytometry. Appendix and cecal tonsil lymphocytes from 17 SPF New Zealand White rabbits (4 uninfected, 13 HTLV-1 infected) were collected using Percoll isolation and stained with anti-rabbit antibodies against surface markers CD45, CD4, CD8, CD25, and Pan-T cells. No large differences in surface marker staining between uninfected and infected rabbits was observed, with exception of a slight increase in CD4+ cells in HTLV-1 infected rabbits. Further studies will be necessary to characterize the significance of this trend. Anti-rabbit Foxp3 antibodies are unavailable commercially, so anti-mouse/rat and anti-human Foxp3 antibodies were evaluated using flow cytometry. Flow cytometry failed to demonstrate appreciable specificity for rabbit Foxp3, but preliminary western blot results suggest anti-human Foxp3 clone 259D will cross-react with rabbit Foxp3. Staining for intranuclear proteins using a commercially available cell permeabilization kit was established for rabbit T-cells using a Ki67 antibody and flow cytometry. The ability to efficiently profile rabbit T-regulatory cells will facilitate comparison between uninfected and HTLV-1 infected rabbits. Elucidating the T-regulatory cell dynamics in the HTLV-1 rabbit animal model may help clarify fundamental viral pathogenesis and illustrate avenues for therapeutic intervention involving this important T-cell subset.

Keywords: Regulatory T Cells, Rabbit, HTLV-1
AEROSOLIZED NUCLEOTIDE SYNTHESIS INHIBITORS FOR TREATMENT OF INFLUENZA. Rivera, P, Davis, I. Department of Veterinary Biosciences

Introduction: Influenza virus causes highly-contagious acute respiratory disease in man. Most infections are self-limited, but lower respiratory tract and cardiac complications can lead to significant morbidity and mortality. Influenza pandemics elicited devastating loss of life throughout the 20th century, and have the potential to do so in the 21st. Influenza is therefore of major concern to public health.

Rationale: We have shown that influenza inhibits alveolar fluid clearance (AFC, an in vivo measure of bronchoalveolar epithelial ion transport) in BALB/c mice as a consequence of virally-induced synthesis and release of the nucleotides UTP and ATP.

Hypothesis: We hypothesize that, because of its impact on both gas exchange and mucociliary clearance, nucleotide-mediated AFC inhibition may contribute significantly to influenza morbidity and mortality. Furthermore, we propose that nucleotide-mediated AFC inhibition will be amenable to therapeutic blockade with nucleotide synthesis inhibitors, which can therefore readily be developed as novel symptomatic therapies for IV pneumonia. These agents, which are cheap, stable, and likely to have minimal side-effects when administered topically, could have a significant positive impact upon influenza-related morbidity and mortality.

Specific Aims: To test our hypothesis we will determine effects of aerosol administration of the de novo pyrimidine synthesis inhibitor A77-1726 on influenza-mediated inhibition of AFC in vivo. We will also evaluate effects of A77-1726 treatment on influenza-induced hypoxemia, weight loss, and lung injury, as well as viral replication.

Significance: Completion of our objectives may lead to development of novel nucleotide inhibitor-based therapeutic agents to abrogate hypoxemia in life-threatening influenza infection.

Keywords: influenza, pneumonia, therapy
EFFECT OF H1N1 INFLUENZA VIRUS (A/PR/8) ON LUNG FUNCTION IN BALB/C MICE. Sherman, A. Aeffner, F. Traylor, Z. Davis, IC. Department Of Veterinary Biosciences

Introduction: Patients with severe primary influenza pneumonia can exhibit many of the clinical features of acute respiratory distress syndrome (ARDS): progressive, severe hypoxemia; decreased PaO2:FiO2 (P:F) ratios when mechanically ventilated; increased airway resistance and decreased lung compliance; and bilateral thoracic infiltrates; as a result of developing noncardiogenic, high-permeability pulmonary edema.

Rationale of proposed studies: In mouse models, influenza disease severity is often characterized by histopathologic criteria and mortality only. However, our studies indicate that mice infected with a lethal dose (10,000 PFU/mouse) of the mouse-adapted H1N1 influenza strain A/WSN/33 also exhibit clinical and pathophysiologic features of ARDS, including progressive pulmonary edema and hypoxemia, altered lung mechanics, and impaired pulmonary gas exchange. We therefore propose that definition of lung function changes in response to influenza infection can provide more meaningful endpoints for pathogenesis and therapeutics studies in this animal model. To confirm this hypothesis, the proposed studies will characterize the effects of the non-neurovirulent H1N1 influenza strain A/PR/8 on murine lung function.

Specific Aims:
1. To determine effects of the pneumotropic, non-neurovirulent, mouse-adapted H1N1 influenza strain A/PR/8 on lung function and other indices of ARDS in mice.
2. To determine the effects of secondary homo- and heterologous influenza infection on lung function in influenza-immune mice.

Significance: If mice infected with influenza A meet current clinical and pathophysiologic definitions of ARDS, our findings will further validate the mouse influenza model and suggest that analysis of treatment effects on these functional readouts

Keywords - Influenza, mouse model, H1N1, Alveolar Fluid Clearance
EFFECTS OF ESTRIOL (E3) ON REGULATORY DENDRITIC CELLS: A POTENTIAL THERAPEUTIC FOR AUTOIMMUNE DISEASE  A. Bedarf, D. Muth, T. Papenfuss, A. Singh, C. Taylor, A. White, Z. VanGundy. Department of Veterinary Biosciences, The Ohio State University, Columbus, OH 43210

Estrogen has been found to affect the development and function of immune cells. Specifically, estriol (E3), a pregnancy-specific estrogen, has a protective effect in autoimmune disease and has been shown to promote the differentiation of regulatory DCs (regDCs). Such E3 regDC’s, alone, are able to prevent the establishment of the autoimmune disease experimental autoimmune encephalitis (EAE) and do so through the promotion of a protective Th2 response. The mechanisms by which E3 regDCs shift immune responses to a Th2 response are not known. We have found that E3 regDCs have an activated regulatory phenotype but have decreased IL-12 and IL-23. Surprisingly, production of the immunoregulatory cytokine, IL-10, did not differ in DCs exposed to E3, suggesting that other mechanisms may be responsible for their protective effects. We hypothesize that alterations in the activity and balance of other immunomodulatory factors (i.e. arginase, iNOS, and TGF-beta) contribute to the immunoregulatory effects of E3 regDCs. This project used real-time RT-PCR to determine whether E3 alters arginase, iNOS and TGF-beta within DC populations and progenitors. We believe that arginase and TGF-beta will be increased in those cells treated with E3 compared to our control cells while iNOS levels will be decreased. These findings will contribute to our knowledge on how regulatory DCs function to regulate immune responses which has important implications in the treatment of autoimmune and chronic inflammatory diseases.

Keywords: Estriol, autoimmune disease, regulatory dendritic cells, Th2, arginase, TGF-beta, iNOS
SUPPRESSIVE EFFECTS OF THE TUMOR MICROENVIRONMENT ON CANINE MYELOID CELLS. J Wasserman, L Diese, Z VanGundy (Department of Veterinary Biosciences), A Singh, C London (Department of Veterinary Biosciences), T Papenfuss (Department of Veterinary Biosciences)

Comparative oncology has broad application in the development and optimization of novel therapeutics for cancer in both humans and animals. Utilizing the immune system to treat cancer (cancer immunotherapy) is a targeted and potentially more effective and less toxic treatment than stand-alone chemotherapy, but impractical because the patient’s immune system often is significantly suppressed. Soluble factors secreted by the tumor microenvironment (i.e. TDSFs) play a role in this immunosuppression through effects on myeloid cells. Although well documented in other species, the effects of TDSFs on canine myeloid cells are largely unknown. In this study, we developed and applied tools to study canine myeloid cells and subsequently evaluated the effects of TDSFs from canine osteosarcoma (OSA) and melanoma (Mel) tumors on canine macrophages and dendritic cells (DCs). We utilized a canine myeloid cell line (DH82) and primary bone marrow-derived DCs and macrophages as myeloid cells and utilized canine OSA and Mel cell lines from canine oncology patients presenting to The OSU Veterinary Medical Center. Based on CD11b and CD11c expression, DH82 cells are phenotypically most similar to canine bone marrow-derived DCs with a demonstrated phagocytic ability. We found by flow cytometry that both OSA and Mel TDSFs decreased MHC class II expression of DH82 cells, which suggests that antigen presenting abilities may be diminished following exposure to TDSFs. We next evaluated the effects of TDSFs on myeloid cell function. Utilizing fluorescently-labeled beads, we determined the OSA and Mel TDSFs decreased the percentage of phagocytosing cells as well as the amount of beads able to be phagocytosed. These results show that, similar to humans and mice, the tumor microenvironment suppresses myeloid cell function in dogs. These data have important implications both in understanding canine cancer immunology and for translational and therapeutic applications utilizing the dog as a model for human cancer.

Keywords: Cancer Immunotherapy, Myeloid Cells, Canine, Phagocytosis, TDSFs
ACUTE LPS TREATMENT INDUCES CCAAT/ENHANCER BINDING PROTEINδ (C/EBPδ) EXPRESSION AND NUCLEAR FACTOR-KAPPAβ (NF-κB) DNA BINDING ACTIVITY IN THE RAW 264.7 MONOCYTE/MACROPHAGE CELL LINE.  K. Couto, J. Dewille, and X. Yu.  Department of Veterinary Biosciences

The long-term goal of this project is to investigate the potential roles of CCAAT/Enhancer Binding Proteinδ (C/EBPδ) and Nuclear factor-kappaB (NF-κB) in chronic inflammation and cancer promotion. Epidemiological and experimental studies link chronic inflammation and local macrophage infiltration with epithelial cell transformation and cancer progression, but the mechanisms underlying this link are poorly understood. This project is testing the hypothesis that C/EBPδ and NF-κB are coordinately induced in acutely activated macrophages, but chronic activation (inflammation) results in “loss of function” alterations in C/EBPδ, uncontrolled NF-κB activation and the production of tumor promoting NF-κB gene products, such as IL-6. This hypothesis is supported by preliminary studies in which chronic inflammatory treatments induced “loss of function” alterations in C/EBPδ and “loss of function” alterations in C/EBPδ enhanced NF-κB transcriptional activation. This study investigated the influence of acute LPS (0-200 ng/mL) treatments on the C/EBPδ expression and NF-κB activation in the mouse RAW 264.7 monocyte/macrophage cell line. The results indicate that acute (2-6 hours) LPS treatment increases C/EBPδ expression and also increases the nuclear accumulation of NF-κB family members RelA and RelB. In addition, electromobility shift assays (EMSAs) demonstrate that LPS treatment increases NF-κB DNA binding activity. RAW 264.7 cells treated with LPS for 24 hours exhibited a decline in C/EBPδ protein levels, consistent with the induction of a state of “endotoxin tolerance”. In addition to changes in gene expression, RAW 264.7 cells treated with LPS exhibit a dendritic-like morphology. These findings demonstrate that acute LPS treatment increases C/EBPδ expression and NF-κB DNA binding activity. In contrast, chronic LPS treatment results in reduced C/EBPδ protein levels. Future experiments will extend these results and determine the influence of chronic LPS treatment on C/EBPδ target genes, NF-κB activation, and the expression and release of tumor promoting NF-κB target gene products into the microenvironment.

Keywords: C/EBPδ, LPS, macrophage, inflammation

Historically, oncology research has been directed towards identifying genes expressing messenger RNAs and proteins expressed in cancer cells, with the aim of understanding and predicting tumor behavior, improving cancer diagnosis, and identifying new targets for therapy. Recent studies, however, have begun to focus on non-protein coding genes that express short sequences of RNA, known as microRNAs. The purpose of the present study is to examine the expression of a specific microRNA, microRNA-9 (miR-9), in canine mast cell tumors (MCTs). We hypothesize that miR-9 will be over-expressed in biologically high-grade mast cell tumors as compared to their low-grade counterparts.

Previous microRNA expression profiling of a relatively small number of primary canine MCTs identified miR-9 as being differentially expressed in biologically low-grade and high-grade MCTs. To independently confirm these results, we used quantitative RT-PCR to determine miR-9 expression in a different, larger, cohort of primary canine MCTs for which clinical outcomes were available. Canine mast cell tumors for which formalin fixed paraffin embedded (FFPE) tissue blocks were available were retrieved from the Ohio State University veterinary pathology archive. Total RNA was isolated from FFPE tissue cores, cDNA was synthesized, and quantitative real-time PCR was performed using TaqMan probes for the mature miR-9 sequence. Data were analyzed using the comparative Ct method with snU6 as the endogenous control. Although the difference was not statistically significant, the mean expression (0.00183) of miR-9 in the high-grade group was nearly 6 times greater than the mean expression (0.000325) in the low-grade group (p=0.114). These data suggest that miR-9 may play a role in metastasis and invasion that is characteristic of malignant MCTs. However, additional research is required to confirm that miR-9 is over-expressed in biologically high-grade canine MCTs.

Keywords: MicroRNA, miR-9, Canine Mast Cell Tumors
EVALUATION OF A PROTOTYPE NEEDLE TO CONCENTRATE AND ISOLATE STEM CELLS FROM BONE MARROW ASPIRATES. H. Helbig, B.S., A. Ishishara, DVM, PhD, R. Sanchez- Hodge, M. Wellman DVM, MS, PhD, DACVP, and A. L. Bertone, DVM, PhD, DACVS. Depts. of Veterinary Clinical Sciences and Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University.

Bone marrow has been shown to contain progenitor cells (stem cells) that may provide powerful therapies to a wide variety of disorders. Autologous stem cell therapies utilize concentrated bone marrow aspirate (cBMA) to deliver adult mononuclear stem cells to the patient. Concentration of the mononuclear cell-rich fraction (CRF) is limited by the available number of recovered mononuclear cells in the aspirate. We evaluated two bone marrow aspiration needle designs, the traditional Jamshidi needle and a prototype test needle, for the ability to increase mononuclear CRF and isolate pluripotent stem cells.

Sixty-four, 60 ml, bone marrow aspirates [BMA] were collected into 6mls of heparin from each of four sternebra from each of eight 5 yr old mares in two replicates four weeks apart. At each collection time point, two sternebrae were used for the control needle and the other two sternebrae for the test needle. Bone marrow was collected, rocked to prevent clotting, and then concentrated using manufacturer's recommended centrifugation protocol and the MarrowStim Concentration Kit [BioMet, Inc]. Baseline peripheral venous blood, bone marrow, and cBMAs were evaluated for the number of platelets, mononuclear cells, granulocytes, and total nucleated cells with the CellDyn blood analyzer and hemacytometer microscopy. Cell viability was evaluated with flow cytometry [7-AAD] and trypan blue exclusion stain microscopy. The cells were cultured for two weeks in media and conditions established to drive and contain progenitor cells into Colony Forming Units [CFU] of mature fibroblasts, (CFU)-Fibroblast-, and mature endothelial cells, (CFU)-Endothelial Progenitor Cells. Sample aspiration time was recorded for each needle.

Our results confirmed that both test and control needles aspirated raw bone marrow that contained 4.2 +/- 1.1 CFU (per 1-million WBC) of precursor cells not found in the corresponding autologous peripheral venous blood samples. Use of the MarrowStim Concentration Kit significantly increased the number of platelets and white blood cells in the equine bone marrow aspirates (p<0.05) from a mean +/- SEM of 62.000 to 294.000 for platelets and 15.000 to 101.000 for white blood cells. The volume of aspirate was reduced from a mean of 59 +/- 3ml to 6.8 +/- 1.5ml. The test needle and the Jamshidi control needle demonstrated comparable number of platelets, white blood cells, and red blood cells. In conclusion, the prototype bone marrow needle was equivalent to an established bone marrow needle in the isolation and concentration of platelets, white blood cells and pluripotent stem cells. The MarrowStim Concentration Kit effectively concentrated key biologic blood elements that would subsequently have medical use at a platelet-rich concentrate or stem cell-rich concentrate.

Keywords: horse, bone marrow, stem cells, cytology, flow cytometry
Potomac horse fever (PHF) is an acute equine disease caused by a gram-negative intracellular bacterial pathogen, *Neorickettsia risticii*. Clinical signs range from fever, diarrhea, anorexia and colic to severe laminitis, abortion and death. The aim of this study is to determine levels of reactivity of five major Neorickettsial surface proteins by sera from horses having clinical signs of PHF. Native proteins were separated from whole bacteria isolated from *N. risticii*-infected P388D1 host cells, and recombinant proteins: P51, Nsp2, Nsp3, and GroEL were produced in *E. coli* and isolated. All proteins were separated by gel electrophoresis and transferred to membranes. These membranes were cut into strips and western immunoblot analysis was performed using equine clinical blood samples confirmed positive by PHF immunofluorescence assay. Reacting protein bands were compared to quantify the intensity of antisera recognition. Our data indicate that *N. risticii* native proteins, rP51, rNsp2, rNSP3 and rGroEL were significantly recognized by sera from PHF immunofluorescence assay-confirmed horses. Clinical presentations were compared to western blot results, and it was found that an antiserum reaction to rGroEL is inversely related to diarrhea development in PHF patients and correlated to the onset of colic. Strong recognition of *N. risticii* native proteins by antisera is also correlated to the presence of fever. These results for the first time demonstrated reactivity of sera from naturally-infected horses to defined major surface proteins of *Neorickettsia* species and the relationship of reactivity with some PHF clinical signs. The study could help in developing new and more effective diagnostics and vaccines for PHF.

Keywords: Potomac Horse Fever, *Neorickettsia risticii*, gram-negative bacteria, antigenicity
ENHANCING ANTIPROLIFERATIVE EFFECTS OF CALCITRIOL ON CANINE TRANSITIONAL CELL CARCINOMA OF THE URINARY BLADDER BY CURCUMIN IN VITRO E. Shull, W. Smith, N. Inpanbutr, Department of Veterinary Bioscience

Transitional cell carcinoma is the most common canine bladder cancer and treatment is difficult. Studies indicate that calcitriol, the biologically active form of vitamin D, inhibits growth of canine transitional cell carcinoma of the urinary bladder (cbTCC) in a dose dependent manner. High doses of calcitriol ($10^{-6}$ M) are required to significantly reduce cbTCC cell growth, however these doses risk a hypercalcemic effect. Identifying treatments that enhance the antiproliferative effects of calcitriol is integral in treating cbTCC. In this study, we hypothesize that curcumin potentiates the antiproliferative effects of calcitriol on cbTCC. CbTCC cells are treated with calcitriol, curcumin, and the combination of both, and are analyzed for growth by MTT assay, apoptosis by flow cytometry, and the proteins involved in apoptosis, including p73, BAX, Bcl-2, and Survivin, by western blot analysis. We expect these proteins will be altered in treated cells in a way that supports the method of apoptosis induction determined by flow cytometry. CbTCC cells were grown on 96-well plates, allowed to adhere for 24 hours, then treated with 10, 20, 30, 40, and 50 µM of curcumin and incubated for 24, 48, or 72 hours. The cells were then analyzed for growth inhibition compared to control using MTT assay. At 48 and 72, but not 24 hours post-treatment, curcumin significantly inhibited cbTCC growth in a dose dependent manner. Cells treated for 48 hours with 30 µM, 40 µM, and 50 µM of curcumin showed growth averaging 81%, 69%, and 43% of control, respectively. Cells treated for 72 hours with 30 µM, 40 µM, and 50 µM of curcumin showed growth averaging 82%, 52%, and 28% of control, respectively. Ongoing studies are expected to show that curcumin and calcitriol work synergistically to inhibit cell growth and promote the induction of apoptosis.

Keywords: Vitamin D, calcitriol, curcumin, canine transitional cell carcinoma, bladder cancer, p73, Bcl-2, BAX, surviving
ENHANCING RADIOSENSITIVITY OF CANINE TRANSITIONAL CELL CARCINOMA OF URINARY BLADDER BY CURCUMIN IN VITRO
Smith, W., Shull, B., Green, E., Inpanbutr, N. The Ohio State University College of Veterinary Medicine, Department of Veterinary Biosciences

Canine Transitional Cell Carcinoma of the urinary bladder (cbTCC) is a disease commonly affecting dogs, and has shown much resistance to treatment when compared with human TCC. Previous research in our lab has shown calcitriol, the hormone form of vitamin D, and curcumin to inhibit cell growth and differentiation of canine osteosarcoma and cbTCC. The objective of our research was to enhance the effects of irradiation (IR) by pretreating cbTCC cells with curcumin. We hypothesized that curcumin would enhance the sensitivity of IR on cell growth. CbTCC cells were grown for 24 hours, treated with curcumin, grown for an additional 24 hours, then irradiated with 6mV photons ranging from 0–6 GY. The cells were then allowed to grow for 24, 48, and 72 hours before being tested. Cells were analyzed for growth using MTT assay. Preliminary results showed 24 hours post treatment to be inconsistent compared to 48 and 72 hrs. Analysis showed an IC50 of curcumin on cbTCC cell growth at concentrations of 62.1µM and 47.8uM at 48 and 72 hours respectively. Student’s t-Test showed treatment at 30uM to be well within 95% confidence. MTT assay results of cbTCC cells pretreated with 30uM curcumin showed that curcumin potentiated the efficiency of IR at the range of 0-6 GY in a dose-dependent manner. Continuing research will examine cbTCC cells treated with both calcitriol and curcumin for cell cycle arrest and apoptosis using flow cytometry and for protein expression (p73, BAX, Bcl-2, Survivin) using Western Blot.

Keywords: Canine transitional cell carcinoma (cbTCC), curcumin, radiosensitivity
GENETIC CHARACTERIZATION AND CHEMOTHERAPEUTIC STUDIES ON FELINE ORAL SQUAMOUS CELL CARCINOMA. D. Yanik; S. P. S. Pillai; C. K. Martin; T. J. Rosol. Dept of Veterinary Biosciences.

We determined the levels of expression of 3 genes, EGFR1 (ErbB1/HER1), EGFR2 (ErbB2/HER2), and p16 (CDKN2a/INK4a) in three feline oral squamous carcinoma (FOSCC) cell lines (SCCF1, SCCF2, SCCF3) using real time RT-PCR. The family of ErbB (EGFR) proteins is an important regulator of cell proliferation and migration with a list of other functions including regulation of apoptosis. p16 gene regulates cell cycle progression. Real time RT-PCR showed that the 3 FOSCC cell lines expressed EGFR1 and EGFR2, the expression of p16 was limited to SCCF2 and SCCF3. Our further interests are to investigate if the levels of expression of these 3 genes in the 3 FOSCC cell lines correlate with their bone invasiveness and metastatic potential. We also evaluated the therapeutic efficacy of arsenic trioxide (ATO) on the 3 cell lines in vitro by determining viable cell counts using trypan blue dye exclusion. Arsenic trioxide, a chemotherapeutic agent being developed for several solid tumors in man, is a potential therapeutic target for FOSCC. Arsenic trioxide induces oxidative damage and apoptosis in cells by reducing intracellular glutathione peroxidase levels. We also determined ascorbic acid, a pro-oxidant in presence of arsenic, potentiated the effects of ATO on FOSCC. Arsenic trioxide and ascorbic acid were found to be effective pro-apoptotic agents on FOSCC cells even at a low concentration of 0.6 uM ATO and 100uM ascorbate. We further plan to evaluate the therapeutic efficacy of ATO and ascorbate on a nude mouse model of FOSCC. Our long term goal is to find an effective therapy for this debilitating feline cancer.

Keywords: squamous cell carcinoma, arsenic trioxide, ascorbic acid, EGFR, EGFR2, p16
IN-VITRO EFFECTS OF BILIRUBIN ON PANCREATIC ISLET CELL VIABILITY
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Diabetes is a chronic debilitating disease, affecting 24 million Americans and resulting in 116 billion dollars in medical associated costs each year. Pancreatic islet cell transplantation is currently the only non-invasive, curative treatment for diabetes. However, success of islet transplantation has been limited by loss of up to 60% of islet mass secondary to isolation and hypoxic stresses experienced during cell transplantation. Our hypothesis was that bilirubin, a compound with strong antioxidant and anti-apoptotic effects, would decrease cell death during islet transplantation. AJ mouse donors were assigned to two treatment groups and administered either 20μmol/kg bilirubin IP (Bili IP), or vehicle control, 1 hr before harvest. Islets were harvested via digestion of the pancreas using collagenase solution and isolated using a gradient separation technique. Cell viability was compared between groups using propidium iodide staining to quantify percent cell death after isolation and hypoxic stresses. Bili IP caused significant decreases in cell death at 0, 24 and 48 hours after isolation (P <0.0001, 0.002, and <0.0001) when compared to control. Donor pre-treatment with Bili IP also conveyed cytoprotective effects in a model of hypoxic injury when compared to control (P= 0.03); however, addition of 20μmol/L to the media provided the most effective protection from hypoxic injury (P <0.0001). Results support the beneficial effects of bilirubin on pancreatic islet cell viability. Further research will explore the use of bilirubin to improve islet harvest protocols in clinical islet transplantation.

Keywords: Pancreas, Islets, Transplantation, Bilirubin, Hypoxia
LAMINAR INFLAMMATORY EVENTS AND EPITHELIAL STRESS AT OBEL GRADE 3 LAMENESS IN THE CARBOHYDRATE OVERLOAD MODEL OF EQUINE LAMINITIS. M. Hensel¹, L.A. Fugler¹, B. Leise¹, M. Watts¹, S. Eades², and J. Belknap¹.

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Pathologic events reported in the laminae in sepsis-related equine laminitis include leukocyte extravasation into the laminar interstitium, pro-inflammatory cytokine expression, and epithelial stress. While these events are documented early in the disease process (Obel Grade 1 [OG1] laminitis) in the carbohydrate overload (CHO) model of laminitis, the later events occurring at onset of structural failure of the laminae (OG3) have not been determined. We hypothesized that the inflammatory events described above are sustained through OG3 laminitis, culminating in laminar failure. Archived laminar tissue samples were used from a previous CHO study (Control group [n=6, water], CHO group [n=7, corn starch]). Calprotectin (CP) immunohistochemistry (IHC) was used to assess both laminar myeloid leukocyte numbers and epithelial stress; RT-qPCR was used to assess inflammatory gene expression. Minimal inflammatory changes were present at OG3 compared to published values at OG1 stage including decreased mRNA concentrations of cytokines (i.e. 20-fold increase in IL-6 at OG3 vs. >2000-fold increase at OG1, no increase in IL-1 at OG3 vs. 11-fold increase at OG1), chemokines (no change in MCP-1 at OG3 vs. >30-fold increase at OG1, 8-fold increase in IL-8 at OG3 vs. 95-fold increase at OG1) and adhesion molecules (no change in E-selectin at OG3 vs. 10-fold increase at OG1). Laminar leukocyte concentrations at OG3 were increased compared to control tissue; the concentrations were less than reported at OG1. Interestingly COX-2, underwent a greater increase at OG3 (approx. 50-fold) compared to that reported at OG1 lameness (35-fold). Finally, epithelial stress at OG3 evidenced by CP IHC was present in focal areas in which secondary epidermal laminae on either side of a common primary dermal vascular supply demonstrated increased CP signal. The events at OG3 appear more focal and possibly due to vascular dysregulation rather than diffuse inflammatory events observed at OG1 lameness.

Keywords: laminitis, inflammation, epithelial stress, pro-inflammatory cytokine, leukocyte, calprotectin
Digital hypothermia is a therapy to decrease the incidence of sepsis-related equine laminitis, a disease causing structural failure of digital laminae resulting in crippling lameness. Due to the fact that hypothermia was recently reported to decrease laminar expression of inflammatory molecules including pro-inflammatory cytokines, chemokines and COX-2 in equine laminitis, our laboratory is investigating the effect of hypothermia on upstream signaling cascades which may induce expression of these diverse inflammatory molecules. The p38 MAPK pathway has recently been reported to be a central component of inflammatory signaling in multiple diseases including human sepsis, and is currently being assessed as a therapeutic target. We thus hypothesized that 1) p38 MAPK is upregulated in affected laminae in equine laminitis and 2) digital hypothermia blocks p38 MAPK phosphorylation (indicator of p38-MAPK activation). Western hybridization using a phospho-p38 MAPK antibody was performed on archived pooled laminar samples from black walnut extract (BWE) model (3H CON, 12H CON, 1.5H, 3H, 12H [n=5 each]) and carbohydrate overload (CHO) models (CON [n=8], DEV [n=6], OG1[n=6]) of laminitis, and individual laminar samples from two groups of horses from a digital hypothermia (DH) study. In the DH study, one forelimb of each horse was kept at approximately 4°C in ice water and the other at ambient temperature following administration of 10g/kg oligofructose (OF). Dorsal laminae were harvested for snap freezing at either 24 hours after OF administration (DEV, n=7) or at the onset of lameness (OG1, n=6) using protein extracted from treated and untreated digital laminae of each horse. Increased laminar concentrations of phospho-p38 MAPK were present in both the BWE and CHO laminitis models. However, digital hypothermia had no effect on laminar phospho-p38 MAPK concentrations. Thus, although digital hypothermia decreases laminar expression of pro-inflammatory cytokines, it does not appear to work through the p38 MAPK signaling cascade.

Keywords: equine, laminitis, p38 MAPK, digital hypothermia