Comparative & Experimental Medicine and Public Health Research Symposium

May 20 & 21, 2013

PROGRAM & SCHEDULE

Sponsored by the College of Veterinary Medicine, Center for Health Policy and Services Research, Tennessee AgResearch, UTK Office of Research, and the UT Graduate School

WELCOME

For the seventh consecutive year, the University of Tennessee (UT) Agricultural Campus is hosting a symposium for UT investigators with animal and human health interests. This symposium has grown explosively and has become a calendar event for the Knoxville campuses of UT. Comparative and Experimental Medicine (CEM), an intercollegiate graduate program with shared governance by the College of Veterinary Medicine and the Graduate School of Medicine, initiated this symposium in 2007 as an event to showcase the research of CEM student investigators. In 2008, the symposium was opened to participants throughout the Knoxville campuses, and there was a four-fold increase in presentations with representation from 19 different UT departments and programs. For the fifth year, the Center for Health Policy and Services Research has teamed with CEM to produce a joint *Comparative & Experimental Medicine and Public Health Research Symposium* hosting a large group of scientists including 72 presenters representing 18 different UT departments and programs.

The *Comparative & Experimental Medicine and Public Health Research Symposium* has gained both a reputation and recognition for providing an excellent venue for students and new investigators to gain experience showcasing their work as oral presentations. In addition, the gathering of UT investigators with related and varying interests provides opportunities for the creation of new ideas, collaborations, and networking that will enhance health-related research at the UT Knoxville campuses. The joint sponsorship of the symposium by the College of Veterinary Medicine, the UT Center for Health Policy and Services Research, Tennessee AgResearch, the UT Graduate School, and the UT Knoxville Office of Research signifies both a shared recognition of the need for such a symposium and a cooperative spirit in bringing this exciting event to reality.

We are happy to welcome all participants and attendees and hope the experience will be as positive as it is promising.

Larry Arrington, Chancellor
University of Tennessee
Institute of Agriculture





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We wish to acknowledge the following university programs and individuals, without whom this event would not be possible:

College of Veterinary Medicine

Center for Health Policy and Services Research

Tennessee AgResearch

UTK Office of Research

UT Graduate School

Misty Bailey
Debra L Butenko
Michael Cunningham
Paul Campbell Erwin
Stephen Kania
Lisa Lindley
Michael McEntee
Carole Myers
Kim Rutherford
Anik Vasington

We appreciate the contributions of session moderators and judges.

Thanks also to the UTCVM chapter of Phi Zeta, the UTIA chapter of Gamma Sigma Delta, the UT chapter of Sigma Theta Tau International, 2013 Center of Excellence Summer Student Research Program participants, and our sponsors and exhibitors.

Jimmy Cheek, *Chancellor*
UT Knoxville

Larry Arrington, *Chancellor*
UTIA

James Thompson, *Dean*
College of Veterinary Medicine
Taylor Eighmy, *Vice Chancellor for Research*
UT Knoxville Office of Research

William F. Brown, *Dean*
Tennessee AgResearch

Robert Rider, *Dean*
College of Education, Health & Human Sciences

Carolyn Hodges, *Dean*
UT Graduate School

SCHEDULE AT A GLANCE

Monday, May 20

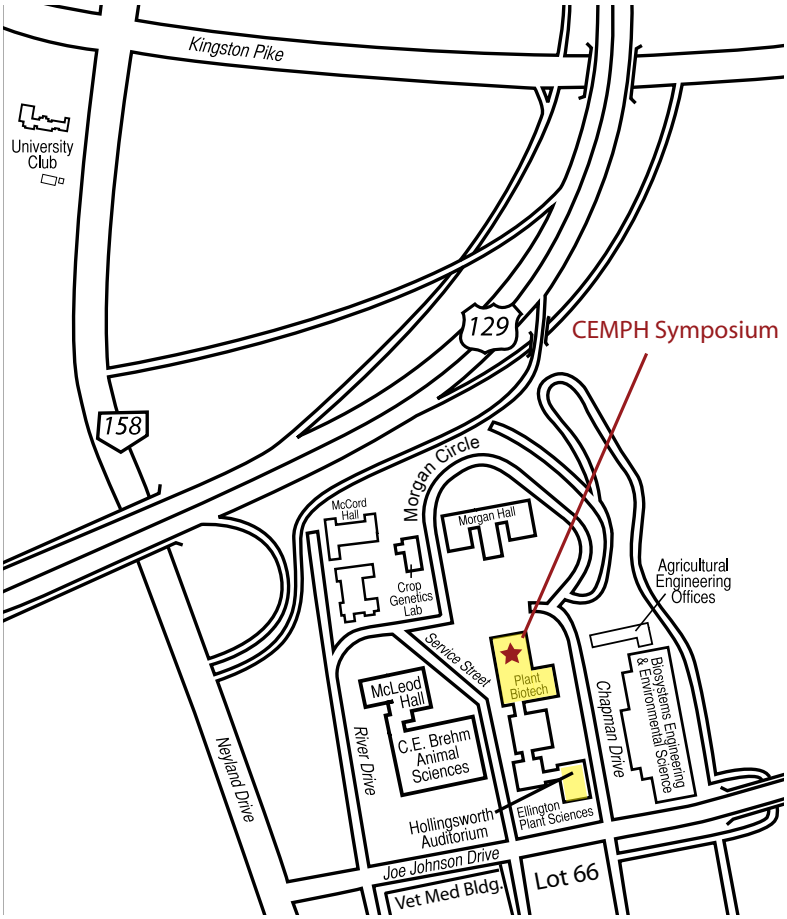
	Room	Event
8:30-9:00	PBB	Morning refreshments
9:00-10:00	156/157 PBB	Keynote address: Marion Kainer, MD, MPH, FRACP, "A Fungus Among Us: Anatomy of an Outbreak Investigation"
10:30-12:00	See session matrix (p. 6)	New investigator presentations
12:30-1:30	Hollingsworth Auditorium	Featured speaker: David Bemis, PhD, "Laboratory Diagnosis of Fungal Infections: Mycology in the Trenches"
2:30-4:45	See session matrix (p. 7)	New investigator presentations

Tuesday, May 21

	Room	Event
8:30-9:00	PBB	Morning refreshments
9:00-10:00	156/157 PBB	Plenary address: Ralph Tripp, PhD, "Translational Disease Intervention Strategies Based on Studies of Virus-Host Interactions"
10:30-12:00	See session matrix (p. 8)	New investigator presentations
12:30-1:30	Hollingsworth Auditorium	Featured speaker: Channa Palmer, "Postdoctoral Opportunities"
2:00-4:15	See session matrix (p. 9)	New investigator presentations
6:00	Calhoun's on the River	Awards banquet & after-dinner address: Marcy Souza, DVM, MPH, "One Health – What the HELLbenders Have to Do with It"

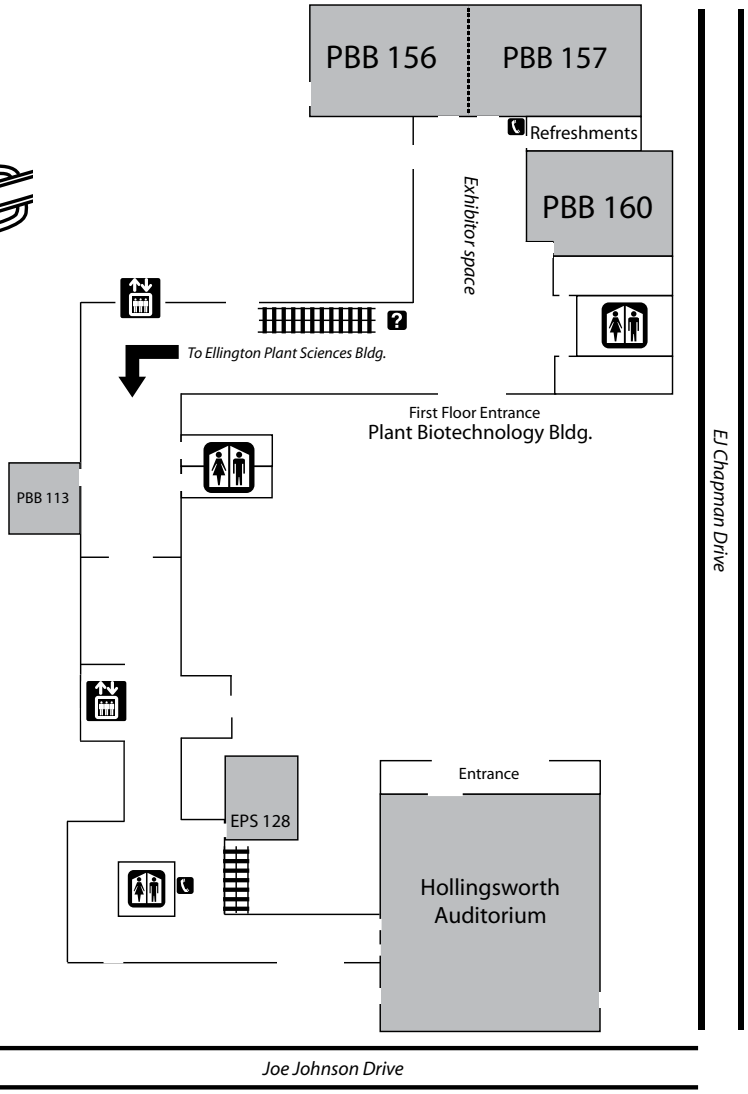
PBB, Plant Biotechnology Building (*see map on p. 5*)

LOCATION INFORMATION



University of Tennessee Agricultural Campus

Plant Biotechnology Building



Parking

A valid student, faculty, or staff parking permit is required to park in university lots. Faculty, staff, and students with disabilities may ride a paratransit service offered by "The T" (free for all UT faculty, staff, and students) by using the Blue Phone system and requesting the Access Service. All other bus service on campus is suspended during the symposium due to no classes being in session.

__Notice__: Lot 66 may no longer be used by those without permits for that specific lot (violators may be ticketed or towed). All visitors will need a temporary parking permit.

SESSION MATRIX

Monday, May 20

(Abstracts on pp. 14-44)

9:00 **Keynote address: Marion Kainer, MD, MPH, FRACP, "A Fungus Among Us: Anatomy of an Outbreak Investigation" (PBB 156/157)**

	Molecular Cell Biology	Microbial Virulence & Transmission	Public Health, Fitness, & Nutrition
	Rm. PBB* 113	**Rm. PBB* 156/157**	**Rm. PBB* 160**
10:30	1. Death Receptor 6 Induces Apoptosis Not through Type I or Type II Pathways, but via a Unique Mitochondria-Dependent Pathway by Interacting with Bax Protein **(Zeng)**	15. Role of SUAM in the Pathogenesis of *Streptococcus uberis* Mastitis (**Kerro Dego**)	28. Comparison of Breastfeeding Knowledge, Attitudes, and Intention between Chinese and U.S. Undergraduates (**Lou**)
10:45	2. Pen-2 is Dispensable for Endoproteolysis of PS1 but is Required for Gamma Secretase Activity (**Hu**)	16. Use of Microfluidics to Study *Streptococcus uberis* Adhesion to Bovine Mammary Epithelial Cells Under Conditions of Shear Flow (**Prado**)	29. UT Moves: Use of Blackboard Internet Technology to Promote Walking among University Faculty and Staff (**Monroe**)
11:00	3. PSAP Induces a Unique Apaf-1 and Smac-dependent Mitochondrial Apoptotic Pathway Independent of Bcl-2 Family Proteins (**Li**)	17. Protective Effect of Anti-SUAM Antibodies on *Streptococcus uberis* Mastitis (**Kerro Dego**)	30. A Pilot Study of the Efficacy and Program Cost-Effectiveness of Prevention Plus for Childhood Obesity (**Looney**)
11:15	4. Matricellular Protein Cyr61 Bridges the LPA/GPCR Pathway with the Integrin Pathway Leading to LPA-Induced Pathological Response (**Zhang**)	19. Fc-Mediated Binding of Canine Immunoglobulin G to Protein A on the Surface of *Staphylococcus pseudintermedius* (**Balachandran**)	31. Influence of a Nutraceutical on Treatment of Obesity in Research Beagles (**Murphy**)
11:30	5. Mechanism study of LPA-Induced Lipid Uptake Effect in Macrophages (**Xu**)	20. Cysteine Proteases of Feline *Tritrichomonas foetus* Mediate Adhesion-dependent Cytotoxicity to Intestinal Epithelial Cells (**Tolbert**)	32. Determining the Composition of Lipid Molecules in Lipid Droplets Associated with Obesity (**Shumaker**)
11:45	6. Protein Kinase D2 Mediates LPA-Induced Tissue Factor Expression and Activity through the MAPK Pathway in Mouse Aortic Smooth Muscle Cells (**Hao**)		33. Body Composition of Outdoor-Intact Cats Compared to Indoor-Neutered Cats using Dual Energy X-Ray Absorptiometry (**Cline**)

12:00 **BREAK for Lunch – Featured speaker: David Bemis, PhD, "Laboratory Diagnosis of Fungal Infections: Mycology in the Trenches" (Hollingsworth Auditorium)**

*PBB, Plant Biotechnology Building

Monday, May 20

	Innovative Technologies	Microbial Virulence & Transmission	Public Health, Fitness, & Nutrition
	Rm. PBB 113	**Rm. PBB 156/157**	**Rm. PBB 160**
2:00	9. Engineering Biodegradable Microporous Bacterial Cellulose Scaffolds and Biomimetic Composites for Bone and Cartilage Tissue Regeneration (**Favi**)	21. Use of Probiotics to Reduce *Salmonella* Infection in Mice (**Andino**)	34. Mechanisms of Population-Level Variation in Fatness and Leanness (**Wells**)
2:15	10. Developing a Non-Surgical Contraceptive Method for Female Dogs (**Papu John**)	22. *Yerba mate* Enhances Probiotic Bacteria Growth In Vitro but as a Prebiotic Does Not Reduce *Salmonella enteritidis* Colonization In Vivo (**Gonzalez-Gil**)	35. Challenges to Refugee and Immigrant Well-Being (**Allen**)
2:30	11. Diagnostic Accuracy of a Novel Point-of-Care Urinary Culture Test in Dogs (**Olin**)	23. Identification of Chemical Compounds to Inhibit Phosphatidylserine Synthesis in *Candida albicans* (**Cassilly**)	36. Survey of Pharmaceutical and Personal Care Products in Effluent Wastewater Treatment Plants in Tennessee (**Johnson**)
2:45	12. Transmission and Expansion of Major Clonal Lineages of *Toxoplasma gondii* using Multilocus Microsatellite Markers (**Saraf Dogra)**	24. Inhibition of Foodborne Microorganisms by White Mustard Extract, Nisin, and Laurice Arginate Applied Individually and in Combination (**Techathuvanan**)	8. Identification and Distribution of *Ehrlichia* and *Rickettsia* species within *Amblyomma americanum* at Ames Plantation in Western Tennessee (**Hendricks**)
3:00		25. The Role of Phosphatidylserine in *Candida albicans* Virulence (**Davis**)	18. *Ixodes scapularis* as a Vector of Concern in Human Tick-borne Diseases in Western Tennessee (**Mays**)
BREAK	**Innovative Technologies**	**Microbial Virulence & Transmission**	**Clinical Sciences**
3:30	13. Neutron Radiography and Neutron Computed Tomography: Novel Imaging Technologies for Detection of Cancers (**Cekanova**)	26. Effect of COMT-Knockdown Switchgrass of Rhizosphere Microbiome (**Chauhan**)	37. Differentiation of Equine Mesenchymal Stromal Cells into Neurons and Transdifferentiation into Schwann Cells: Potential for Clinical Applications (**Cruz Villagran**)
3:45	14. Early Exposure to Triclocarban During Lactation Alters Survival Rate in the Female Rat Neonate (**Kennedy**)	27. Use of Whole Genome Sequencing in the Discovery, Identification, and Functional Characterization of a Novel Linear Plasmid in a Clinical Isolate of Methicillin-Resistant *Staphylococcus pseudintermedius* (**Riley**)	38. Platelet-Rich Plasma Enhances In Vitro Proliferation and Osteogenesis of Equine, Bone Marrow-Derived Mesenchymal Stem Cells within Age- and Gender-Matched Horses (**Carter-Arnold**)
4:00			39. Effects of Environmental Carcinogens on Canine Adipose Tissue-Derived Mesenchymal Stem Cells (**Rathore**)
4:15			40. The Effects of Stem Cells and Platelet-Rich Plasma on Healing of Full-Thickness Cutaneous Wounds on the Distal Limb of Horses (**Caruso III**)
4:30			41. Evaluation of the Ability of a DACC-Impregnated Antimicrobial Wound Dressing to Reduce Bacterial Concentration and Enhance Healing of Equine Wounds (**Boswell**)

9:00 **Plenary address: Ralph Tripp, PhD, "Translational Disease Intervention Strategies Based on Studies of Virus-Host Interactions" (PBB 156/157)**

	Immunology & Viral Pathology	Oncology & Cancer Cell Biology	Clinical Sciences
	Rm. PBB 113	**Rm. PBB 156/157**	**Rm. PBB 160**
10:30	42. Targeting Robo4/UNC5b Pathway Reduces Corneal Angiogenesis Induced by Herpes Simplex Virus (**Gimenez**)	55. GABA but not Baclofen Prevents Gemcitabine Resistance Induced by Low-Dose Nicotine in Pancreatic Cancer Xenografts (**Banerjee**)	66. Kinetic and Kinematic Analysis of Hind Limb Joints Following Immobilization of the Tarsus (**Tobias**)
10:45	43. Gut Bacteria Modulates Angiogenesis and Corneal Immunopathology after Herpes Simplex Virus Infection (**Richardson**)	56. Short-Term Direct Electric Current Exposure Increases Caspase-3/7 Activity in Colon Cancer Cells (**Fleming**)	67. Sensitivity and Specificity of Tissue Impedance Determination for Correct Needle Placement in the Coxofemoral Joints of Cadaveric Dogs (**Applegate**)
11:00	44. Neuroprotectin D1 Reduces the Severity of Herpes Simplex Virus-Induced Corneal Immunopathology (**Rajasagi**)	57. Aberrant Alternative Splicing in Colorectal Cancer is Attenuated by a Conventional Non-Steroidal Anti-Inflammatory Drug, Sulindac Sulfide (**Smolensky**)	68. Biometry, Keratometry, and Calculation of Intraocular Lens Power for the Bald Eagle (*Haliaeetus leucocephalus*) (**Kuhn**)
11:15	45. siRNA Combinations Inhibit Feline Coronavirus Replication and Expression in Cell Culture (**Anis**)	58. A Secreted Protein NAG-1 Plays a Role in the Nucleus (**Min**)	69. Pharmacokinetics of Cefovecin (Convenia) in White Bamboo Sharks (*Chiloscyllium plagiosum*) and Atlantic Horseshoe Crabs (*Limulus polyphemus*) (**Steeil**)
11:30	46. Temperature Affects Anuran Suceptibility to Ranavirus (**Brand**)	59. A Novel COX-Independent Mechanism of Sulindac Sulfide Facilitates Cleavage of Epithelial Cell Adhesion Molecule Protein (**Liggett**)	70. Pharmacokinetics of Gabapentin in Hispaniolan Amazon Parrots (*Amazona ventralis*) (**Baine**)
11:45	47. At Both High and Low Concentrations of Platelets, Leukocyte-Reduced Platelet-Rich Plasma Reduces Evidence of Inflammation in Equine Tendon Explant Culture (**Boswell**)	60. FK228 and Cisplatin Synergistically Induce Cell Death and Reduce Clonogenic Resistance in Human Bladder Cancer Cells (**Choudhary**)	71. The Effect of Propofol on Sevoflurane Minimum Alveolar Concentration Preventing Motor Movement (MACNM) in Dogs (**Singsank**)

12:00 **BREAK for Lunch–Featured speaker: Channa Palmer, "Postdoctoral Opportunities" (Hollingsworth Auditorium)**

Tuesday, May 21

	Immunology & Viral Pathology	Oncology & Cancer Cell Biology	Clinical Sciences
	Rm. PBB 113	**Rm. PBB 156/157**	**Rm. PBB 160**
2:00	48. Regulation of the CXCL10 Gene by Pro-inflammatory Cytokines Interleukin 1-Beta and Gamma-Interferon in Pancreatic Beta Cells (**Goff**)	61. Acquisition of Carcinogenic Properties in Human Breast Epithelial Cells Induced by Sequential Exposure Versus Co-exposure to Environmental Carcinogens (**Pluchino**)	72. Correlation and Repeatability of Glucose and D-Xylose Intestinal Absorption Tests in Normal Horses (**Cruz Villagran**)
2:15	49. In Vivo Testosterone Supplementation Decreases Circulating Interleukin Family Isoforms and Differentially Regulates MMPs (**Freeman**)	62. Curcumin in the Intervention of Triclocarban-Induced Human Breast Cell Carcinogenesis (**Sood**)	73. Effects of Butorphanol Versus Dexmedetomidine Sedation on Intradermal Allergy Test Results in Dogs with Atopic Dermatitis (**Milosevic**)
2:30	50. Transmission of Ranavirus Between Ectothermic Vertebrate Hosts (**Brenes**)	63. Role of Fibrin Inhibitory Peptides in Cancer Immunotherapy (**Adams**)	74. Use of the T2*-Weighted GRE Sequence for MRI Examination of the Canine and Feline Brain (**Hodshon**)
2:45	51. Ranavirus Suceptibility in Checkered Gartner Snakes (*Thamnophis marcianus*) (**Chaney**)	64. Lighting up COX-2-Expressed Carcinomas by Fluorocoxib A (**Cekanova**)	75. Magnetic Resonance Imaging (MRI) of the Liver in Normal Dogs Using the Specific Contrast Agent Eovist (**Marks**)
3:00	52. Examining Heterologous T Cell Suppression in Plasmodium-Infected Mice (**White**)	65. Characterization of Four New Canine Transitional Cell Carcinoma Cell Lines (**Rathore**)	76. Hormonal Regulation of Lysyl Oxidase in Vascular Remodeling (**Chapman**)
3:15	**BREAK**		
3:45	53. MicroRNA-155: Regulates HSV-1 Encephalitis but Promotes Stromal Keratitis (**Bhela**)		7. Prevalence of Ranavirus and Bd in Hellbender Populations in Tennessee and Arkansas (**Hardman**)
4:00	54. Does Geographic Distance Between Host Population and Isolate Location Equate to Ranavirus Pathogenicity? (**Reilly**)		
6:00	**Awards banquet & after-dinner address: Marcy Souza, DVM, MPH, "One Health – What the HELLbenders Have to Do with It" (Calhoun's on the River)**		

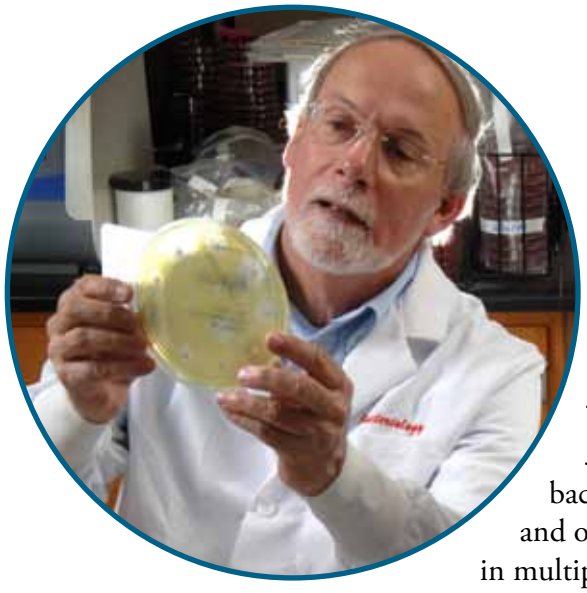
FEATURED SPEAKERS

Marion Kainer, MD, MPH, FRACP

Director, Healthcare Associated Infections and Antimicrobial Resistance Program
Tennessee Department of Health

"A Fungus Among Us: Anatomy of an Outbreak Investigation" – Monday Keynote Address

Marion Kainer, MD, MPH, FRACP, is an adult infectious diseases physician and healthcare epidemiologist for the Tennessee Department of Health (TDH). Additionally, Dr. Kainer is the chair of the Healthcare Associated Infections [HAI] subcommittee of the Council of State and Territorial Epidemiologists [CSTE]. She co-chairs the CSTE HAI standards committee and the antimicrobial resistance reporting working group. Dr. Kainer led the recent fungal meningitis outbreak investigation at the TDH and was named Tennessean of the Year by the Nashville-based *Tennessean* newspaper. Before joining the TDH, Dr. Kainer was an Epidemic Intelligence Service officer at the U.S. Centers for Disease Control and Prevention (CDC), in the Division of Healthcare Quality Promotion.



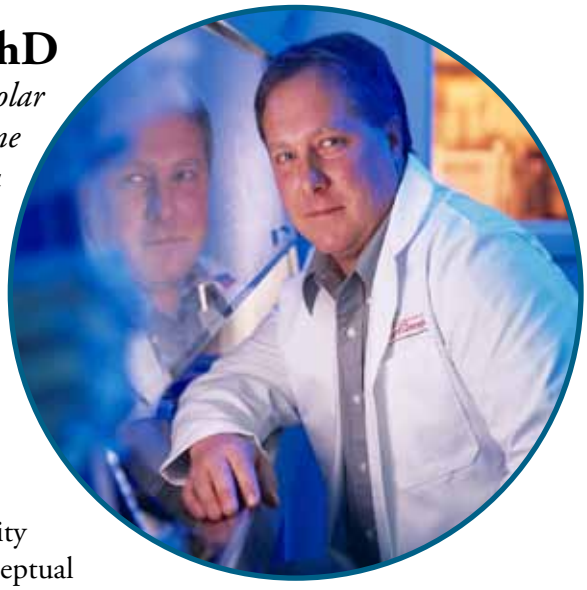
David Bemis, PhD

Professor & Director, Clinical Bacteriology and Mycology Laboratory
Department of Biomedical and Diagnostic Sciences
College of Veterinary Medicine
University of Tennessee

"Laboratory Diagnosis of Fungal Infections: Mycology in the Trenches" – Monday Featured Speaker

David Bemis, PhD, teaches bacteriology and mycology in the professional veterinary curriculum and directs a full service clinical bacteriology and mycology laboratory that receives specimens from both within and outside of the UT Veterinary Medical Center. He conducts applied research in multiple areas of the discipline and provides laboratory collaborative support for clinical investigators. Dr. Bemis has published more than 70 peer-reviewed journal articles in his field. He is also a recipient of the Trek Award for Excellence in Veterinary Microbiology given by the American Association of Veterinary Microbiologists and was awarded honorary diplomate status in the American College of Veterinary Microbiologists.

FEATURED SPEAKERS



Ralph Tripp, PhD

Professor & Georgia Research Alliance Eminent Scholar
Department of Infectious Diseases, College of Veterinary Medicine
University of Georgia

"Translational Disease Intervention Strategies Based on Studies of Virus-Host Interactions" – Tuesday Plenary Address

Ralph Tripp, PhD, earned his terminal degree in immunology from Oregon State University. His research interests are to develop translational disease intervention strategies for important human viruses and emerging infectious diseases of zoonotic origin. His labs investigate the mechanisms of immunity and disease pathogenesis associated with infection to better understand the conceptual and functional differences between innate and adaptive immune responses that provide the foundation necessary to facilitate vaccine and antiviral therapeutic protocols. His research vision is to understand the dynamics of host response to virus infection that will provide new strategies for resolving disease through the development of treatments or vaccines. Before joining the faculty at UGA, Dr. Tripp led a research team in vaccine studies for human viral diseases in the Respiratory and Enteric Viruses Branch at the CDC. He has been recognized for outstanding service to UGA and the State of Georgia, and he has been a recipient of the the NIAID Special Recognition Award for Extraordinary Work in H1N1 Influenza Research. In addition, he was named Georgia BioBusiness Academic Entrepreneur of the Year in 2011.

Channa Palmer

University Recruiting
Oak Ridge National Laboratory

"Postdoctoral Opportunities" – Tuesday Featured Speaker

Channa Palmer is the lead university recruiter at Oak Ridge National Laboratory. Ms. Palmer works to recruit top talent for ORNL distinguished doctoral fellowships, post-graduate positions, internships, and early career employment. She also recruits for the ORNL-UT graduate program, the Bredesen Center for Interdisciplinary Research in Graduate Education (The Bredesen Center). Prior to joining the recruiting team, Ms. Palmer worked as an HR Administrator for the Computing and Computational Sciences organization at ORNL. She joined ORNL after completing a bachelor's degree in Business Administration and Human Resource Management at the University of Tennessee. Ms. Palmer is a member of the ORNL Early Career Professionals Group and ORNL African American Affinity Resource Council. She also serves as the College Relations Board Member for the Tennessee Valley Human Resources Association.

FEATURED SPEAKERS

Marcy Souza, DVM, MPH

Assistant Professor of Public Health and Wild Animal Medicine
Department of Biomedical and Diagnostic Sciences
College of Veterinary Medicine
University of Tennessee

"One Health – What the HELLbenders Have to Do with It"
Featured After-dinner Address

Marcy Souza, DVM, MPH, graduated with a BS from the University of Maryland in 1995 and a DVM from North Carolina State University in 2004. She worked in a small and exotic animal private practice outside Houston, TX, for one year after graduation and then moved to Knoxville to complete a residency in Avian & Zoological Medicine and a Masters in Public Health. Dr. Souza is a boarded specialist in both avian medicine and veterinary preventive medicine. Her research interests include infectious and zoonotic diseases of exotic pets and wildlife and using wildlife as sentinels for human health. She has worked with a variety of species in a research capacity including hellbenders, little brown bats, raccoons, and Hispaniolan Amazon parrots.

Dr. Souza recently spent a month in India teaching a course on avian medicine at the Madras Veterinary College in Chennai. This program was supported by a Fulbright Specialist Grant. She also teaches veterinary and other graduate-level courses, including Wildlife Diseases and Infectious Diseases. She shares her home with one human, three dogs, one rabbit, one parrot, and two finches. Ady is one of the dogs in the house and has been a HABIT (Human Animal Bond in Tennessee) dog for about 1 ½ years. Ady enjoys her weekly visits to the UT Cancer Institute.

Abstracts

THE UNIVERSITY of TENNESSEE

Awards Descriptions

- **Graduate Student Category**: Travel awards for the top 3 presentations. 1st Place – $1,000; 2nd Place – $750; 3rd Place – $500
- **Intern/Resident Category**: Travel award for the top presentation. $1,000
- **Research Associate Category**: Travel award for the top presentation. $1,000
- **Assistant Professor Category**: Travel award for the top presentation. $1,000
- **Gamma Sigma Delta Award for Excellence in Agricultural & Related Sciences**: Top graduate student presentation representing Gamma Sigma Delta's high standards of scholarship in agricultural and related sciences. $250
- **Phi Zeta Award for Excellence in Animal Health Research**: Top presentation representing Phi Zeta's goal to excel in scholarship and research in matters pertaining to the welfare and diseases of animals. $250
- **Sigma Theta Tau International Award for Excellence in Human Health Research**: Top presentation that represents STTI's mission to lead using knowledge, scholarship, service, and learning to improve the health of the world's people.

1. Death Receptor 6 Induces Apoptosis Not through Type I or Type II Pathways, but via a Unique Mitochondria-Dependent Pathway by Interacting with Bax Protein

Linlin Zeng, Ting Li, Derek C. Xu, Jennifer Liu, Guozhang Mao, Mei-Zhen Cui, Xueqi Fu, Xuemin Xu

Department of Biomedical and Diagnostic Sciences (Zeng, Li, Mao, Cui, X. Xu); Edmond H. Fischer Signal Transduction Laboratory, College of Life Sciences, Jilin University, Changchun, China (Fu); Farragut High School, Knoxville (D. Xu, Liu)

Cells undergo apoptosis through two major pathways, the extrinsic pathway (death receptor pathway) and the intrinsic pathway (the mitochondrial pathway). These two pathways can be linked by caspase-8-activated truncated Bid formation. Very recently, death receptor 6 (DR6) was shown to be involved in the neurodegeneration observed in Alzheimer's disease. DR6, also known as TNFRSF21, is a relatively new member of the death receptor family, and it was found that DR6 induces apoptosis when it is overexpressed. However, how the death signal mediated by DR6 is transduced intracellularly is not known. To this end, we have examined the roles of caspases, apoptogenic mitochondrial factor cytochrome c, and the Bcl-2 family proteins in DR6-induced apoptosis. Our data demonstrated that Bax translocation is absolutely required for DR6-induced apoptosis. On the other hand, inhibition of caspase-8 and knockdown of Bid have no effect on DR6-induced apoptosis. Our results strongly suggest that DR6-induced apoptosis occurs through a new pathway that is different from the type I and type II pathways through interacting with Bax.

2. Pen-2 is Dispensable for Endoproteolysis of PS1 but is Required for Gamma Secretase Activity

Chen Hu, Guozhang Mao, Linlin Zeng, Ting Li, Xuemin Xu

Department of Biomedical and Diagnostic Sciences

Beta-amyloid peptide (Aβ) is produced from a large type I transmembrane protein called amyloid precursor protein (APP) by β-secretase and ɣ-secretase. ɣ-secretase cleaves APP at the C terminal and produces Aβ 49, 46, 43, 42, 40, and 38. These C-terminuses are important because longer Aβ tends to be more amyloidogenic and in turn more pathogenic. Based on the "amyloid cascade hypothesis," the ratio of Aβ42 verses Aβ40 plays a key role in Alzheimer's disease (AD). Hence, the investigation on the biological and biochemical nature of gamma secretase is very important. ɣ-secretase is composed of four components: presenilins (PS1 or PS2), nicastrin (NCT), anterior pharynx-defective 1 (Aph-1), and presenilin enhancer 2 (pen-2). PS functions as the catalytic subunit; NCT may serve as a receptor for the substrate; Aph is assumed to stabilize the other three components; and pen-2 is hypothesized to access PS1 endoproteolysis into C (PS1C) and N-terminal (PS1N) fragments. However, our data demonstrate that in the absence of pen-2, PS1 is still processed into PS1C and PS1N. More importantly, knockout of pen-2 causes ɣ-secretase to lose its APP cleavage ability. These results strongly indicate that pen-2 is dispensable for endoproteolysis of PS1 but required for ɣ-secretase activity. Our data also demonstrate that NCT is necessary for ɣ-secretase activity. We also examined the function of Aph-1. Taken together, our results provide a novel view about the properties of all four components in ɣ-secretase structure and function.

3. PSAP Induces a Unique Apaf-1 and Smac-Dependent Mitochondrial Apoptotic Pathway Independent of Bcl-2 Family Proteins

Ting Li, Linlin Zeng, Wei Gao, Mei-Zhen Cui, Xueqi Fu, Xuemin Xu

Department of Biomedical and Diagnostic Sciences (Li, Zeng, Gao, Cui, Xu); Department of Microbial and Biochemical Pharmacy, Jilin University (Fu)

Presenilin-associated protein (PSAP) has been identified as a mitochondrial proapoptotic protein. However, the mechanism by which PSAP induces apoptosis remains unknown. To this end, we have established an inducible expression system. Using this system, we have examined the roles of B-cell lymphoma 2 (Bcl-2) family proteins, cytochrome c, Smac (Smac/Diablo, second mitochondria-derived activator of caspases/direct IAP binding protein with low PI), and Apaf-1 (apoptotic protease-activating factor) in PSAP-induced apoptosis. Our results demonstrate that knockdown of Apaf-1 abolished PSAP-induced caspase activation and poly(ADP ribose) polymerase (PARP) cleavage, indicating that the apoptosome formation triggered by cytochrome c is crucial for PSAP-induced apoptosis. Our data also demonstrate that knockdown of Smac abolished PSAP-induced caspase activation and PARP cleavage, indicating that, in addition to Apaf-1 or apoptosome formation, Smac is also essential for PSAP-induced apoptosis. However, interestingly, our data demonstrate that overexpression of Bcl-2 and Bcl-xL did

not protect cells from PSAP-induced apoptosis, and that knockdown of Bid, Bax, and Bak had no effect on PSAP-induced cytochrome c and Smac release, indicating that PSAP-induced apoptosis is not regulated by Bcl-2 family proteins. These results strongly suggest that PSAP evokes mitochondrial apoptotic cascades via a novel mechanism that is not regulated by Bcl-2 family proteins, but that both the formation of cytochrome c-Apaf-1 apoptosome and the presence of Smac are absolutely required for PSAP-induced apoptosis.

4. Matricellular Protein Cyr61 Bridges the LPA/GPCR Pathway with the Integrin Pathway Leading to LPA-Induced Pathological Response

Fuqiang Zhang, Daniel D. Wu, Feng Hao, Kan Xu, Xuemin Xu, Mei-Zhen Cui

Department of Biomedical and Diagnostic Sciences

Lysophosphatidic acid (LPA), a potent bioactive lipid, markedly induces smooth muscle cell (SMC) migration. We identified a novel function of the de novo synthesized matricellular protein Cyr61, which bridges LPA and integrin signaling pathways leading to cell migration. LPA transiently and markedly induced Cyr61 mRNA expression and the temporal and spatial expression of Cyr61 proteins in SMCs. The de novo synthesized Cyr61 proteins promptly accumulate in the Golgi apparatus and then translocate to the extracellular matrix. Using primary SMCs from LPA receptor knockout mice, we identified that LPA1 receptor is required for LPA-induced Cyr61 expression. Neutralization or knockdown of Cyr61 protein expression blocked LPA-induced cell migration, indicating the novel regulatory role of the induced matricellular protein Cyr61 in LPA-induced cell migration. LPA induces the activation of intracellular focal adhesion kinase (FAK) in SMCs. Interestingly, 1) knockdown of Cyr61 blocked LPA-induced cellular FAK activation and cell migration, and 2) knockdown of the expression of integrins alpha6, beta1, and beta3 prevented LPA or Cyr61-induced FAK activation and cell migration. These data reveal for the first time that a novel LPA/Cyr61 pathway controls cell migration and that the matricellular protein Cyr61 bridges LPA signaling and integrin signaling, which, in turn, activate FAK leading to cell migration.

5. Mechanism Study of LPA-Induced Lipid Uptake Effect in Macrophages

Kan Xu, Feng Hao, Fuqiang Zhang, Xuemin Xu, Mei-Zhen Cui

Department of Biomedical and Diagnostic Sciences

Atherosclerosis is driven by cardiovascular risk factors that cause the recruitment of circulating immune cells beneath the vascular endothelium. Infiltrated monocytes differentiate into different macrophage subtypes with both protective and pathogenic activities in vascular lesions. So the study of macrophage cell activities during atherosclerotic lesion formation becomes quite crucial in understanding the disease progression. Lysophosphatidic acid (LPA) is a potent bioactive lipid in oxidized-LDL, proved by previous reports, regulating a broad range of cellular functions in various cell types. According to our observations, LPA-induced macrophages may augment the lipid (oxidized LDL) uptake effects both in the J774A.1 cell line and bone-marrow derived macrophages directly isolated from mice. In order to determine more detail in the mechanism, a complete micro-array analysis of RNA samples collected at different time points was done, and several other validation methods as real-time PCR was also performed to confirm some interesting findings. Since macrophages are believed to play an important role in the tissue inflammation process, through carefully examining the protein kinase activation by Western blotting, we surprisingly saw p-Erk inhibition and p-PKD activation during the early phase of LPA-induced macrophages. All the emerging data led us to the hypothesis that LPA, through regulating cellular functions, may expedite macrophage-foam cell formation in atherosclerotic lesions.

6. Protein Kinase D2 Mediates LPA-Induced Tissue Factor Expression and Activity through the MAPK Pathway in Mouse Aortic Smooth Muscle Cells

Feng Hao, Fuqiang Zhang, Kan Xu, Xuemin Xu, Mei-Zhen Cui

Department of Biomedical and Diagnostics Sciences

Lysophosphatidic acid (LPA), a potent bioactive lipid in oxidized-LDL, modulates vascular cell functions in vitro and in vivo via regulating the expression of specific functional genes. In the current study, we found that LPA markedly induced protein kinase D (PKD)

activation in mouse primary aortic smooth muscle cells (MASMCs). Protein kinase D (PKD), a family of serine and threonine protein kinases, has been implicated in various cellular functions. We found that LPA-induced PKD phosphorylation is via a PKC-dependent pathway and that knockdown of PKD2 expression blocked LPA-induced tissue factor (TF) expression and activity, indicating that PKD2 is the key intracellular mediator for TF. Tissue factor is the principal initiator of blood coagulation and is important for thrombosis. Our data also demonstrate that PKD2 mediated LPA-induced TF expression via the mitogen-activated protein kinase pathway. Using primary MASMCs isolated from LPA receptor knockout mice, our data revealed that LPA receptor 1 was responsible for LPA-induced TF expression. Therefore, these data provide the first evidence that PKD is a component in LPA signaling in vascular SMCs, leading to gene expression, as evidenced by TF expression. Our data provide new insights into the mechanisms of coagulation and possible therapeutic targets for atherothrombosis.

7. Prevalence of Ranavirus and Bd in Hellbender Populations in Tennessee and Arkansas

Rebecca Hardman, William Sutton, Dale McGinnity, Kelly Irwin, Sherri Reinsch, Benjamin Fitzpatrick, Philip Colclough, Marcy Souza, Michael Freake, Matthew Gray, Debra Miller

Center for Wildlife Health (Hardman, Sutton, Souza, Gray, Miller); Nashville Zoo (McGinnity, Reinsch); Arkansas Game and Fish Commission (Irwin); Department of Ecology and Evolutionary Biology (Fitzpatrick); Knoxville Zoo (Colclough); Lee University Department of Biology (Freake); Department of Biomedical and Diagnostic Sciences (Souza, Miller)

The Hellbender, *Cryptobranchis allenganiensis*, is a large aquatic salamander containing two subspecies (Ozark Hellbender, *C. a. bishopi* and Eastern Hellbender, *C. a. alleganiensis*) from the Ozark Mountains and eastern United States, respectively. Both subspecies have seen population declines over the past 25 years, especially in *C. a. bishop,* which is federally endangered. Habitat degradation and possibly low genetic diversity may lead to secondary infections with amphibian pathogens such as ranavirus and *Batrachochytrium dendrobatidis* (Bd). The objective of this study was to determine prevalence of these pathogens in both subspecies as a first step in understanding the role of emerging amphibian pathogens in *C. alleganiensis* declines. We collected tail tissue and skin swabs from *C. a. bishopi* and *C. a. alleganiensis* individuals from Arkansas and Tennessee, respectively, during the summers of 2011 and 2012. We used qPCR analysis to determine presence of ranavirus and Bd from tail samples and skin swabs, respectively. Overall, for *C. a. bishopi*, we detected 20% prevalence of Bd and no cases of ranaviral infections; for *C. a. alleganiensis*, we detected 7% prevalence of Bd and 5% prevalence of ranavirus. Additionally, we observed leeches parasitizing many individuals and identified one *C.a. allenganiensis* leech positive for ranavirus. These data reveal that Bd is present in these populations and, as Bd is known to favor keratinized tissue, may play a role in causing physical deformities (e.g., missing and fused toes) seen in *C. a. bishopi*. Furthermore, early analyses of ranavirus suggest a link to watershed and ectoparasitism.

8. Identification and Distribution of *Ehrlichia* and *Rickettsia* Species within *Amblyomma americanum* at Ames Plantation in Western Tennessee

Brian Hendricks, Dave Paulsen, Graham Hickling, Allan Houston, Rebecca T. Trout Fryxell

Departments of Entomology and Plant Pathology (Hendricks, Paulsen, Trout Fryxell); Forestry, Wildlife and Fisheries (Hickling, Houston)

The status of tick-borne diseases (TBD) in the southeastern United States is uncertain due to a number of factors but not limited to emerging pathogens, misdiagnoses, and modifications to landscapes. Human monocytic ehrlichiosis and Rocky Mountain spotted fever are two of the most common TBDs in Tennessee, and Tennessee cases account for 5.7% and 2.4% of all cases reported in the United States. Consequently, the objective of this study was to identify different species of *Ehrlichia* and *Rickettsia* associated with *Amblyomma americanum* and each pathogen's temporal and spatial distribution in western Tennessee. Ticks were collected from May to September 2012, using vegetation drags and CO_2 traps, and only adult *A. americanum* (n = 926) were screened to determine the prevalence of *Ehrlichia* and *Rickettsia* in the most prevalent tick of Tennessee. A total of 353 *A. americanum* (38%) were PCR positive for *Rickettsia* spp. and were collected from May through August from 81 different sites. All the *Rickettsia* species were identified as non-pathogenic *R. amblyommii*. Conversely, 18 *A. americanum* (1.9%) were PCR positive for *Ehrlichia* species and were collected in May, June, and August from 13 different sites; all identified species

were pathogenic and included *E. ewingii*, *E. chaffensis*, and Panola Mountain *Ehrlichia*. While we did not screen all life stages of each potential vector, this study does demonstrate the need for continued research in TBDs including *Ehrlichia*.

9. Engineering Biodegradable Microporous Bacterial Cellulose Scaffolds and Biomimetic Composites for Bone and Cartilage Tissue Regeneration

Pelagie Favi, Roberto Benson, Nancy Neilsen, Madhu Dhar

Department of Materials Science and Engineering (Favi, Benson); College of Veterinary Medicine(Neilsen); and Department of Large Animal Clinical Sciences (Dhar)

We have recently reported that native bacterial cellulose (BC) supports in vitro proliferation and osteogenic and chondrogenic differentiation of equine bone marrow-derived adult mesenchymal stem cells (EqMSCs). The objective of this study was to generate biodegradable, microporous and surface-modified BC scaffolds to mimic native bone and cartilage tissues for in vivo application. Microporous BC scaffolds were synthesized and oxidized to render the BC degradable. To mimic bone tissue, calcium-deficient hydroxyapatite (CdHAP) was deposited on the BCs. Surface amination and carboxylation of BCs were performed to simulate the glycosaminoglycans present in the native cartilage tissue. In vitro proliferation and differentiation potentials of EqMSCs were evaluated. EqMSCs formed multilayers on the microporous BC scaffolds. The various forms of BC hydrogel scaffolds exhibited distinct differences in the rate of proliferation and in vitro differentiation. Compared to native BC, non-degradable modified BCs did not enhance the rate of cell proliferation after 7 days; whereas the biodegradable and biodegradable-microporous forms did enhance the rate of cell proliferation approximately 1.5 to 2 fold after 7 days. Although the rates of cell proliferation and differentiation potential varied between the scaffolds, cells were viable and underwent osteogenesis and chondrogenesis on the modified surfaces. Based on these in vitro data, the biodegradable microporous CdHAP BC is recommended for in vivo bone tissue regeneration applications. These findings demonstrate that specifically modified BC scaffolds are promising constructs for bone and cartilage tissue engineering therapies.

10. Developing a Non-Surgical Contraceptive Method for Female Dogs

A. M. Sashi Papu John, Tulio M. Prado, Kellie A. Fecteau, Robert L. Donnell, S. M. L. Chamindrani Mendis-Handagama

Department of Biomedical and Diagnostic Sciences

Developing an innovative non-surgical sterilization product for cats and dogs is a challenge. Ideally, a contraceptive product would induce permanent sterilization and be effective in male and female cats and dogs of all ages. For the present study, dogs were chosen because they out-number cats as pets in the United States. Nicotine was chosen as the contraceptive agent based on previous experimental studies. The hypothesis of this study is that nicotine can maintain the anestrus state, with no sexual receptivity status in female dogs. Two experimental groups of female dogs were studied (n = 4 per group). Placebo and nicotine pellets that release 0.5 mg of nicotine per kg body weight per dog, over a 90-day period were implanted subcutaneously, followed by two more rounds of implants, for a total of 270 days. Vaginal cytology was performed to characterize the estrous cycle. Animals were euthanized, followed by plasma and tissue collections. Circulating estrogen and progesterone levels were significantly reduced in nicotine-treated females ($P < 0.05$). No significant change in body weight was observed between the placebo and experimental females. However, ovary and uterus weights were lower in nicotine-treated females, compared to their age-matched placebo females. Microscopic studies of the ovaries showed the structures of anestrus stages in nicotine-treated females, when compared with the placebo females, which existed in either estrus/diestrus. These preliminary observations suggest that nicotine treatment has the potential to maintain female dogs in anestrus, without undesirable side-effects. (Supported by Alliance of Women Philanthropists)

11. Diagnostic Accuracy of a Novel Point-of-Care Urinary Culture Test in Dogs

Shelly Olin, Joseph Bartges, Rebekah Jones, David Bemis

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The study's purpose was to determine diagnostic accuracy of a compartmented culture and susceptibility

plate (CCS) for detection of urinary tract infection and antimicrobial susceptibility in dogs. The CCS is an agar plate with one compartment for quantitative analysis using a chromogenic substrate allowing for bacterial identification and five antibiotic impregnated compartments: ampicillin, amoxicillin plus clavulanate, cephalothin, enrofloxacin, and trimethoprim-sulfamethoxazole. For assay validation (phase 1), frozen canine isolates of previously characterized bacteria (n = 62) were revitalized and tested with CCS and standard aerobic microbiological culture (SAMC). For the clinical study (phase 2), urine samples collected for routine diagnostics from clinical canine patients (n = 147) were tested in parallel and blinded fashion with CCS and SAMC. For phase 1 at 24 h, 46/62 (74%) samples had growth on CCS and 100% on SAMC. For samples with growth, CCS correctly identified 45/46 (97.8%) bacteria. Bacteria with no growth were gram-positive cocci (*Staphylococcus* sp. [n = 7, 100%], *Enterococcus* sp. [n = 7, 58 %]). In phase 2, the overall sensitivity of CCS was 81% and specificity was 99%. There was 94% accuracy with 98% positive predictive value and 92% negative predictive value. Enrofloxacin and trimethoprim-sulfa susceptibilities had greatest concordance with those determined by SAMC (71% and 95.8%, respectively). In conclusion, CCS accurately excluded urinary tract infection but was less reliable for diagnosing infection, especially with gram-positive cocci. The biggest study limitation was small sample size.

12. Study Transmission and Expansion of Major Clonal Lineages of *Toxoplasma gondii* using Multilocus Microsatellite Markers

Pooja Saraf, Chunlei Su

Department of Microbiology

Toxoplasma gondii is the most successful zoonotic pathogen known today. It has a complex life cycle that propagates sexually in definitive host (cats) and asexually in intermediate host (mammals and birds). Cats contaminate the environment by shedding *T. gondii* oocyts in the feces. Intermediate host acquire infection by ingesting oocysts from contaminated food and water or by consuming tissue cysts in other infected animals. One third of people are chronically infected worldwide. Infection in humans may cause ocular, congenital, or severe acute disseminated toxoplasmosis; in immunocompromised patients, reactivation of latent infection can cause life threatening encephalitis. PCR-RFLP genotyping analysis of *T. gondii* isolates shows a worldwide distribution of Type II and III clonal lineages. In Europe, Africa, Asia, and North America, several lineages (including Types II and III and a few others) predominate in the population; however, *T. gondii* isolates are highly diverse, and there is a lack of predominant lineages in Central and South America. It is not clear what factors lead to such difference in these geographical regions. We hypothesize that genetic diversity and population structure of *T. gondii* are influenced by human agricultural environment in which domestic cats and house mice play a central role in *T. gondii* transmission. To test this hypothesis, we will use 15 microsatellite markers to analyze Type II and III strains collected worldwide to determine their within-lineage diversity. Accomplishment of this study will allow us to better understand transmission patterns and the role of agricultural environment on the expansion of *T. gondii*.

13. Neutron Radiography and Neutron Computed Tomography: Novel Imaging Technologies for Detection of Cancers

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Neutron radiography (NR) combined with neutron computed tomography (nCT) are novel imaging modalities that could provide additional information about tumors. NR using cold and thermal neutron is often described as being complementary to conventional X-ray imaging in the sense that imaging with X-rays detects heavy elements, while imaging with neutrons detect light elements. Hydrogen is a primary contributor to neutron contrast of biological specimens. We used NR combined with nCT to evaluate the transmission of neutrons in normal tissue and compared it with dissected cancer tissues from dogs with naturally-occurring tumors. The normal and adenocarcinoma tissues were fixed in formalin and imaged by NR and nCT at the CG-1D neutron imaging prototype beam-line located at the Oak Ridge National Laboratory High Flux Isotope Reactor. The data obtained from neutron radiographs were evaluated using custom-made code based on MATLAB. Octopus software

was used to reconstruct the data from sinograms and thus provided nCT 3D images. NR of biospecimens detected cancer at a spatial resolution of 50 µm and showed correlation with the obtained histology from hematoxylin and eosin-stained tissue sections. NR and nCT are novel imaging technologies for cancer research to obtain additional information about tumors at very high spatial resolution (50 µm) with the ability to differentiate tumors from surrounding normal tissues. NR and nCT are complementary to currently used microMRI and microCT to obtain additional information about tumor structure.

14. Early Exposure to Triclocarban During Lactation Alters Survival Rate in the Female Rat Neonate

Rebekah C. Kennedy, Laura N. Healy, Kellie Fecteau, Ling Zhao, Pan Hu, Nancy A. Gee, Kurt Benirschke, Bill L. Lasley, Jiangang Chen

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Triclocarban (TCC), a bactericide in personal care products, has been shown to impact sex and thyroid hormone function in vitro and influence adult sex organ accretion in vivo. This study investigates the critical period of TCC exposure in utero and during lactation through a cross-fostering design. Time-pregnant Sprague Dawley rats were fed control or TCC supplemented chow (0.2% or 0.5% [w/w]) ad lib from gestational day (GD) 5 until weaning/post natal day (PND) 21. On PND 0, litters were culled to six females per dam followed by cross-fostering of pups between the three dam groups. After cross-fostering, a reduced survival rate was shown among neonates nursed by TCC-exposed dams, while 100% of pups nursed by control dams survived to PND 21. Length of survival among pups raised by treated dams was dose dependent as no pups nursed by 0.5% (w/w) TCC treated dams survived at PND 6 and 57% of pups nursed by 0.2% (w/w) TCC treated dams survived at PND 9. Only 13% of pups raised by 0.2% (w/w) TCC-treated dams survived after weaning, with an average reduced body weight of 51% compared to the body weight of control nursed pups. Histological evaluation of breast tissue collected from treated dams at necropsy (PND 21) revealed evidence of involution, with inflammatory cell infiltration or inflammation compared to control lactating tissue. Our data suggest the critical exposure window affecting neonate survival is related to lactation and provide supporting evidence for the potential adverse effects of TCC exposure during early life.

15. Role of SUAM in the Pathogenesis of *Streptococcus uberis* Mastitis

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Streptococcus uberis adheres to and internalizes into mammary epithelial cells where humoral host defenses and antimicrobials in milk are ineffective, thus allowing persistence of this pathogen in the mammary glands. We showed that *S. uberis* expresses a surface adhesion molecule (SUAM) that forms a molecular bridge through binding to lactoferrin, which in turn binds to its putative host cell surface ligand. This binding enhances adherence to and internalization of *S. uberis* into bovine mammary epithelial cells. To better define the role of SUAM an sua gene-deletion mutant clone of *S. uberis* UT888 (Δsua *S. uberis* UT888) was created. When tested under in vitro conditions, significantly fewer mutant clones adhered to and internalized into host cells than the isogenic wild type strain. To prove that the absence of SUAM affects bacterial attachment to and subsequent colonization and infection of bovine mammary glands under in vivo conditions, a wild type or its isogenic SUAM mutant were infused into udder quarters of dairy cows. Results from these challenge studies showed that fewer mammary glands of cows infused with the mutant become infected than mammary glands of cows infused with the isogenic wild type strain. Infected quarters of cows infused with the mutant had less severe clinical symptoms compared to infected quarters of cows infused with isogenic wild type strain. These results suggest that the SUAM mutant clone was less virulent than the isogenic wild type strain, which further substantiates the role of SUAM in the pathogenesis of *S. uberis* mastitis.

16. Use of Microfluidics to Study *Streptococcus uberis* Adhesion to Bovine Mammary Epithelial Cells Under Conditions of Shear Flow

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Streptococcus uberis is an important environmental pathogen that causes mastitis in dairy cows. Infections caused by this pathogen can be clinical or subclinical in nature, and sometimes become chronic, which has been associated with the ability to form biofilms in other bacterial pathogens. We have previously shown that *S. uberis* can form biofilms under in vitro conditions, but whether this occurs in vivo remains to be seen. The objective of this study was to develop an in vitro model to study adhesion and biofilm formation of a *S. uberis* bioreporter under conditions that mimic the bovine mammary gland. An *S. uberis* bioreporter consisting of mCherry gene linked to the luxS promoter was used in these experiments. Wells of a microfluidic plate were pre-coated with bovine collagen, and bovine mammary epithelial cells were seeded into the microchannels. After reaching confluence, the *S. uberis* bioreporter was inoculated into the wells, and a 0.2 dine shear flow was applied. The bioreporter was monitored in real time, and images showed successful attachment to the mammary cells and formation of biofilms under shear flow conditions. Results suggest that *S. uberis* is capable of forming biofilms under conditions that more closely resemble the bovine mammary gland. In addition, use of a *S. uberis* bioreporter is an effective means for assessing biofilm formation under flow conditions.

17. Protective Effect of Anti-SUAM Antibodies on *Streptococcus uberis* Mastitis

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For many pathogens capable of causing intramammary infections (IMI), including *Streptococcus uberis*, adherence is a prerequisite for subsequent internalization into intracellular milieu and establishment of persistent IMI. These mechanisms allow pathogens to overcome host defenses and colonize mammary glands. A common strategy used by *S. uberis* is through the formation of a molecular bridge by a *S. uberis* cell surface adhesin molecule (SUAM) binding to lactoferrin (LF), which in turn binds to its putative host cell surface ligand. Research results from our laboratory showed that vaccination of dairy cows with SUAM or natural infection with *S. uberis* induces anti-SUAM antibodies in serum and colostrum. We also showed that anti-SUAM antibodies inhibited adherence to and internalization of *S. uberis* into bovine mammary epithelial cells under in vitro conditions. In this study the protective effect of anti-rSUAM antibodies against *S. uberis* IMI was evaluated using a passive protection model. *Streptococcus uberis* UT888 was opsonized with affinity purified anti-rSUAM antibodies or hyperimmune sera and subsequently infused into mammary quarters of healthy cows. As a control, the non-opsonized *S. uberis* UT888 was infused into healthy mammary quarters. Results showed that mammary quarters infused with *S. uberis* UT888 opsonized with anti-rSUAM antibodies showed mild to undetectable clinical symptoms of mastitis, with lower number of bacteria in milk, and less IMI than quarters challenged with non-opsonized *S. uberis* UT888. These findings suggest that anti-rSUAM antibodies enhanced clearance of *S. uberis* through opsonophagocytic mechanism and/or blocked adherence to and internalization into mammary gland cells.

18. *Ixodes scapularis* as a Vector of Concern in Human Tick-Borne Diseases in Western Tennessee

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The status of tick-borne diseases (TBD) in the southeastern United States is uncertain due to the identification of new pathogens, the spread of existing diseases, the misdiagnosis of disease, and warming weather trends. *Ixodes scapularis* (the black-legged tick) is known to transmit the causal agents of

anaplasmosis (*Anaplasma phagocytophilum*), babesiosis (*Babesia microti*), Lyme disease (*Borrelia burgdorferi*), and rickettsiosis (*Rickettsia montanensis*). Recently, incidences of TBD have been reported at Ames Plantation Research and Education Center (AMES) located in western Tennessee, and personal correspondence suggests TBD incidence may be on the rise. The objective of this study was to determine if future *I. scapularis* research in western Tennessee is warranted; consequently, we screened 47 adult *I. scapularis* collected over a 2-year period from white-tailed deer for five common TBD genera. In fall of 2011 and 2012, adult *I. scapularis* were collected from 15 white-tailed deer harvested at AMES, and were PCR screened for infection with *Anaplasma*, *Babesia*, *Borrelia*, *Ehrlichia*, and *Rickettsia* species with genera-specific primers. None of the ticks tested positive for *Borrelia* or *Babesia* species. A total of 29 ticks (62%) tested positive for non-pathogenic *Rickettsia* species. Four ticks (9%) tested positive for both a *Rickettsia* species (uncultured *Rickettsia*) and *Anaplasma*/*Ehrlichia* species. Finding human pathogens in this limited sample size merits additional studies. Such studies should include continued *I. scapularis* pathogen prevalence surveys at AMES, both because of the risk of human exposure to disease agents and to aid in accurate diagnosis of TBD cases in western Tennessee.

19. Fc-Mediated Binding of Canine Immunoglobulin G to Protein A on the Surface of *Staphylococcus pseudintermedius*

Manasi Balachandran, David Bemis, Stephen Kania

Comparative and Experimental Medicine (Balachandran); Biomedical and Diagnostic Sciences (Bemis, Kania)

Staphylococcus pseudintermedius is an opportunistic pathogen in canines and the most frequent cause of canine pyoderma. Much of what is known today about *S. pseudintermedius* comes from comparative research with *Staphylococcus aureus*. *S. pseudintermedius* possesses genes analogous to those encoding Protein A in *S. aureus*, but whether they are expressed is still unanswered. If they are expressed, it is worth investigating if these proteins are functional. The ability of Protein A produced by *S. pseudintermedius* to bind to canine IgG has not been investigated. The purpose of this study was to measure the binding of canine IgG to the surface of *S. pseudintermedius* as a potential virulence factor. Several strains of *S. pseudintermedius* with diverse genetic backgrounds were incubated with different fractions of canine IgG, i.e., whole IgG, Fab, and Fc to determine which portion binds Protein A effectively. Binding of commercially available anti-Protein A antibody to Protein A in *S. pseudintermedius* was also investigated. The amount of binding was determined by flow cytometry. *S. aureus* Cowan I strain was used as the positive control for all experiments. Our results showed that isolates representing the major clonal populations in the United States and Europe bound significantly more IgG than isolates with other genetic backgrounds. Also, our results showed that canine IgG binds to Protein A on *S. pseudintermedius* via its Fc region and not via Fab. Taken together, our results suggest that *S. pseudintermedius* shares *S. aureus's* ability to bind to the Fc region of canine IgG and may serve as a means of evading the host immune system.

20. Cysteine Proteases of Feline *Tritrichomonas foetus* Mediate Adhesion-Dependent Cytotoxicity to Intestinal Epithelial Cells

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Trichomonads are obligate protozoan parasites most renowned as venereal pathogens of the human and bovine reproductive tract. Recently, a trichomonad highly similar to bovine venereal *Tritrichomonas foetus*, but having a unique tropism for the intestinal tract, was recognized as a significant cause of colitis in domestic cats. Despite a high prevalence, worldwide distribution, and lack of consistently effective drugs for treatment of the infection, the cellular mechanisms of *T. foetus* pathogenicity in the intestinal tract have not been examined. The aims of this study were to determine the pathogenic effect of feline *T. foetus* on intestinal epithelial cells, the dependence of *T. foetus* pathogenicity on adhesion of *T. foetus* to the intestinal epithelium, and the identity of mediators responsible for these effects. Using an in vitro co-culture approach to model feline *T. foetus* infection of the intestinal epithelium, these studies demonstrate that *T. foetus* promotes a direct contact-dependent activation of intestinal epithelial cell apoptosis signaling and progressive monolayer destruction. Moreover, these pathologic effects were demonstrated to be dependent on *T. foetus*

cell-associated cysteine protease activity. Finally, *T. foetus* cysteine proteases were identified as mediating cytopathic effects by promoting adhesion of *T. foetus* to the intestinal epithelium. The present studies are the first to examine the cellular mechanisms of pathogenicity of any trichomonad towards the intestinal epithelium. By identifying cysteine proteases as key adhesion-dependent mediators of *T. foetus* pathogenicity, these studies pinpoint a potential therapeutic target for ameliorating the pathological effects of intestinal trichomonosis.

21. Use of Probiotics to Reduce *Salmonella* Infection in Mice

Ana Andino, Sandra Diaz-Sanchez, Nan Zhang, Sean Pendleton, Francisco Gonzalez-Gil, Carrie Yard, Irene Hanning

Department of Food Science and Technology

Salmonella is a leading cause of foodborne illness. To prevent salmonellosis, much work has been conducted using probiotic bacteria. Probiotic strains of bacteria can prevent intestinal disease, including salmonellosis, by preventing the pathogen from colonizing the intestines. The objective of the study focused on the efficacy of the probiotics *Lactobacillus* and *Bacillus* to prevent *Salmonella* colonization in the intestinal tract of mice. A total of 15 mice were divided into three groups: 1) control; 2) *Lactobacillus* and *Pediococcus* (LP; 9:1 concentration); and 3) *Bacillus subtilus* (106 Log CFU per g of feed). The mice were treated with LP in water for the first 2 days of the experiment, while *Bacillus* was given in the feed for the duration of the experiment. All mice were challenged with *Salmonella* on day 3. After challenge with the *Salmonella*, mice droppings were collected daily and cultured for *Salmonella*. At day 20 post challenge all mice were sacrificed and the intestinal tracts and organs removed and cultured for *Salmonella*. The data indicate that the two probiotic treatments did not protect mice from *Salmonella* infection. In fact, the *Bacillus* treatment group was more susceptible to infection as compared to the control group. It appears that the probiotics may have disrupted the established normal microflora, leaving the mice more susceptible to *Salmonella* infection.

22. Yerba Mate Enhances Probiotic Bacteria Growth in vitro but as a Prebiotic does not Reduce *Salmonella enteritidis* Colonization in vivo

Francisco Gonzalez-Gil, Sandra Diaz-Sanchez, Sean Pendleton, Ana Andino, Nan Zhang, Nate Crilly, Carrie Yard, Wei Chen, Irene Hanning

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Poultry are leading sources of *Salmonella*. Horizontal transmission occurs rapidly among the flock after one bird is initially colonized. Yerba mate, a tea with antimicrobial activity, was evaluated as a prebiotic feed additive to reduce horizontal transmission of *Salmonella* in broiler chicks. For in vitro evaluation, the biocidal activity of yerba mate on *Salmonella enteritidis* (SE) and *Lactobacillus acidophilus* and *pediococcus* (LP) was evaluated. For in vivo evaluation, 80 day-of-hatch chicks were divided into five treatments: 1) control, 2) yerba mate (1% inclusion rate), 3) yerba mate (5% inclusion rate), 4) probiotic (LP at a ratio of 9:1; 107 CFU; gavaged at day 0), or 5) probiotic and yerba mate (1% inclusion rate). Horizontal transmission was evaluated using five seeder chicks per treatment, challenged with SE (106 CFU) at day 3; at day 10, all birds were euthanized and weighed; ceca and gizzards were collected and contents enumerated for SE. For the in vitro evaluation, antimicrobial activity was observed against SE (MIC 7.38–9.80 mg/mL), while the same treatment enhanced LP growth. For in vivo evaluation, the yerba mate did not reduce SE transmission, while the probiotic treatment significantly reduced transmission. Yerba mate significantly reduced performance in terms of body weight and affected the treatment with probiotic. Because yerba mate enhanced probiotic bacteria growth in vitro, we expected the yerba mate to enhance the probiotic effects in vivo. However, no synergism was observed, which could be due to decreased feed intake or reduced feed passage rate caused by the yerba mate.

23. Identification of Chemical Compounds to Inhibit Phosphatidylserine Synthesis in *Candida albicans*

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Departments of Microbiology (Cassilly, Reynolds); Chemistry (Campagna)

Candida albicans is a pathogenic fungus that causes vaginal, oral, and systemic infections, most notably in immunocompromised individuals. Although there are three different classes of antifungals typically used against systemic *C. albicans* infections, including azoles, polyenes (e.g. Amphotericin B), and Caspofungin, a combination of drug resistance and toxicity makes these antifungals less effective. New drugs are needed to combat *C. albicans*. Previous studies in a mouse model of systemic or oral infection have shown that the phosphatidylserine (PS) synthase mutant of *C. albicans* (cho1 Δ/Δ) is avirulent. The role of PS in virulence is currently unknown. However, because of the avirulent phenotype seen in the cho1 Δ/Δ mutant, PS synthase is a good potential target for chemical inhibitors. In addition, cho1 PS synthase is conserved among fungi, while it is absent within mammals, further implicating cho 1 as a specific and efficient drug target. Identifying chemical compounds that inhibit PS synthesis is an ideal starting point for drug development against yeast infections. Papaumide A is a peptide that recognizes PS and subsequently kills cells. Cells with inhibited PS synthase, and thus no PS, show resistance to the effects of Pap-A. Over 5,500 compounds were screened with Pap-A and two, SB-224289 and MG-624, that may inhibit PS synthesis in *C. albicans* were identified. Recent assays confirmed that SB-224289 confers Pap-A resistance to *C. albicans* in a dose-dependent manner. Further study, including lipid analysis by TLC and mass spectrometry, is required to determine the mechanism of SB-224289 action in *C. albicans*.

24. Inhibition of Foodborne Microorganisms by White Mustard Extract, Nisin, and Lauric Arginate Applied Individually and in Combination

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Food Science and Technology (Techathuvanan, Reyes, Davidson)

Microbial control strategies are needed in the food industry to prevent foodborne illnesses/outbreaks and prolong product shelf life. The aim of this study was to investigate the antimicrobial efficacy of white mustard extract (WME), nisin, and lauric arginate (LAE) against foodborne pathogens and spoilage organisms. Minimum inhibitory concentrations (MICs) of individual and combined antimicrobials against *Escherichia coli, Salmonella Enteritidis*, *Enterobacter aerogenes*, *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus* were determined by broth dilution assay at pH 6.0 and 25 °C. WME was most effective against *B. cereus* and *S. aureus* with MICs at 250 and 500 mg/l, respectively. An inhibitory effect with nisin was obtained only against gram-positive bacteria with MICs ranging between 400–1,200 mg/l. LAE was effective at low concentrations and required only 20–50 mg/l to cause inhibition to all tested microorganisms. When WME was combined with nisin, an additive antimicrobial effect was demonstrated against *B. cereus* and *S. aureus*. For *L. monocytogenes*, synergistic activity was shown with the WME and nisin combination. However, there was still no inhibitory effect against gram-negative bacteria with nisin even when combined with WME. For the WME and LAE combinations, additive antimicrobial activity was observed against all tested microorganisms. These findings suggest that WME, nisin, and LAE have potential to enhance food safety by inhibiting foodborne pathogens as well as to extend product shelf life.

25. The Role of Phosphatidylserine in *Candida albicans* Virulence

Sarah E. Davis, Robert Wheeler, Todd B. Reynolds

Department of Microbiology (Davis, Reynolds); Molecular and Biomedical Sciences, University of Maine (Wheeler)

Systemic fungal infections caused by the yeast *Candida albicans* have approximately a 30% mortality rate. Treatment of these infections can be problematic due to a combination of drug toxicity and resistance, creating a need for new drug targets. The phosphatidylserine synthase of *C. albicans*, Cho1p, appears to be a good drug target, as a mutant lacking this enzyme (cho1Δ/Δ) is a virulent in a mouse model of *Candida* infections, and the protein is not conserved in humans. We discovered that a loss of phosphatidylserine affects *C. albicans'* virulence trait expression, cell wall integrity, and immune evasion. These phenotypes may affect virulence and are being explored to determine the role of phosphatidylserine in fungal virulence. Agglutinin-like sequence (ALS) proteins are cell wall adhesins that are vital for *C. albicans* attachment to host ligands expressed on epithelial cells, aiding in tissue invasion and damage. We discovered

that the cho1Δ/Δ mutant has reduced Als3p protein expression, correlating with reduced adhesion to mammalian epithelial cells. *C. albicans'* cell wall contains mannoproteins that mask it from innate immunity recognition. An important PAMP that is recognized by phagocytic cells of the innate immune system is β-1,3-glucan, a polysaccharide located beneath this mannoprotein layer. This β-1,3 glucan can be recognized by the Dectin-1 receptor expressed by phagocytic cells, mediating recognition and killing of *C. albicans*. We discovered the cho1Δ/Δ mutant has increased exposure of β-1,3 glucan and an increased binding by Dectin-1, suggesting that the cho1Δ/Δ mutant is susceptible to immune system recognition.

26. Effect of COMT-Knockdown Switchgrass of Rhizosphere Microbiome

Archana Chauhan, Abby Smart, Jun Wang, Sagar Utturkar, Ashley Frank, Bi, Meng, Jiang Liu, Daniel Williams, TingTing Xu, Melanie Eldridge, Andres Ignacio Arreaza, Alexandra Rogers, Hector Castro Gonzalez, Mitra Mazarei, Holly Lauren Baxter, Jennifer Mary DeBruyn, Neal Stewart, Steven D. Brown, Gary S. Sayler

Center for Environmental Biotechnology (Chauhan, Smart, Wang, Williams, Xu, Eldridge, Rogers, Sayler); UT-ORNL Joint Institute of Biological Sciences (Utturkar, Brown); Departments of Microbiology (Frank, Meng, Liu, Gonzalez); Biological Sciences (Arreaza); Plant Sciences (Mazarei, Baxter, Stewart); Biosystems Engineering & Soil Science (DeBruyn)

Switchgrass (*Panicum virgatum L.*) is a perennial warm-season forage grass indigenous to North America. Currently, switchgrass is planted extensively for bioenergy production and has also been genetically engineered to reduce the expression of the caffeic acid 3-O-methyltransferase (COMT) gene in the lignin biosynthetic pathway. The increased use of transgenic switchgrass plants to get enhanced ethanol yields with reduced processing costs is a promising method, but its wide-scale planting may carry risk of invasiveness and extinction of wild relative populations. Although some research is being carried out addressing some of these issues, little to no information is available on the effect of using genetically modified switchgrass on the microbial community structures, despite the important role played by microorganisms in maintaining soil health and biogeochemical cycles. In our study, we used a high throughput sequencing approach to carry out comparative analysis of genetically modified versus wild-type COMT-knockdown switchgrass rhizosphere microbiome.

27. Use of Whole Genome Sequencing in the Discovery, Identification and Functional Characterization of a Novel Linear Plasmid in a Clinical Isolate of Methicillin-Resistant *Staphylococcus pseudintermedius*

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Comparative and Experimental Medicine (Riley); Department of Biomedical and Diagnostic Sciences (Bemis, Kania); United States Army (Riley)

Staphylococcus pseudintermedius is a gram-positive bacterial pathogen commonly associated with infection in dogs. Multidrug-resistant isolates, particularly methicillin-resistant isolates (MRSP), are becoming more prevalent throughout North America, and eight major sequence types have been identified in our collection. Whole genome sequencing (WGS) was performed on one representative from each major type to investigate genetic content that may be responsible for clinical and phenotypic differences, and to detect any mobile genetic elements that may be present. WGS reads from three platforms (454, Illumina, and Ion Torrent) were assembled and compared to Whole Genome Optical Maps. One isolate contained a ~43 kilobase contig unable to be placed into the genomic scaffold with 10 times higher read coverage than the average for its genome. It resolved as a separate genetic element from chromosomal DNA during gel separation and was therefore hypothesized to be an extrachromosomal, linear, high copy number plasmid. Coding sequences (CDS) were predicted using Genemark and automatically annotated using RAST. Of 48 predicted CDS, only five were able to be functionally annotated, while several had unique hits to hypothetical proteins of unknown function in *Escherichia coli*, *Enterococcus faecium,* and *Bacillus subtilis*. Manual annotation was able to resolve eight additional predicted functions, which were used as the basis to test for drug resistance, biofilm formation, and cell adhesion. Additionally, other isolates from the North American collection were screened, and a range of sequence types tested positive for the plasmid using a unique multiplex PCR based on the WGS contig.

28. Comparison of Breastfeeding Knowledge, Attitudes, and Intention between Chinese and U.S. Undergraduates

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The objective of this study was to compare breastfeeding knowledge, attitudes, prior exposure, and intent between Chinese and U.S. undergraduates. This was a cross-sectional study, conducted in 2012 in two large public universities: one in China and one in the United States. A bilingual questionnaire was completed by 595 students (394 in China; 201 in the United States). The samples (China vs. United States, respectively) were similar in terms of gender (% female: 64.3 vs. 66.1), age (% < 20 years: 33.5 vs. 29.6), and major (% health-related: 53.4 vs. 60.0). However, there were significantly more freshmen Chinese students than freshmen U.S. students (62.1% vs. 13.3%, $P = 0.00$). Mean knowledge scores were 9.98 (Chinese) and 10.47 (United States), out of a possible 13. The attitude scale was based on a 5-point Likert Scale. Responses ranged from 1 to 5, with 5 being the most positive. Both groups expressed concerns regarding limited freedom and embarrassment associated with breastfeeding in public (scores ranged from 2.5–2.9). Chinese students were less likely to consider breastfeeding to be cheaper (3.45 vs. 4.31, $P = 0.00$), but more likely to perceive infant enjoyment of breastfeeding (3.9 vs. 3.24, $P = 0.00$) and to believe that breastfed infants grow better than formula-fed infants (4.05 vs. 3.42, $P = 0.00$). Despite modest knowledge levels and a relatively high rate of intent to breastfeed (or support a partner to breastfeed) a future child (~75% in both groups), breastfeeding attitudes were of concern. Though somewhat similar, results highlight some areas of difference that may inform future, targeted interventions.

29. UT Moves: Use of Blackboard Internet Technology to Promote Walking among University Faculty and Staff

Courtney Monroe, Dixie Thompson

Kinesiology, Recreation, and Sport Studies

The purpose of this pilot study was to examine the efficacy of a Blackboard Internet technology intervention grounded in social cognitive theory (SCT) for increasing steps in university faculty and staff. A sample of 36 sedentary/insufficiently active University of Tennessee faculty and staff members (48.8 ± 10.1 y) participated in an 8-week, Internet-delivered walking intervention. Participants received a pedometer, individualized step goals, and access to a Blackboard webpage composed of SCT-based components. Participants reported daily steps online and completed baseline and post-intervention measurements of psychosocial variables. Paired *t* tests were used to compare baseline and post-intervention variables of interest. Average daily step counts for each week (weeks 0–8) were compared using a repeated measures ANOVA. Participants significantly increased their average daily steps between baseline and each week of the intervention ($P < 0.001$). An increase of 1803 ± 240 steps/day ($P < 0.001$) was observed from baseline (5210 ± 232 steps/day) to week 1 (7013 ± 279 steps/day). The magnitude of improvement in average daily steps between baseline and all other weeks was similar, as no significant differences in average daily steps were found among each week of the intervention ($P > 0.05$). Perceived social support and self-regulation significantly improved ($P < 0.05$). The results suggest that a Blackboard Internet technology intervention can significantly increase physical activity by approximately 2,000 steps/day from baseline among sedentary/insufficiently active university faculty and staff. The intervention appeared to result in the improvement of social support and self-regulation. A larger-scale, similarly designed Internet technology intervention is warranted.

30. A Pilot Study of the Efficacy and Program Cost-effectiveness of Prevention Plus for Childhood Obesity

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Department of Nutrition

In 2007, recommendations for the treatment of childhood obesity in the primary care setting, using a staged-approach, were published. Limited research has evaluated the efficacy of these recommendations. Thus, this study tested the efficacy and program cost-effectiveness of Prevention Plus (stage 1), using a tiered approach, for the treatment of childhood overweight and obesity in a primary care setting. Twenty-two overweight/obese children (8.0 ± 1.8 years; 2.34 ± 0.48 z-BMI; 68.2% female, 72.7% white, 90.9% non-Hispanic)

were randomized to one of three, 6-month conditions: 1) newsletter (N); 2) newsletter and growth monitoring (N+GM); 3) newsletter and growth monitoring plus family-based behavior counseling (N+GM+BC). Primary outcomes were z-BMI and program cost-effectiveness based on medical professionals that may deliver the intervention in the primary care setting. Outcomes were analyzed using linear mixed-factor analysis of variance (ANOVA) and one-way ANOVA using the intent-to-treat principle. There was a significant ($P < 0.05$) main effect of time for z-BMI (ΔN+GM+BC: -0.16 ± 0.22, ΔN+GM: -0.08 ± 0.15, ΔN: -0.06 ± 0.24). Cost-effectiveness was significantly ($P < 0.001$) different between conditions. While there was a greater decrease in z-BMI in the N+GM+BC condition, it was the most expensive condition due to high personnel costs. The N condition had the smallest decrease on z-BMI, but since it was the least costly condition due to low personnel costs, it was the most cost-effective. N+GM+BC promoted the greatest change in z-BMI, but personnel costs should be considered during implementation.

31. Influence of a Nutraceutical on Treatment of Obesity in Research Beagles

Maryanne Murphy, Joseph W. Bartges, Claudia A. Kirk, Angela L. Witzel

Comparative and Experimental Medicine (Murphy); Department of Small Animal Clinical Sciences (Murphy, Bartges, Kirk, Witzel)

Obesity is the most common nutritional disorder of pet dogs (prevalence of 23–59%). This 31-week study investigated a nutraceutical containing 1 g leucine and 13 mg pyridoxine designed to maintain lean muscle mass while decreasing adiposity when compared with positive and negative controls. After determining individual maintenance calorie requirements over 4 weeks, 18 healthy adult beagles were fed a canned adult diet (CAD) in calories sufficient to maintain a lean body weight with excess calories supplied by oil and kitten food to induce a body fat of at least 30% over 15 weeks. During a weight loss phase, excess calories were removed, and dogs were followed for 12 weeks on nutraceutical with CAD (N), placebo with CAD (P), or a therapeutic weight loss diet (WLD). During the weight loss phase, ANOVA revealed no significant difference in food intake (kcal/BWkg) between groups. The N group lost a higher percentage of their body weight (21.0% vs. 6.9%, $P = 0.085$) and lost more body fat compared to P (12.1% vs. 2.6%, $P < 0.001$), but did not differ from WLD. Change in lean mass did not differ between N and WLD (11.4% vs. 15.0%), but both lost more lean mass compared to P (2.4%, $P < 0.001$). These data show dogs eating maintenance levels of CAD performed better when receiving N when compared with P. Without changing to WLD or restricting calories below lean maintenance levels, N dogs were able to lose similar amounts of weight and body fat as WLD dogs, while maintaining similar lean mass.

32. Determining the Composition of Lipid Molecules in Lipid Droplets Associated with Obesity

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Lipid droplets are organelles primarily responsible for neutral lipid storage but may have other roles in cellular function. These structures are directly associated with obesity, and the number of lipid droplets per cell is largely dependent on the growth phase. It is hypothesized that the lipid composition of lipid droplets will change in type and abundance across droplets from unique growth stages. In this study, *Schizosaccharomyces pombe* was grown to log and stationary phase, and both yeast cells and lipid droplets purified from stationary phase cells were subjected to membrane lipid analysis. For determining membrane lipid composition, the samples underwent solvent extraction to recover total lipids, which were subsequently fractionated by salicic acid column chromatography into neutral, glycol-, and polar lipid fractions. The focus of this study was on the polar lipids. Polar lipids were subjected to mild alkaline methanolysis, resulting in phospholipid fatty acid methyl esters (FAMEs), which were then separated, quantified, and identified by gas chromatography-mass spectrometry. The yeast cell FAME profiles showed a 1.7 order of magnitude higher lipid concentration in the stationary phase than in the log phase. Despite the difference in concentration, the general proportions of individual FAMEs remained similar. The major FAMEs were 16:0 (12.42%), 18:1 (83.07%), and 18:0 (3.84%), with trace FAMEs at 0.67%. The lipid droplet samples gave similar composition results of 16:0 (4.43%), 18:1 (87.70%), and 18:0 (7.87%). Future experiments will

investigate lipid droplets of varying sizes and from various stages of cellular growth to identify changes in composition.

33. Body Composition of Outdoor, Intact Cats Compared to Indoor, Neutered Cats Using Dual Energy X-ray Absorptiometry

Martha G. Cline, Angela L. Witzel, Tamberlyn D. Moyers, Joseph W. Bartges, Claudia A. Kirk

Department of Small Animal Clinical Sciences

Most domestic house cats differ from feral cats in that house cats are confined indoors and neutered. Indoor, neutered cats have reduced activity and hormonal alterations that may result in lower muscle mass and higher body fat percentages compared to outdoor, intact cats. We hypothesize that adult, outdoor, intact (OI) cats will have a lower body fat percentage and higher lean body mass compared to indoor, neutered (IN) cats with similar body condition scores (BCS) (4/9 or 5/9) using dual energy x-ray absorptiometry (DXA). Twenty-one OI (10 male/11 female) and 16 IN (10 male/6 female) were enrolled. The two groups did not differ by BCS. OI had a lower HCT than IN (mean IN 40.4 ± 5.00%, OI 32.4 ± 5.81%, $P < 0.001$). OI had a lower albumin and creatinine than IN; however, both of these parameters were normal. IN had a greater total body mass than OI (median IN 4.23 kg [IQR = 3.66–4.94 kg], OI 3.32 kg [IQR = 2.89–3.72 kg], $P = 0.004$). IN had a higher body fat percentage (median IN 22.1% [IQR = 19.75–25.4%], OI 17.3% (IQR = 15.32–19.43%], $P = 0.002$) and lower lean body mass percentage than OI (median IN 74.6% [IQR = 71.41–76.95%], OI 79.9% [IQR = 77.16–81.53%], $P < 0.001$). IN had a higher bone mineral content (BMC) percentage than OI (mean IN 3.46 ± 0.37%, OI 3.07 ± 0.30%, $P = 0.001$). Differences in blood work parameters may reflect parasitic disease or poorer nutrition status of OI. Differences in BMC may be due to access to higher quality nutrition in IN. Our findings support our hypothesis that IN have a higher body fat percentage and lower mean lean body mass percentage when compared to OI with similar BCS.

34. Mechanisms of Population Level Variation in Fatness and Leanness

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Genome Science and Technology (Wells, Voy); Department of Animal Science (Spence, Das, Voy)

Obesity represents the extreme of fat deposition. Adipose tissue from a host of obese models has been well-characterized in comparison to normal weight controls. However, much less is known about differences in adipose tissue between leaner and fattier individuals within a population. The objective of this study was to determine if fatness can be attributed to changes in expression of key lipogenic and adipogenic genes, in the context of a heterogeneous genetic background. The C57BL/6J * DBA/2J (BXD) recombinant inbred mouse strain panel was used to select two pools of leaner and fattier individuals, based on percent abdominal fat pad weight. Expression of genes controlling adipogenesis and lipogenesis were profiled using PCR Arrays. Using Pearson's correlation, genes correlated with adiposity were identified, and the Student *t* test was used to identify differentially expressed genes between lean and fatty mice. Thirteen of the 84 genes measured differed significantly between groups, including expected genes such as leptin and Cdkn1a.

35. Challenges to Refugee and Immigrant Well-Being

Chenoa Allen, Clea McNeely

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Much of the research on refugee and immigrant psychosocial adjustment has been guided by three theoretical frameworks, each of which emphasizes different challenges to well-being. The acculturative stress framework emphasizes the stresses of cross-cultural contact. The conservation of resources theory focuses on the loss of social, cultural, and economic resources. The psychological trauma model centers on the psychological effects of traumatic events. We used qualitative data from refugee and immigrant parents and their service providers to describe challenges to well-being and to examine the three models in light of this data. This study uses data from an evaluation of The Robert Wood Johnson Foundation's Caring Across Communities (CAC) program to describe the challenges faced by refugees and immigrants. Interviews were conducted with 75 refugee and immigrant parents, educators, and other service providers in five cities across the United States. Providers and parents described a wide array of challenges in all domains. The data suggest that: (1) all three models describe challenges that are

relevant to newcomers, but no one model is complete by itself; (2) the three domains of challenges vary over time, with acculturation and resources more relevant at first, sometimes later giving way to trauma; and (3) challenges in one domain exacerbate challenges in other domains. These results suggest that more integrative interventions that address challenges in all domains should be more effective at supporting immigrant and refugee adaptation.

36. Survey of Pharmaceutical and Personal Care Products in Effluent Wastewater Treatment Plants in Tennessee

Kimberly Johnson, Fu-Min Menn, Melanie Eldridge

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Pharmaceuticals and other chemical compounds have been detected in ground and surface waters nationwide. In 2012, the Tennessee Department of Environment and Conservation (TDEC) and the Center for Environmental Biotechnology (CEB) at the University of Tennessee began surveying for Pharmaceuticals and Personal Care Products (PPCPs) in the effluents from wastewater treatment facilities among all Tennessee counties. Analyses of this nature had never been previously conducted in Tennessee, and little was known about the concentration of contaminants in the effluents prior to their release into the environment. Therefore, effluent samples were collected in order to further understand their potential impact upon Tennessee streams and waterways. Effluent samples collected from every county in Tennessee were tested for 15 chemicals, including DEET (insect repellant), triclosan (disinfectant), fluoxetine (Prozac), ibuprofen, caffeine, etc. Chemicals were chosen to range from household and industrial cleaners, herbicides, prescription and over the counter drugs, and fecal indicators. An Environmental Protection Agency (EPA)-implemented solid phase extraction (SPE) method was used to concentrate samples 1,000 fold, and samples were analyzed by gas chromatography/ mass spectrometry (GC/MS) for detection of chemicals. An in-house method was developed to detect the 15 compounds in a single GC run, and MS was operated in select ion monitoring (SIM) mode. In addition, four estrogens, including 17α-ethinylestradiol (EE2), a synthetic hormone, were also monitored in the study. The most frequently detected chemicals among ten tested effluents were DEET, diethyl phthalate, bisphenol A (plasticizer), caffeine, carbamazepine (anticonvulsant), and coprostanol (fecal steroid).

37. Differentiation of Equine Mesenchymal Stromal Cells into Neurons and Transdifferentiation into Schwann Cells: Potential for Clinical Applications

Claudia Cruz Villagran, Madhu Dhar

Comparative and Experimental Medicine (Cruz Villagran); Department of Large Animal Clinical Sciences (Cruz Villagran, Dhar)

Peripheral nerve injuries are a cause of decreased musculoskeletal function and performance, and long recovery time in the horse. Spinal cord injuries can result from infectious or traumatic events and are often associated with devastating outcomes. Both conditions are difficult to manage, and clinicians mostly rely upon physical therapy and anti-inflammatories to treat them, but the long-term effects of such treatments are time- and personnel-consuming, and may also be associated with adverse effects. The purpose of these experiments was to demonstrate the capability of equine mesenchymal stromal cells (MSCs) to differentiate into neuronal lineage in vitro, to transdifferentiate into mature Schwann cells, and, finally, to test this potential in clinical cases. Bone marrow-derived MSCs were obtained from three young (1–3 years) and two adult (10–14 years) horses. Passage 2 cells were induced to differentiate into neuronal lineage in 24 well plates. Cells were fixed and morphologically assessed at 3, 6, 12, and 24 h post-differentiation. Transdifferentiation into Schwann cells was then induced, and morphological assessment was done at days 2 and 8. Preliminary results show that a commercially available tissue culture plate, PRIMARIA, supported proliferation and neuronal differentiation. Morphological changes in equine MSCs were observed as early as 3 h after differentiation. Preliminary results suggest that equine MSCs are capable of differentiation into neuronal lineage and, more specifically, into mature Schwann cells. Future experiments using immunofluorescence to detect the presence of nestin. S100B, Krox-20, and CD104 proteins as neuronal and Schwann cell markers are ongoing.

38. Platelet-Rich Plasma Enhances In Vitro Proliferation and Osteogenesis of Equine, Bone Marrow-Derived Mesenchymal Stem Cells within Age- and Gender-Matched Horses

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Previous reports characterize stem cells from younger horses; however, middle-aged horses may be involved in athletic performance and also experience degenerative diseases. Thus, mesenchymal stem cells (MSCs) from this group should be characterized, and methods of enhancing their performance should be investigated. Our main objectives in this study were to describe differences in in vitro proliferation and potential for differentiation of equine, bone marrow-derived, mesenchymal stem cells (eBMMSCs) harvested from middle-aged female donors, and secondly, to enhance in vitro characterization with the addition of platelet rich plasma (PRP) to eBMMSC cultures. Equine BMMSCs from six horses were evaluated for proliferation, differentiation, cluster-of-differentiation markers, and gene expression. An optimal concentration of PRP to promote eBMMSC proliferation was identified. In vitro analysis of eBMMSCs cultured with PRP was evaluated using MTS assay, cell staining, and qPCR. Equine BMMSCs from all six donors varied markedly in all assays. One of six donors demonstrated increased proliferation and a more robust in vitro differentiation. Comparatively, eBMMSCs from two donors demonstrated decreased proliferation and lack of osteogenic and chondrogenic differentiation. The addition of PRP increased proliferation and, very significantly, osteogenesis in all donors. This study confirms our hypothesis that donor-to-donor variation exists and can be documented using in vitro assays. Complete characterization may be relevant to autologous therapies because the molecular character of the cells may determine their effectiveness in vivo. The addition of PRP improves the in vitro characterization of eBMMSCs and could enhance eBMMSC function in vivo.

39. Effects of Environmental Carcinogens on Canine Adipose Tissue-Derived Mesenchymal Stem Cells

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Department of Small Animal Clinical Sciences

The exposure to certain environmental substances plays an important role in the initiation of cancer. Dogs with naturally-occurring tumors offer a unique opportunity to study human cancers given the spontaneous development of the tumors, and their similarities with human cancers in physiological development and in exposure to environmental carcinogens. Mesenchymal stem cells (MSCs) are multipotent cells that can differentiate into a variety of cell types, including osteoblasts, chondrocytes, and adipocytes. In this study, we evaluated the effects of two carcinogens, benzo-a-pyrene (BaP) and bisphenol A (BPA), on adipose tissue-derived MSCs (ADMSCs) isolated from dogs of different ages to assess the effects of these carcinogens on cell proliferation and differentiation. Tested carcinogens did not affect proliferation of canine ADMCS after short-time exposure; however, BaP inhibited differentiation of canine MSCs into adipocytes in a dose-dependent manner. We identified that the aryl hydrocarbon receptor (AhR) signaling pathway negatively regulated ADMSC adipogenesis. In addition, AhR expression was higher in ADMSCs of older dogs compared to ADMSCs from young dogs. AhR in ADMSCs was up-regulated by both tested carcinogens with age independence. In conclusion, our data suggest that ADMSC isolated from young dogs might be more susceptible to carcinogen exposure by decreasing adipogenesis and increasing AhR expression.

40. The Effects of Stem Cells and Platelet-Rich Plasma on Healing of Full-Thickness Cutaneous Wounds on the Distal Limb of Horses

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Departments of Large Animal Clinical Sciences (Caruso III, Schumacher, Cruz, Dhar); Biomedical and Diagnostic Sciences (Donnell); College of Veterinary Medicine (Neilsen)

Slow-healing wounds of the distal aspect of the equine limb are common. Many wounds are slow to heal because they develop exuberant granulation tissue, which adversely affects epithelialization and contraction.

Based on results of studies of wounds of humans and horses, we hypothesized that a combination of mesenchymal stem cells (MSCs) and platelet-rich plasma (PRP) would accelerate the rate of epithelialization and contraction. We intended to evaluate the effects of allogenic MSCs and PRP on healing and production of exuberant granulation tissue. In this pilot study, two full-thickness, circular, cutaneous wounds (4-cm diameter and 2-cm diameter) were created on the distal aspect of each limb of one horse. Wounds of each limb were treated with isotonic saline solution, allogenic MSCs, allogenic PRP, or a combination of MSCs and PRP. Treatments were initiated 2 days after wounding, and a biopsy was obtained from the 2-cm diameter wound on days 7 and 15 for histological analyses. Using a 3-D Wound Measurement System, the 4-cm diameter wound was imaged at each bandage change for 30 days. The images obtained were used to calculate the rates of epithelialization and contraction. Preliminary results showed that wounds injected with a combination of MSCs and PRP produced less exuberant granulation tissue and healed faster than did the other wounds. Based on the results of this pilot study, investigation is warranted to determine if the combination of MSCs and PRP can accelerate wound healing in horses.

41. Evaluation of the Ability of a DACC-impregnated Antimicrobial Wound Dressing to Reduce Bacterial Concentration and Enhance Healing of Equine Wounds

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Departments of Large Animal Clinical Sciences (Boswell, Hines, Schumacher); Biomedical and Diagnostic Sciences (Bemis, Rohrbach)

Wound healing may be hindered by bacterial infection. Dialkyl-carbamoyl-chloride (DACC) is hydrophobic and adsorbs to the outer capsule of pathogenic bacteria. The purpose of this study was to investigate the ability of DACC-impregnated wound dressings to promote healing by reducing the concentration bacteria on wounds. We hypothesized that DACC-impregnated gauze would reduce bacterial concentration and enhance healing. Two full-thickness, standardized cutaneous wounds were created on the metacarpi/tarsi of six horses. The wounds of three limbs of each horse were infected with 5 x 107 colony-forming units of *Staphylococcus aureus*. The wound on three limbs infected were each treated with sterile physiological saline solution, topical triple antibiotic ointment, or DACC-impregnated gauze. The concentration of bacteria in a biopsy of one wound on each limb was quantified by serial dilution of bacterial cultures on days 13, 17, 21, 25, and 29 of the study. Bacterial concentrations of tissue were determined, and wound area and 3-D volume were assessed with a specialized camera. Bacterial counts were made in 10-fold dilutions. A mixed-model ANOVA was used to determine the effect of treatment on bacterial counts and wound measurements. The rate of healing among treatment groups did not differ. The DACC-impregnated gauze tended to reduce the bacterial concentration more than did the other treatments. This reduction was not significant, although it suggests DACC-impregnated gauze may be effective in bacterial removal.

42. Targeting the Robo4/UNC5b Pathway Reduces Corneal Angiogenesis Induced by Herpes Simplex Virus

Fernanda Gimenez, Sachin Mulik, Barry T. Rouse

Comparative and Experimental Medicine (Gimenez); Department of Biomedical and Diagnostic Sciences (Gimenez); Idem (Mulik, Rouse)

The cornea is a complex tissue that must preserve its transparency to maintain optimal vision. Eye infections such as with herpes simplex virus (HSV) can result in a chronic immuno-inflammatory syndrome and pathological neovascularization that impairs vision. One useful therapeutic approach is controlling pathological angiogenesis. Recent reports demonstrate that angiogenic sprouting shows morphological similarities to axon growth. Moreover, there are several key molecules that, upon binding to receptors, regulate the direction of both capillary and axon guidance. In this study, we show that Roundabout 4 (ROBO4) and UNC5β, two endothelial guidance receptors, play a significant role in reducing angiogenesis. We demonstrate that ROBO4 -/- mice are hyper-susceptible to HSV-induced corneal angiogenesis compared to wild type mice. Moreover, providing additional soluble ROBO4 protein by subconjunctival administration significantly diminished the extent of corneal angiogenesis in wild type animals. Finally, by administration of soluble ROBO4 protein to ROBO-/- HSV-infected mice, it was possible to rescue the wild type phenotype. In conclusion, the administration of soluble ROBO4 protein promotes the activation of the ROBO5/UNC5β pathway. This could represent a valuable

therapeutic tool to control corneal angiogenesis related to HSV-induced stromal keratitis.

43. Gut Bacteria Modulates Angiogenesis and Corneal Immunopathology after Herpes Simplex Virus Infection

Raphael Richardson, Sachin Mulik, Sid Bhela, Barry Rouse

Comparative and Experimental Medicine (Richardson); Department of Biomedical and Diagnostic Sciences (Mulik, Bhela, Rouse)

The ocular disease herpetic stromal keratitis results in blindness due to an immunopathological attack mainly orchestrated by T cells and neutrophils. Recently, it has become apparent that lesion severity at local sites may be influenced by the balance of microbes in distal locations such as the gut, respiratory tract, and skin. Reasons appear to involve an influence on both innate and adaptive immunity. Since HSK is an uncommon outcome of ocular HSV infections, we sought to determine if the nature of enteric flora would influence the expression of HSK. Mice were ocularly infected with HSV and separated into two groups. Controls received only sucralose, and the experimental group received sucrolose and a combination of antibiotics (ampicillin, gentamicin, metronidazole, neomycin, and gentamicin) 3 weeks before infection to deplete gut flora. Treatment continued until termination of the experiment at day 15. Significant decreases in corneal angiogenesis and HSK was evident in the antibiotic-treated group. Analysis of inflammation cell numbers and molecules involved in angiogenesis also showed significant differences between treated and control groups. Further mechanistic studies to explain for the differences observed will be reported and discussed.

44. Neuroprotectin D1 Reduces the Severity of Herpes Simplex Virus Induced Corneal Immunopathology

Naveen K. Rajasagi, Pradeep B.J. Reddy, Sachin Mulik, Barry T. Rouse

Department of Biomedical and Diagnostic Sciences

Stromal keratitis (SK) is a chronic immunopathological lesion of the eye caused by herpes simplex virus-1 (HSV) infection and is a common infectious cause of blindness in humans. The inflammatory lesions are primarily caused by neutrophils with the active participation of CD4+ T cells. Consequently, targeting these immune cell types and their products represents a potentially valuable form of therapy to reduce the severity of the disease. Neuroprotectin D1 (NPD1) is an anti-inflammatory and pro-resolving lipid mediator biosynthesized from the omega-3-polyunsaturated fatty acid docosahexaenoic acid (DHA). In the present report, we investigated if NPD1 administration after ocular infection of mice with HSV could control the severity of SK lesions. Topical treatment with pro-drug NPD1 significantly reduced SK lesions and corneal neovascularization when compared with untreated control animals. The mechanisms by which NPD1 acts appear to be multiple. These included reducing the infiltration of neutrophils and pathogenic CD4+ T cells into the cornea, increasing the production of the anti-inflammatory cytokine IL-10, and inhibitory effects on the production of pro-inflammatory cytokines, chemokines, and angiogenic molecules such as IL-6, CXCL1, CXCL10, CCL-20, VEGF-A, MMP-2, and MMP-9, that are involved in SK pathogenesis. Furthermore, NPD1 reduced the expression of microRNA-132, which is involved in pathological angiogenesis. These findings are the first to show that NPD1 treatment could represent a novel approach to control lesion severity in a virus induced immunopathological disease.

45. siRNA Combinations Inhibit Feline Coronavirus Replication and Expression in Cell Culture

Eman Anis, Rebecca Penrose-Wilkes, Stephen Kania, Alfred Legendre, Melissa Kennedy

Comparative and Experimental Medicine (Anis); Departments of Biomedical and Diagnostic Sciences (Anis, Wilkes, Kania, Kennedy); Small Animal Clinical Sciences (Legendre)

Feline infectious peritonitis (FIP) continues to be a significant cause of mortality in cats. Feline coronavirus (FCoV), the agent of FIP, primarily targets intestinal epithelial cells, but in certain cats, virus mutation may occur that allows the virus to replicate efficiently in monocytes and macrophages, resulting in FIP development. Once afflicted, there is no treatment to prevent progression to death. In this study, we evaluated the ability of siRNAs combinations to inhibit FCoV replication and expression in vitro. Different combinations of siRNAs that target different regions

of the FCoV genome were tested for their antiviral effects against two different strains of FCoV. Efficacy of the siRNAs was determined by 1) quantification of the inhibition of intracellular viral genomic RNA using real time RT-PCR, 2) evaluation of the reduction of viral protein expression in infected cells using flow cytometry, and 3) assessment of virus replication inhibition in cell culture via titration of extracellular virus using TCID50 infectivity assay. siRNAs that were tested included combinations that targeted (1) the leader and the 3 UTR, (2) the leader region and the nucleocapsid gene, and (3) the leader and the 3UTR regions as well as the nucleocapsid gene. These combinations resulted in more than 97.9%, 97.3%, and 97.2% reduction in viral replication, respectively, in comparison to siRNA negative control cells, based on virus titration results. These preliminary findings show that FCoV replication can be specifically inhibited using siRNAs combinations targeting various regions of the viral genome, suggesting a potential therapeutic application of siRNA in treating FIP.

46. Temperature Affects Anuran Susceptibility to Ranavirus

Mabre D. Brand, Matthew J. Gray, Rebecca P. Wilkes, Jennifer Tucker, Roberto Brenes, Debra L. Miller

Center for Wildlife Health (Brand, Gray, Tucker, Brenes, Miller); College of Veterinary Medicine (Wilkes, Miller)

Ranaviruses are emerging pathogens that cause morbidity in fish, reptiles, and amphibians. Surveillance studies suggest that winter coincides with a high infection prevalence, but die-offs are often reported during summer or fall. Several factors vary seasonally (e.g., temperature) that could affect susceptibility to ranavirus. There are two competing hypotheses that might drive relationships between host susceptibility to ranavirus and temperature. Ranavirus virulence could be positively correlated with water temperature because virus replication in vitro increases with temperature; thus, warmer temperatures could lead to more outbreaks. Alternatively, water temperature may function as a stressor; thus, larvae of summer breeding species may be more susceptible to ranavirus at colder temperatures, while spring breeding species may be more susceptible to ranavirus at warm temperatures. We tested the relative susceptibility of three amphibian species (*Lithobates sylvaticus, L. clamitans, Ambystoma maculatum*) whose larvae typically develop during winter and spring in North America compared to one species whose larvae develop during summer only (*Hyla chrysoscelis*). Larvae were challenged with ranavirus in a water bath under controlled conditions at two temperatures (10 and 25 °C). After 28 days, susceptibility to ranavirus was lower at 10 °C compared to 25 °C for *L. sylvaticus, L. clamitans*, and *A. maculatum*, whereas susceptibility was greater at 10 °C compared to 25 °C for *H. chrysoscelis*. Additional species will need to be tested to determine if these trends are consistent. However, preliminary results suggest that temperature may act as a stressor; thus, abnormal temperatures during larval development may increase susceptibility to ranavirus and result in an outbreak.

47. At Both High and Low Concentrations of Platelets, Leukocyte-Reduced Platelet-Rich Plasma Reduces Evidence of Inflammation in Equine Tendon Explant Culture

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Platelet-rich plasma (PRP) is used for treatment of musculoskeletal injuries. Systems available for PRP generation result in products with different platelet and leukocyte concentrations. The purpose of this study was to determine the effect of varying platelet concentrations within leukocyte-reduced PRP (lrPRP) preparations for the treatment of tendinopathy. Blood and control bone marrow aspirate (BMA) were obtained from five horses. Three groups of lrPRP were made with equivalent platelet:leukocyte ratios but different platelet concentrations. Explants of superficial flexor tendon were cultured in DMEM, BMA, or lrPRP groups 1–3 for 96 h. PDGF-BB, TNF-α, TGF- β1, and IL-1β concentrations in media were determined at 0 and 96 h. RNA was extracted from tendon explants and qRT-PCR used to determine gene expression of COL1A1, COL3A1, MMP-3, MMP-13, COMP, and IL-1β. Complete blood counts on lrPRP verified leukocyte reduction and platelet enrichment. The mean platelet:leukocyte ratio was 2478:1 and did not vary significantly between groups, while the absolute concentration of platelets varied significantly between groups: lrPRP-1 (mean = 642,800 plt/µl), lrPRP-2 (mean= 275,600 plt/µl), and lrPRP-3 (mean = 96,200 plt/µl). Platelet concentration was positively correlated with growth factor concentration. IL-1β

protein concentration was significantly increased in BMA compared to lrPRP groups at 96 h. Although tendon gene expression was different in lrPRP groups compared to controls, it was not significantly different among lrPRP groups. These findings support the use of lrPRP for the treatment of tendinopathy and suggest that as long as the platelet:leukocyte ratio is high, variable numbers of platelets exert similar biologic effects.

48. Regulation of the CXCL10 Gene by Pro-inflammatory Cytokines Interleukin 1-Beta and Gamma-Interferon in Pancreatic Beta Cells

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The pro-inflammatory cytokines interleukin 1-beta (IL-1beta) and gamma-interferon (gamma-IFN) increase the expression of genes within pancreatic beta-cells, including the chemokine CXCL10. CXCL10 is a chemoattractant for various immune cells (e.g., T-cells, macrophages, etc) and is expressed in pancreatic islets isolated from individuals with Type 1 and Type 2 diabetes mellitus. However, the transcriptional mechanisms regulating the expression of the CXCL10 gene are incompletely understood. Using the 832/13 rat insulinoma cell line, we investigated the regulation of the CXCL10 gene in response to IL-1beta and gamma-IFN. We found that CXCL10 gene expression increased 47-fold by treatment with 1 ng/mL of IL-1beta, an effect potentiated another 8-fold by the addition of gamma-IFN. The gamma-IFN-mediated potentiation of IL-1beta-induced gene expression and promoter activity was decreased by silencing STAT1. Additionally, the Y701 site was inducible by gamma-IFN, and the S727 site was constitutively phosphorylated. We further found that a JAK1 inhibitor dose-dependently reduced phosphorylation of STAT1 at Y701, which in turn diminished gamma-IFN mediated induction of the CXCL10 gene and promoter activity. We thus generated STAT1 single mutant (Y701F and S727A) and double mutant (Y701F/S727A) adenoviruses. Using these molecular approaches, we discovered that overexpressing either the Y701F or the Y701F/S727A decreased the gamma-IFN mediated potentiation of the CXCL10 gene, while the S727A had no effect. We conclude that the gamma-IFN mediated potentiation of IL-1beta-expression of the CXCL10 gene is controlled by phosphorylation of STAT1 at Tyr 701, while Ser727 is dispensible.

49. In Vivo Testosterone Supplementation Decreases Circulating Interleukin Family Isoforms and Differentially Regulates MMPs

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Androgen deficiency (AD) is associated with increased risk of vascular disease, yet the molecular mechanisms remain unclear. Our group has previously demonstrated female sex hormones influence hyperplasia development via inflammatory-modulated matrix metalloproteinase (MMP) regulation. Here, we investigated the effect of testosterone on circulating MMPs and inflammatory cytokines. We hypothesize that AD is acting as a pro-inflammatory modulator contributing to dysfunctional vascular remodeling. Aged orchiectomized rats were implanted with testosterone pellets (TST; 0.5–150 mg). Serum was collected at 0–28 d. TST levels in young, aged intact (AI), and placebo controls were 2.72 ± 0.35, 2.31 ± 0.66, and 0.15 ± 0.07ng/ml per ELISA. Sub-physiological, physiological, and supra-physiological levels were achieved at 14 days with 0.5-, 2.5-, and 35-mg pellets (0.37 ± 0.09, 2.89 ± 0.44, 13.45 ± 0.65ng/ml). Inflammatory cytokine arrays indicated interleukin family isoforms IL-1α, IL-2, IL-6, IL-10, and IL-1R6 were elevated in the absence of TST, while TST supplementation decreased interleukins in a dose-dependent manner, approaching basal young and AI levels. MMP inhibitor TIMP-1 was decreased in AI and placebo vs. young, while TST increased TIMP-1 in a dose-dependent manner. ELISA indicated MMP-9 was significantly decreased in AI vs. young (25.2 ± 2.4, 53.1 ± 8.6 ng/ml). Placebo, sub-physiological, and physiological TST had no significant effect, while supra-physiological levels significantly elevated MMP-9 (72.0 ± 17.9ng/ml) compared to all aged groups. Testosterone downregulates interleukins and differentially affects circulating MMP isoforms in vivo. Future studies will examine the role of AD in inflammatory cytokine and MMP-modulated hyperplasia development in animal models of vascular disease. AD could be playing a role in vascular disease via the regulation of inflammatory signaling cascades.

50. Transmission of Ranavirus between Ectothermic Vertebrate Hosts

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Transmission among hosts can be an essential process to perpetuate the survival of pathogens. Ranaviruses are known to infect different classes of lower vertebrates including amphibians, fishes, and reptiles. Differences in capacity of infection among ectothermic hosts could partly explain the successful annual persistence of ranaviruses in aquatic environments. The goal of this study was to determine the capacity of transmission of a highly virulent FV3-like ranavirus isolate among three commonly sympatric ectothermic classes (Cope's gray treefrog larvae [*Hyla chrysocelys*], mosquito fish [*Gambusia affinis*], and red-eared slider [*Trachemys scripta*]). We housed naïve and infected individuals in containers divided by mesh screening to permit water flow between subjects. Our results showed that infected gray treefrog larvae were capable of transmitting the pathogen to naïve larval conspecifics, turtles, and fish (70%, 30%, and 15%, respectively). Also, infected turtles and fish transmitted the pathogen to 50% and 20% of the naïve gray treefrog larvae, respectively. Although infection of turtles and fish was observed when naïve individuals were housed with infected gray treefrog larvae, no mortality was observed. Our results demonstrate that ranaviruses can be transmitted through water among syntopic ectothermic classes. The capacity of ranavirus to infect multiple ectothermic vertebrate hosts, added to the differences in susceptibility of the tested species, demonstrates that both fish and reptiles could serve as reservoirs for ranavirus. Persistence of ranaviruses in permanent residents of the aquatic environment might sustain its survival when highly susceptible hosts like amphibians are absent as a result of breeding phenology.

51. Ranavirus Susceptibility in Checkered Garter Snakes (*Thamnophis marcianus*)

Jordan Chaney, Matthew Gray, Tom Waltzek, Rebecca Wilkes, Jennifer Tucker, Rebecca Hardman, Debra Miller

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Preliminary evidence supports that ranaviruses can infect a variety of vertebrate classes; however, no studies have tested susceptibility of snakes to the pathogen. Our objective was to test the relative susceptibility of juvenile checkered garter snakes (*Thamnophis marcianus*) to ranavirus via three routes of exposure: intra-muscular (IM) injection, oral (OR) exposure, and transdermal (TD) exposure in a water bath. We designed a controlled experiment in 12-L tubs, fed snakes every 3 days following exposure treatments, and monitored mortality for 28 days. We observed 40% mortality in the TD exposure treatments within the first 8 days, while other treatment groups maintained relatively low mortality rates. Our results indicate that garter snakes are suitable hosts for ranavirus. Future research needs to explore the relative susceptibility of other snake species and determine the role of snakes in the persistence of ranavirus in the environment.

52. Examining Heterologous T Cell Suppression in Plasmodium-Infected Mice

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Department of Microbiology

Malaria is a deadly disease caused by a parasite of the genus *Plasmodium*. This parasite infects approximately 300 million people annually, with 1 million of those infections being lethal. The parasite's complex life cycle enables it to evade the immune system extremely well, making it difficult to develop an efficacious vaccine. The blood stage of the *Plasmodium* life cycle has been demonstrated to be immunosuppressive, which could alter immune responses to other infectious agents or vaccines. At present, it is not known to what extent *Plasmodium* blood stage infections suppress immune responses to heterologous infections. To address this question, we used two rodent-specific *Plasmodium* species, *P. chabaudi* and *P. yoelii* that cause chronic and acute infections, respectively, to determine to what extent they suppress T cell responses to a subsequent *Listeria monocytogenes* infection. We show here that both *P. chabaudi* and *P. yoelii* suppress *L. monocytogenes*-specific T cell responses. Immunosuppression is most

dramatic during the initial 2 weeks of the *Plasmodium* infection. We also demonstrate that immunosuppression is not limited to *Plasmodium* infections. Using the lymphocytic choriomeningitis virus, we show that both a chronic and acute strain induce immunosuppression in a subsequent *Listeria* infection. Therefore, immunosuppression may occur with multiple chronic infections. Given a recent recommendation that vaccines be administered in malaria-endemic areas, even in the presence of clinical malaria, our data suggest this policy may not be beneficial, especially for those that aim to induce T cell responses.

53. MicroRNA-155: Regulator of HSV-1 Encephalitis but Promoter of Stromal Keratitis

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Herpes simplex virus (HSV) infection in humans can lead to life-threatening herpes simplex encephalitis (HSE), and on rare occasions, leads to blinding ocular lesions—stromal keratitis (SK). In this paper, we show that mice with a deficiency of miR-155 are highly susceptible to HSE, with a majority of mice (75–80%) dying after ocular infection with HSV. Acyclovir treatment, provided the day after the virus reaches the brain, reduced brain viral levels and protected miR-155KO mice from HSE, thus supporting the role for virus replication in the brain as the cause of encephalitis. HSV-infected miR-155 deficient mice generated compromised virus-specific CD8 T cell responses in lymphoid organs and in trigeminal ganglia (TG). The defective TG CD8 T cell responses were also associated with increased herpes virus reactivation in miR-155 null mice. Of note, miR-155KO survivors developed attenuated ocular lesions and revealed significant reduction in pathogenic Th1 cells in corneas and lymphoid organs. Local delivery of antagomir-155 did not increase incidence of HSE but led to profound reduction in pro-inflammatory milieu and significantly diminished SK lesions. In conclusion, we have discovered a dual role for miR-155, a regulator of brain damage while a promoter of ocular immunopathology after HSV infection.

54. Does Geographic Distance between Host Population and Isolate Location Equate to Ranavirus Pathogenicity?

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Hosts that coevolve with pathogens presumably have a greater immune response when infected. Ranaviruses are an emerging pathogen and have been associated with die-offs in wood frog (*Lithobates sylvaticus*) populations from Georgia to Alaska, USA. We hypothesized that pathogenicity of a ranavirus would increase as distance between isolate and host population locations increased. We are testing pathogenicity of two FV3-like ranaviruses isolated from morbid amphibians in Minnesota and Tennessee, among four populations of wood frog tadpoles collected from Tennessee; Michigan; Manitoba, Canada; and Alaska. Inasmuch as temperature affects amphibian immune response and viral replication, we are performing our experiments in environmental chambers at 15 and 25 °C. If our predictions hold true, pathogenicity of the Tennessee isolate should decrease in the following order: Alaska, Manitoba, Michigan, and Tennessee populations. Similarly, pathogenicity of the Minnesota isolate should decrease in the following order: Alaska, Tennessee, Manitoba, and Michigan populations. We also anticipate that pathogenicity will be greater in the 25 °C treatment, because rate of ranavirus replication increases with temperature. Additionally, tadpole development increases with temperature, which may compromise immune response to ranavirus. By the time of the symposium, we will have completed the Tennessee experiment. Our results have potential implications in host-pathogen evolutionary theory and conservation relevance regarding the transport of amphibians sub-lethally infected with ranavirus across large geographic distances. Additionally, our temperature results may provide insight into possible effects of global climate change on ranavirus emergence.

55. GABA but not Baclofen Prevents Gemcitabine Resistance Induced by Low Dose Nicotine in Pancreatic Cancer Xenografts

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Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer deaths in developed countries. Our laboratory has shown that proliferation and migration of PDAC and pancreatic duct epithelial cells in vitro is regulated by nicotinic receptor-mediated synthesis and release of stress neurotransmitters that bind to beta-adrenoreceptors (beta-ARs). We have additionally shown that nicotine in drinking water at a high dose (432 µmol/L) comparable to nicotine exposure in heavy smokers significantly stimulated the growth of PDAC xenografts, whereas identical exposure of mice to low-dose nicotine (1 µmol/L) reduced gemcitabine-induced apoptosis, thus significantly increasing resistance to gemcitabine. In the current study, we have investigated the potential prevention of nicotine-induced gemcitabine resistance by gamma-aminobutyric acid (GABA) and Baclofen in PDAC xenografts. We found that GABA significantly reduced nicotine-induced drug resistance. By contrast, Baclofen failed to reduce nicotine-induced resistance to gemcitabine while even slightly increasing xenograft growth in mice not exposed to nicotine. Investigation of xenograft tissues for expression levels of the GABA-B-R, intracellular cAMP, and signaling proteins associated with cell proliferation, apoptosis, and metastasis by immunoassays and Western blots revealed effective inhibition of cAMP-dependent signaling in xenografts of mice treated with GABA. By contrast, Baclofen did not inhibit cAMP-dependent signaling and decreased the protein expression of the GABA-B-R, suggesting downregulation of the receptor. Our findings identify GABA as a promising agent for the prevention of nicotine-induced resistance to gemcitabine in PDAC. On the other hand, our data suggest that treatment of alcohol dependence by Baclofen should be avoided in PDAC patients.

56. Short-Term Direct Electric Current Exposure Increases Caspase-3/7 Activity in Colon Cancer Cells

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Center for Environmental Biotechnology (Large, Fleming, Sayler); Department of Biomedical and Diagnostic Sciences (Baek); Fulton High School (Tolbert)

Recently, electric current has been shown to induce apoptosis in human leukemic and oral mucosa cancer cells. Previously, we demonstrated anti-proliferative and apoptotic effects of direct electric current (DC) exposure in human SW480 colon cancer cells. Here, we investigated the effects of DC exposure on another human colon cancer cell line, HCT116. Our experimental system uses microfabricated platinum electrodes on silica chips in a three-electrode configuration interfaced with an electrochemical potentiostat. Cells were grown on electrode chips using McCoy's media contained within silicon gasket frames. Folded Pt wire functioned as the counter electrode. Cells were exposed for 300 sec (six controls, six tests) to a DC field strength of 1.6–2.3 V/cm with current densities ranging from 0.05–5 µA/cm2. After 24 h the cells were tested for: 1) cell viability (2 x 104 cells/chip) using a tetrazolium/formazan assay, and 2) apoptosis (2 x 104 cells/chip) using a caspase 3/7 assay. We found that caspase activities in HCT116 cells increased directly in proportion to DC exposure, and significant increases occurred at currents below those that adversely affect cell viability (< 600 µA). Interestingly, significant differences in DC effects were observed between the two cell lines: SW480 and HCT116 cells exhibited 5-fold and 3-fold caspase induction peaks at 100 and 800 µA, respectively. SW480 and HCT116 cells began to demonstrate loss of cell viability at 200 and 800 µA, respectively. Compared to SW480 cells, HCT116 tolerate higher currents before exhibiting caspase induction or loss of cell viability.

57. Aberrant Alternative Splicing in Colorectal Cancer is Attenuated by a Conventional Non-steroidal Anti-inflammatory Drug, Sulindac Sulfide

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Alternative splicing of pre-mRNA increases protein diversity from a relatively limited amount of genes by making multiple transcripts from a single pre-mRNA strand. Production of different variants can have various effects on the protein produced, including loss of function, gain of function, expression control, and antagonistic function to another splice variant. Alternative splicing is often observed in tumorigenesis, leading to cancer development. Final product of alternative splicing is determined by several factors,

including, but not limited to, competing splice site recognition by serine-arginine rich (SR) proteins. It has been known that tumor suppressor protein KLF4 (Kruppel-like factor 4) is responsible for cell differentiation in the gastrointestinal tract and pancreas and is under-expressed in cancer, compared to normal tissue. Interestingly, cells produce wildtype KLF4 and several KLF4 spliced forms. During the investigation of SR proteins in KLF4 splicing, we found that SF2/ASF expression results in increased production of the spliced form of KLF4. SF2/ASF attenuates the expression of wild type KLF4 by inducing exon skipping of exon III, the largest exon in KLF4. Non-steroidal anti-inflammatory drugs have been shown to be effective as an anti-cancer agents as well as excellent cancer preventive agents. We also found that sulindac sulfide (SS) can attenuate aberrant splicing of KLF4, thereby producing more wildtype KLF4 genes in cancer cells. This is the first report that SS affects the alternative splicing mechanism, leading to anti-tumorigenic activity of SS. Thus, the discovery of the mechanism behind the effect of SS on alternative splicing in cancer could drive new treatment strategies.

58. Secreted Protein NAG-1 Plays a Role in the Nucleus

Kyung-Won Min, Seung Joon Baek

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Nonsteroidal anti-inflammatory drug (NSAID)-activated gene (NAG-1) is a divergent member of the transforming growth factor-beta (TGF-β) superfamily that consists of key regulators of metazoan embryo development and adult tissue homeostasis. Misregulation of TGF-β signaling can result in tumor development. NAG-1 is up-regulated by several tumor suppressor pathways, but, paradoxically, NAG-1 expression is substantially increased in various tumors, including prostate and glioblastoma brain tumors, leading to poor prognosis and patient survival rates. Therefore, dissecting of the mechanistic bases of NAG-1 is required to understand its dual role in cancer development. Here, we have discovered that NAG-1 is not only secreted as seen in other TGF-β proteins, but also translocated into the nucleus. Nuclear-cytoplasmic shuttling of NAG-1 through the nuclear pore complex (NPC) is energy-dependent. A nuclear export signal (NES) was characterized in the N-terminus of NAG-1, and the deletion of NES resulted in the accumulation of NAG-1 protein in the nucleus. Furthermore, NAG-1 interacts with CRM1, which is an exportin receptor. Treatment of cells with the CRM1-specific export inhibitor shifts NAG-1 from the cytoplasm to the nucleus. In the nucleus, NAG-1 binds to the phosphorylated Smad2 and blocks ubiquitination of Smad2 by E3 ubiquitin ligase Smurf2, resulting in enhanced half-life of TGF-β-activated Smad2. This is the first report indicating that NAG-1 translocates into the nucleus and contributes to the stabilization of Smad2 activity by blocking ubiquitination. NAG-1 seems to play a unique role as modulator of active Smad2 turnover to increase the amplitude and duration of TGF-β signaling.

59. A Novel COX-Independent Mechanism of Sulindac Sulfide Facilitates Cleavage of Epithelial Cell Adhesion Molecule Protein

Jason Lee Liggett, Seung Joon Baek

Comparative and Experimental Medicine (Liggett); Department of Biomedical and Diagnostic Sciences (Liggett, Baek)

Non-steroidal anti-inflammatory drugs (NSAIDs) are extensively used over the counter to treat headaches and inflammation as well as clinically to prevent cancer among high-risk groups. The inhibition of cyclooxygenase (COX) activity by NSAIDs plays a role in their anti-tumorigenic properties. NSAIDs also have COX-independent activity, which is not fully understood. Our lab has previously shown that NSAIDs induce other anti-tumorigenic genes, including NSAID activated gene (NAG-1) and activating transcription factor 3 (ATF3). In this study, we report a novel COX-independent mechanism of sulindac sulfide (SS), a conventional NSAID, which facilitates a previously uncharacterized cleavage of epithelial cell adhesion molecule (EpCAM) protein. EpCAM is a type I transmembrane glycoprotein that has been implemented as an over-expressed oncogene in many cancers including colon, breast, pancreas, and prostate. We found EpCAM to be down-regulated by SS in a manner that is independent of COX activity, transcription, de novo protein synthesis, and proteasomal degradation. Our findings demonstrate that SS drives cleavage of the extracellular portion of EpCAM near the N-terminus. This SS-driven cleavage is blocked by deleting amino acids 55–74 as well as simply mutating

arginine residues at positions 80 and 81 to alanine. Proteolysis of EpCAM by NSAIDs may provide a novel mechanism by which NSAIDs affect anti-tumorigenesis at the post-translational level.

60. FK228 and Cisplatin Synergistically Induce Cell Death and Reduce Clonogenic Resistance in Human Bladder Cancer Cells

Shambhunath Choudhary, Shilpa Sood, Lenora Pluchino, Hwa-Chain R. Wang

Department of Biomedical and Diagnostic Sciences

Human urinary bladder cancer is the fifth most common cancer in the United States, and long-term disease-free survival in patients is still suboptimal with current chemotherapeutic regimens that mainly include cisplatin-based multiagent regimens. Development of effective chemotherapeutic regimens is crucial to decrease the morbidity and mortality of this cancer. The goal of this study was to investigate the effectiveness of FK228 in increasing cisplatin's ability to induce bladder cancer cell death and reduce drug resistance. Our study revealed that FK228 combined with cisplatin synergistically induced cell death and reduced clonogenic survival of human urinary bladder cancer cells. The Erk-Nox pathway played an important role in mediating signals highly increased by this combined treatment to induce significantly-elevated levels of reactive oxygen species (ROS), leading to substantially-induced caspase activation and synergistically-increased death in cancer cells. Cisplatin was able to enhance the ability of FK228 to significantly reduce glutathione, indicating a novel activity of combined FK228 and cisplatin in reducing drug resistance. Hence, combined use of FK228 with cisplatin should be considered in development of therapeutic strategies to control urinary bladder cancer development and recurrence.

61. Acquisition of Carcinogenic Properties in Human Breast Epithelial Cells Induced by Sequential Exposure versus Co-exposure to Environmental Carcinogens

Lenora A. Pluchino, Shambhunath Choudhary, Shilpa Sood, Hwa-Chain Robert Wang

Genome Science and Technology (Pluchino, Wang); Biomedical and Diagnostic Sciences (Choudhary, Sood, Wang)

Breast cancer is the most common type of cancer affecting women in North America and Europe. Over 85% of breast cancers are attributable to long-term exposure to low doses of environmental carcinogens, such as those in tobacco and diet. We have developed a chronic breast cell carcinogenesis model wherein we repeatedly expose non-cancerous, human breast epithelial MCF10A cells to bioachievable concentrations of environmental carcinogens, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), benzo[a]pyrene (B[a]P), and 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), to progressively induce carcinogenesis. Previous studies showed that MCF10A cells exposed to NNK and B[a]P (NB) for 20 cycles increasingly acquired cancer-associated properties, such as reduced dependence on growth factors (RDGF), anchorage-independent growth (AIG), and increased cell proliferation, but not tumorigenicity. Exposure to PhIP for 20 cycles caused MCF10A cells to acquire cancer-associated properties, and also tumorigenicity with metastatic capabilities. In this study, we aim to understand whether there is enhanced carcinogenicity in cells co-exposed to NNK/B[a]P/PhIP (NBP) compared to cells sequentially exposed to NB and PhIP. MCF10A cells were exposed to NBP to create co-exposed cell lines while previously NB-exposed cell lines were exposed to PhIP to generate the sequentially-exposed cell lines. We then analyzed the carcinogenic endpoints of RDGF, AIG, ERK pathway activation, ROS elevation, cellular proliferation, and DNA damage to address differences in acquired carcinogenicity. Our results showed that co-exposed cells were more carcinogenic than their sequentially-exposed counterparts since they had more colonies in RDGF and AIG assays, increased ERK pathway activation, elevated ROS, higher cellular proliferation rates and increased DNA damage.

62. Curcumin in the Intervention of Triclocarban-Induced Human Breast Cell Carcinogenesis

Shilpa Sood, Shambhunath Choudhary, Lenora Pluchino and Hwa-Chain Robert Wang

Comparative and Experimental Medicine (Sood); Department

of Biomedical and Diagnostic Sciences (Sood, Choudhary, Pluchino, Wang)

More than 85% of breast cancers are sporadic and attributable to long-term exposure to environmental carcinogens and co-carcinogens. To identify co-carcinogens with abilities to induce cellular pre-malignancy, we studied the activity of triclocarban (TCC), an antimicrobial agent commonly used in household and personal care products. Here, we demonstrated, for the first time, that chronic exposure to TCC at physiologically-achievable nanomolar concentrations resulted in progressive carcinogenesis of human breast cells from non-cancerous to pre-malignant. Pre-malignant carcinogenesis was measured by increasingly-acquired cancer-associated properties of reduced dependence on growth factors, anchorage-independent growth and increased cell proliferation, without acquisition of cellular tumorigenicity. Long-term TCC exposure induced constitutive activation of the Erk-Nox pathway and increases of reactive oxygen species (ROS) in cells. A single TCC exposure resulted in transient induction of the Erk-Nox pathway, ROS elevation, increased cell proliferation, and DNA damage in not only non-cancerous breast cells but also breast and bladder cancer cells. Using these constitutively- and transiently-induced changes as endpoints, we revealed that non-cytotoxic curcumin, a component of turmeric extracted from the Indian herb *Curcuma longa*, was effective in intervention of TCC-induced cellular pre-malignancy. Our results lead us to suggest that the co-carcinogenic potential of TCC should be seriously considered in epidemiological studies. Using TCC-induced transient and constitutive endpoints as targets will also likely help identify non-cytotoxic preventive agents, such as curcumin, effective in suppressing TCC-induced cellular pre-malignancy.

63. Role of Fibrin Inhibitory Peptides in Cancer Immunotherapy

Joleen Adams, Roni Prater, Keith Prater, Patricia Coan, John Biggerstaff

Department of Biomedical and Diagnostic Sciences (Adams, Coan); Center for Environmental Biotechnology (Biggerstaff, K. Prater, R. Prater)

Specific peptide blockade of the leukocyte and tumor cell binding sites (Mac-1 and CD54) on fibrin restores the immune response, suggesting that these peptides are potential therapeutic agents in immunotherapy. Increased levels of circulating soluble fibrin and tissue fibrin polymer have an immunological dampening effect. Soluble and polymerized fibrin bind to macrophages, inhibiting both lymphokine activated killer (LAK) cell activity and preventing leukocyte tumor adherence, all of which significantly suppress immune function. A mouse tumor model using melanoma cells showed that adding soluble fibrin inhibitory peptides (FIPs) both in vivo and in vitro caused an increased leukocyte infiltration to the tumor site. Tumor growth and metastatic rate were significantly decreased after treatment with FIPs. We then used human melanoma cells in RAG-1 knockout mice and combined use of FIPs in conjunction with LAK cells. These results showed that while peptides alone significantly restored the functioning of the immune system against the tumor, using FIPs with LAK completely inhibited tumor initiation and established tumor growth. We also determined there was an association between fibrin and an increase in angiogenesis. The mechanism of this relationship is unknown, but there is a clear correlation both in vitro and in vivo. In the presence of FIPs, there was a significant reduction in microvessel density within the tumor. Future experiments will be tailored to elucidate the mechanism, to determine whether it is a direct effect by fibrin activating CD-54, indirect through the activation of MAC-1, or if both mechanisms are involved.

64. Lighting up COX-2-Expressed Carcinomas by Fluorocoxib A

Maria Cekanova, Md. Jashim Uddin, Kusum Rathore, Gina Galyon, Joseph W. Bartges, Amanda Callens, Alfred M. Legendre, Lawrence J. Marnett

Department of Small Animal Clinical Sciences (Cekanova, Rathore, Galyon, Bartges, Callens, Legendre); Department of Biochemistry, School of Medicine, Vanderbilt University, Nashville, TN (Uddin, Marnett)

The early detection and appropriate staging of cancer are important factors for successful treatment of patients with cancer. In our study, we evaluated an optical imaging probe, fluorocoxib A to detect naturally-occurring canine cyclooxygenase-2 (COX-2)-expressing carcinomas. Our results from previous studies show that fluorocoxib A is well tolerated by dogs and cats after administration, with no clinically relevant adverse events. We evaluated the specificity of the uptake of fluorocoxib A using canine xenograft cancer in athymic nude mice in vivo and dogs and cats with naturally-occurring tumors. The primary K9TCC, with confirmed COX-2 expression by immunocytochemistry and Western blotting, were

subcutaneously implanted in athymic mice to evaluate the specificity of fluorocoxib A in vivo. Fluorocoxib A selectively bound to COX-2-expressing K9TCC xenograft tumors in vivo. Fluorocoxib A uptake by these xenograft tumors was blocked by pre-treatment with the COX-2 selective inhibitor celecoxib (intravenous injection [i.v.], 2 mg/kg) 4 h before i.v. administration of fluorocoxib A (1 mg/kg). The UMUC-3 xenograft tumors that lack expression of COX-2 showed no uptake of fluorocoxib A, confirming in vivo specificity of fluorocoxib A. COX-2 and E-cadherin expression was determined in dissected K9TCC xenograft tumors using immunohistochemistry. We evaluated the specificity of fluorocoxib A to selectively bind to COX-2-expressed bladder and oral squamous cell carcinomas in dogs using a scoping system in vivo. IHC and WB analysis confirmed COX-2 expression in tested carcinoma biopsies. Spontaneous cancer in companion dogs offers a unique model for human cancer biology and helps to validate novel cancer therapeutics and imaging probes.

65. Characterization of Four New Canine Transitional Cell Carcinoma Cell Lines

Kusum Rathore, Maria Cekanova

Department of Small Animal Clinical Sciences

Development of various in vitro and in vivo models of cancers is important for therapy development. Canine bladder cancer closely resembles human cancer in many aspects; thus, cell lines derived from urinary bladder of dogs can be further used to study transitional cell carcinomas in humans. Here, we characterized four canine primary transitional cell carcinoma (K9TCC) cell lines. Four K9TCC cell lines were established from naturally-occurring canine bladder cancer biopsies. Cells were characterized using doubling time, immunocytochemistry (ICC), and Western blotting (WB). Four established K9TCC cell lines: K9TCC#1Lillie, K9TCC#2Dakota, K9TCC#4Molly, and K9TCC#5Lilly were confirmed to have the epithelial-cell origin by morphology analysis, and cytokeratin and E-cadherin expression. All tested cell lines expressed cancer-related markers, such as COX-2, PDGFR, and EGFR, and lacked the expression of VEGFR and p53 detected by ICC and WB. Two representative cell lines, K9TCC#1Lillie and K9TCC#2Dakota, were found to be tumorigenic in athymic mice with 100% tumor incidence. The expressions of the above-mentioned markers in K9TCC cells were compared with already characterized and established human bladder carcinoma cell lines, T24 and UMUC-3 by WB. These new established primary K9TCC cell lines (K9TCC#1Lillie, K9TCC#2Dakota, K9TCC#4Molly, and K9TCC#5Lilly) can be further used to study various molecular pathways involved in bladder cancer and assist in development of new therapeutic approaches for canine and human bladder cancers.

66. Kinetic and Kinematic Analysis of Hind Limb Joints Following Immobilization of the Tarsus

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The aim of this study was to determine pelvic limb kinetic and kinematic characteristics after partial tarsal immobilization. We hypothesized that stifle and hip flexion would increase and peak vertical force (PVF) would decrease. This randomized prospective study evaluated five clinically sound orthopedically normal dogs. Kinetic and kinematic gait analyses were performed on splinted and contralateral pelvic limbs of each dog at a walk and trot before and after placing a commercially available tarsal splint. Statistical analysis was performed using a paired *t* test. Tarsal flexion (FLX) and range of motion (ROM) of the splinted limb were significantly decreased at both the walk and trot ($P < 0.05$). Significantly greater tarsal ROM of the contralateral limb occurred at a walk and trot compared to the splinted limb (ROM $P = 0.01$ and 0.003, respectively). Splinted limb stifle FLX and ROM increased while walking and trotting ($P = 0.05$). Splinted limbs demonstrated significantly more stifle FLX at a trot when compared to contralateral limbs ($P = 0.0004$). Coxofemoral EXT, FLX ,and ROM were not affected after splinting, but ROM of the splinted limb was greater than the contralateral limb at a walk. There were NSD in PVF at a trot before and after splint application. Splinting reduced tarsal FLX and ROM. Limiting tarsal motion resulted in compensatory changes in stifle motion and contralateral tarsal ROM.

67. Sensitivity and Specificity of Tissue Impedance Determination for Correct Needle Placement in the Coxofemoral Joints of Cadaveric Dogs

Rory Applegate, Jacqueline C. Whittemore, Joseph Weigel

Department of Small Animal Clinical Sciences

Access to the coxofemoral joint for arthrocentesis, arthroscopy, and intra-articular injections in people and dogs is challenging; currently used tests of needle tip location have low diagnostic accuracy. A recently developed tissue impedance measurement interpretation (TIMI) device has been shown to accurately identify peritoneal placements of Veress needles during laparoscopic procedures. The purpose of this study was to determine the diagnostic accuracy of TIMI for spinal needle placements in the coxofemoral joints of cadaveric dogs. A surgeon blinded to TIMI placed 20g 2.5" spinal needles in the right and left coxofemoral joints of 20 cadaveric dogs using a dorsolateral approach. An independent observer evaluated impedance measurements to evaluate placement (appropriate vs inappropriate). Placement location was determined by aspiration of joint fluid. If joint fluid was not aspirated, needle locations were marked using India ink and tissues dissected to determine ink locations. One trial was excluded due to a technical error. Impedance measurement interpretation identified 32/37 correct and 0/2 incorrect placements. Sensitivity and specificity of TIMI for correct needle placement in the coxofemoral joint of dogs were 86% and 0%, respectively. Based on results of this study, TIMI had unacceptable diagnostic accuracy for needle placement in the coxofemoral joint, primarily due to poor specificity. This may be due to the intimate anatomy of the joint and the uniformity of the overlying muscular tissues, providing little opportunity to detect differences in tissue impedance. Due to differences in patient size, caution is warranted when extrapolating results from people to animals.

68. Biometry, Keratometry, and Calculation of Intraocular Lens Power for the Bald Eagle (*Haliaeetus leucocephalus*)

Sonia E. Kuhn, Diane V.H. Hendrix, Daniel A. Ward, Michael P. Jones, Katherine H. Baine, Stephen R. Franklin

Department of Small Animal Clinical Sciences (Kuhn, Hendrix, Ward, Jones, Baine); Center for Sight, PC (Franklin)

Our objective was to document intraocular measurements and predict intraocular lens (IOL) power specific to the bald eagle. Eighteen adult captive bald eagles were studied. Axial globe length (AGL), anterior chamber depth (ACD), anterior-posterior lens thickness (APLT), and the distance from the cornea to the posterior lens capsule (CPLC) were measured in 15 adult bald eagles using B-mode with vector A-mode ultrasound. Keratometry was done on four eagles. Two estimates for post-operative ACD were obtained from aphakic eyes from three eagles by measuring from the apex of the anterior cornea to the anterior lens capsule (poACD1) and from the apex of the anterior cornea to halfway between the anterior and posterior lens capsule (poACD2). IOL strength was predicted using the Colenbrander, Binkhorst, and Fyodorov theoretical formulas. Mean ± SD biometry for phakic eyes was AGL = 26.53 ± 0.53 mm, ACD = 4.40 ± 0.22 mm, APLT = 5.57 ± 0.24 mm, and CPLC = 10.02 ± 0.29 mm. Mean predicted poACD1 was 6.1 ± 0.66 mm and poACD2 was 6.4 ± 0.70 mm. Mean horizontal and vertical corneal refractive power was 39.91 ± 0.43 diopters (D) and 40.02 ± 0.08 D, respectively. Calculated IOL power ranged from 16.3–17.1 D. Calculations using ultrasonographic biometry, keratometry, and theoretical IOL formulas suggest that the strength of an IOL necessary to return an aphakic bald eagle to emmetropia is between 16.3–17.1 D.

69. Pharmacokinetics of Cefovecin (Convenia) in White Bamboo Sharks (*Chiloscyllium plagiosum*) and Atlantic Horseshoe Crabs (*Limulus polyphemus*)

James Steeil, Juergen Schumacher, Robert H. George, Frank Bulman, Katherine Baine, Sherry Cox

Department of Small Animal Clinical Sciences (Steeil, Schumacher, Baine); Ripley's Aquariums, Myrtle Beach, SC, and Gatlinburg, TN (George, Bulman); Department of Biomedical and Diagnostic Sciences (Cox)

Cefovecin (Convenia) is a third-generation, long acting injectable cephalosporin labeled for use in domestic dogs with pyoderma caused by *Staphylococcus intermedius* and *Streptococcus canis,* and in domestic cats with wounds/abscesses caused by *Pasteurella multocida*. As a third-generation cephalosporin, it is expected to have an even greater activity against gram-negative bacteria. At present, no pharmacokinetic data for

cefovecin in elasmobranchs and aquatic invertebrates are available. In this pharmacokinetic study, cefovecin was administered to six healthy, adult white bamboo sharks (*Chiloscyllium plagiosum*), and six healthy, adult Atlantic horseshoe crabs (*Limulus polyphemus*). A dose of 8 mg/kg was administered subcutaneously in the epaxial region of the bamboo sharks and the lateral leg region of the horseshoe crabs. Based on results of a pilot study performed on both species, blood samples were collected at 0, 15, 30, and 60 min and at 1, 2, 3, 4, 5, 6, and 7 days for bamboo sharks, and for horseshoe crabs at 0, 15, 30, and 60 min, and at 1, 6, 9, 12, 14, and 18 days. High performance liquid chromatography was performed on plasma samples to determine levels of cefovecin. Cefovecin concentrations were detected for 4 days in white bamboo sharks and for 14 days in Atlantic horseshoe crabs. No adverse effects associated with cefovecin administration were seen in bamboo sharks or horseshoe crabs. Further studies need to be performed to evaluate drug safety and efficacy on aquatic animal pathogens.

70. Pharmacokinetics of Gabapentin in Hispaniolan Amazon Parrots (*Amazona ventralis*)

Katherine Baine, Michael P. Jones, Sherry Cox, Tomás Martín-Jiménez

Departments of Small Animal Clinical Sciences (Baine, Jones); Biomedical and Diagnostic Sciences (Cox, Martín-Jiménez)

Neuropathic pain is a manifestation of chronic pain that arises with damage to the somatosensory system. In avian species, it has been associated with traumatic and self-induced injuries, and in poultry, specifically, with beak trimming. The few reports documenting treatment of suspected neuropathic pain in avian patients describe the use of gabapentin as part of the therapeutic regimen. Although the mechanism of action is not completely understood, the drug likely decreases the release of excitatory neurotransmitters by binding to the alpha 2-delta subunit of voltage-gated calcium channels. Multiple reports have been published evaluating the pharmacokinetics of gabapentin in veterinary species; however, to date there have been no published pharmacokinetic studies in avian patients. In this study, compounded gabapentin suspensions were administered intravenously (IV) at 30 mg/kg to two birds, orally at 10 mg/kg to three birds, and orally at 30 mg/kg to three birds. Blood was collected prior to drug administration and at 5, 15, and 30 min and 1.5, 3, 6, 9, 12, and 24 h after. Plasma samples were analyzed for gabapentin concentration, and pharmacokinetic parameters were calculated. Mild sedation was noted following IV injection in both study birds. The data showed dose proportionality for oral administration and a high systemic bioavailability. The half-life of the drug was approximately 1.55 and 4.5 h after IV and oral administration, respectively. We hypothesize that analgesic plasma concentrations documented in humans can be maintained in Hispaniolan Amazon parrots; however, additional studies need to be performed.

71. The Effect of Propofol on Sevoflurane Minimum Alveolar Concentration Preventing Motor Movement (MACNM) in Dogs

Jill Singsank, Reza Seddighi, Barton W Rohrbach, Sherry Cox, Thomas Doherty, Christine Egger

Departments of Small Animal Clinical Sciences (Singsank, Egger); Large Animal Clinical Sciences (Seddighi, Doherty); Biomedical and Diagnostic Sciences (Rohrbach, Cox)

The aim of the study was to determine the effects of propofol on the minimum alveolar concentration of sevoflurane preventing motor movement (MACNM) in dogs. Six adult beagles (9.20 ± 1.30 kg) were anesthetized with sevoflurane in a crossover design on three occasions at weekly intervals. Baseline MACNM (MACNM-B) was determined on each occasion. The MACNM was defined as the minimum alveolar sevoflurane concentration preventing motor movement in response to noxious stimulation (50V, 50Hz for 10 ms). Propofol treatments were administered as a loading dose (Ld) and constant rate infusion (CRI) as follows: T1- Ld of 2 mg kg-1 and CRI of 4.5 mg kg-1 h-1; T2- Ld of 4 mg kg-1 and CRI of 9 mg kg-1 h-1; T3- Ld of 8 mg kg-1 and CRI of 18 mg kg-1 h-1. Treatment MACNM determination was initiated 60 min after the start of the CRI. A mixed-model ANOVA was used to determine the effect of each treatment on percent decrease in MACNM-B. The data are expressed as LSM ± SEM. All treatments significantly decreased MACNM-B. The percentage decrease in MACNM-B was 20%, 34%, and 68%, for T1, T2 and T3, respectively. There was a strong relationship (r^2 = 0.855) for the correlation of blood propofol concentrations with the decrease in MACNM. Propofol infusion rates in the range of 4.5–18 mg kg-1 h-1 resulted in blood propofol concentrations of approximately 1-6 µg mL-1 and caused

a concentration-dependent decrease ($P \leq 0.05$) in the sevoflurane MACNM.

72. Correlation and Repeatability of Glucose and D-Xylose Intestinal Absorption Tests in Normal Horses

Claudia Cruz Villagrán, Karen Kalck, Sarah Elliott, Nicholas Frank, Kelly Chameroy, Amy Schuver

Comparative and Experimental Medicine (Cruz); Departments of Large Animal Clinical Sciences (Cruz, Kalck); Biomedical and Diagnostic Sciences (Elliott); Clinical Sciences Department, Tufts Cummings School of Veterinary Medicine, North Grafton, MA (Frank)

Inflammatory bowel disease is a cause of weight loss, decreased performance, and colic in horses. This condition is difficult to diagnose, and clinicians rely upon absorption tests to document malabsorption. The purpose of this study was to compare glucose and xylose absorption tests in normal horses and determine their repeatability. Eight horses received 500 mg/kg dextrose or D-xylose powder mixed as a 10% solution in water or water alone via nasogastric intubation on three different occasions within the same week, for 3 consecutive weeks (nine tests/horse). A crossover design was employed, and the order of treatments was randomized. Blood samples were collected at time 0, 30, 60, 90, 120, 150, and 180 min. Data were analyzed by repeated measures ANOVA and *t* tests. Results showed that the xylose response over time differed significantly from the glucose response over time (test × time; $P < 0.001$). Mean time to maximum concentration differed ($P < 0.001$) between tests (glucose 90 min; xylose 60 min). Within-horse area under the curve, maximum concentration, and time to maximum concentration values for dextrose and xylose did not differ significantly when tests were repeated. Results indicate that glucose and xylose absorption tests are repeatable within the same horse, but plotted curves differ between tests, with peak concentrations occurring at a later time point for the glucose absorption test. We conclude that both tests provide repeatable measures of intestinal absorption, but glucose and xylose appear to differ in their rates of absorption and clearance.

73. Effects of Butorphanol versus Dexmedetomidine Sedation on Intradermal Allergy Test Results in Dogs with Atopic Dermatitis

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Sedation is commonly used to restrain patients during intradermal testing (IDT). Morphine and its derivatives have been avoided because of their histamine-releasing effects. Opioid agonists/antagonists, such as butorphanol, may not stimulate histamine release like pure opioid agonists. Ten dogs were randomly sedated with butorphanol (Torbugesic, Fort Dodge Animal Health, New York, NY) (0.4 mg/kg) or dexmedetomidine (Dexdomitor, Pfizer Animal Health, New York, NY) (5–10 µg/kg). Routine IDT along with intradermal injections of various dilutions of histamine were performed on the lateral thorax, followed 7 days later by the alternative sedative and IDT on the opposite side. Injection sites were subjectively scored and objectively measured by one investigator, blinded to the sedative used, and compared between groups, with a value of $P \leq 0.05$ considered significant. When the mean wheal diameters from the objective measurements of all antigens, including saline and histamine dilutions, were compared, butorphanol was associated with significantly smaller reactions than dexmedetomidine ($P = 0.0003$). There was no difference between sedative types when positive reactions subjectively scored as ≥ 3+ were compared ($P = 0.334$). When mean wheal histamine diameters at concentrations of 1:100,000, 1:400,000, and 1:6,400,000 were compared, there were no significant differences between sedative groups; however, at 1:1,600,000, butorphanol was associated with significantly smaller reactions than dexmedetomidine ($P = 0.02$). The subjective interpretations of histamine dilutions between sedation groups were not significantly different ($P = 0.68$). There was only 68% agreement beyond chance between objective and subjective scores. In summary, butorphanol resulted in significantly smaller wheal size as compared to dexmedetomidine but did not affect overall subjective interpretation.

74. Use of the T2*-Weighted GRE Sequence for MRI Examination of the Canine and Feline Brain

Amy Hodshon, Silke Hecht, William Thomas

Department of Small Animal Clinical Sciences

T2*-weighted magnetic resonance imaging is a sensitive means to detect intracranial hemorrhage and is widely used in human neuroimaging. To assess its utility in small animals, results of this sequence were compared to those of paired T2-weighted and FLAIR sequences from 200 dogs and cats that underwent brain magnetic resonance imaging (MRI) for suspected intracranial disease. The two sets of images (T2 + FLAIR and T2*) were reviewed separately in random order unaccompanied by patient information and were evaluated as normal or abnormal based on whether intracranial abnormalities were seen. The number and location of intracranial lesions were recorded. Eighty-five studies were considered normal, and 88 were considered abnormal based on both sets of images, with good agreement ($\kappa = 0.731$) between the two. Susceptibility artifact was present in 33 cases (16.5%) on T2*-weighted images. In 12 cases (6%), a total of 69 lesions were seen on T2*-weighted images that were not seen on T2/FLAIR, all of which were associated with susceptibility artifact. Pseudolesions were seen on T2*-weighted images in five cases, none of which were associated with susceptibility artifact. Abnormalities were seen on T2/FLAIR images that were not seen on T2*-weighted images in 39 cases, confirming that T2* does not replace standard spin echo sequences. These results demonstrate that T2*-weighted imaging is a useful sequence in small animal brain MRI and that a large number of abnormalities (especially hemorrhagic lesions) can go undetected if it is not performed.

75. Magnetic Resonance Imaging (MRI) of the Liver in Normal Dogs Using the Specific Contrast Agent Eovist

Alyce Marks, Silke Hecht, Jennifer Stokes, Gordon Conklin, Katherine DeAnna

Department of Small Animal Clinical Sciences

The liver in humans and animals is frequently affected by a variety of diseases requiring the use of different imaging modalities for diagnosis. Gadoxetate disodium (Gd-EOB-DTPA; Eovist) is a newly developed paramagnetic contrast agent with high specificity for the hepatobiliary system. The purpose of this study was to develop a protocol for the use of Eovist in dogs and characterize normal liver MR images before and after administration of Eovist in healthy dogs. Abdominal MRI was performed on eight healthy, mature dogs. Sequences evaluated in each patient included pre-contrast dorsal and transverse T1-weighted spin echo (T1-W SE) and T2-weighted fast spin echo (T2-W FSE), and transverse T1-weighted 3D gradient echo (VIBE; volume-interpolated body examination). The dogs were divided into two groups based on the contrast dose administered (0.0125 mmol/kg or 0.025 mmol/kg) and sequences performed after contrast administration (T1-W SE and VIBE). Signal intensity ratios (SIR) of post-contrast T1-W SE and VIBE, and SIR ratios between the two dose groups were evaluated using the Student *t* test. A P value < 0.05 was considered significant. Contrast enhancement peaked between 1 and 10 min and plateaued for the remainder of the examination. Post-contrast SIR was significantly higher for VIBE than T1-W SE images ($P = 0.027$). There was no significant difference in degree of enhancement between the contrast dose groups ($P = 0.603$). Administration of Eovist produces homogenous contrast enhancement of the liver in normal dogs with early peak enhancement.

76. Hormonal Regulation of Lysyl Oxidase in Vascular Remodeling

Jason R. Chapman, Deidra J.H. Mountain, Stacy S. Kirkpatrick, Scott L. Stevens, Josh D. Arnold, Mitchell H. Goldman, Michael B. Freeman, Oscar H Grandas

Department of Surgery, Graduate School of Medicine

Lysyl oxidase (LOX) initiates the covalent cross-linking of elastin and collagen in wound healing and has been shown to increase in vascular lesions post-intervention. We have previously demonstrated dysfunctional matrix metalloproteinase regulation in hormone replacement therapy (HRT)-modulated intimal hyperplasia (IH). Likewise, we hypothesize that HRT plays a role in LOX-mediated IH. Here we investigated the effect of estrogen and progesterone on LOX expression in vitro and in vivo. Human vascular smooth muscle cells (VSMCs) were treated with estrogen (Est; 0-500nM) for 24h. LOX expression was measured by qPCR normalized to 18S. Aged ovariectomized (OVX) female rats were implanted with slow-release Est (0.72 mg), progesterone (Prog; 200 mg), combination (EP), or placebo (Plac) pellets and 6 weeks later underwent carotid artery balloon angioplasty. Vessels were stained 14 days post-injury with trichrome-elastin and LOX-specific antibodies. Intima:media (I:M) ratios are used to quantify degree of hyperplasia. Data are reported as mean ± SEM, n=4-7. I:M decreased in OVX rats receiving Plac (0.925 ± 0.046, $P < 0.05$) vs. non-OVX controls (1.345 ± 0.074). I:M slightly increased in OVX animals receiving Est (1.022 ± 0.077) and EP (1.066 ± 0.104) vs. Plac. While neither Est or Prog alone had an effect on LOX expression in vitro or in vivo, EP combination therapy significantly increased LOX expression in vivo (% intima area stained: non-OVX control, 42.25 ± 6.83; OVX – EP, 78.20 ± 6.05; $P < 0.05$). HRT increased I:M ratios, though significance was not reached with the doses given. While LOX expression may be playing a role in EP-mediated IH, other mechanisms should be investigated to delineate the role of HRT in IH development.

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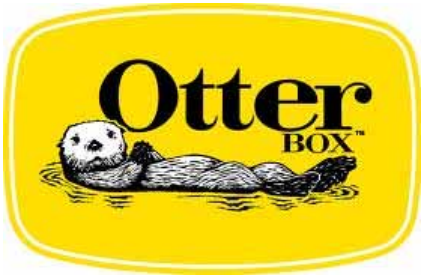
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