Comparative & Experimental Medicine and Public Health Research Symposium

May 18 & 19, 2015

9th Annual

Program & Schedule

UTCVM INSTITUTE OF AGRICULTURE
THE UNIVERSITY OF TENNESSEE

THE UNIVERSITY OF TENNESSEE
KNOXVILLE
DEPARTMENT OF PUBLIC HEALTH

Sponsored by Tennessee AgResearch, the UTK Office of Research & Engagement, and the UT Graduate School of Medicine

Welcome

For the ninth consecutive year, the University of Tennessee (UT) Institute of Agriculture is hosting a symposium for UT investigators with animal and human health interests. As the symposium has grown, it has become a calendar event for the Knoxville campuses of UT. The intercollegiate Comparative and Experimental Medicine (CEM) graduate program 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 seventh year, the Department of Public Health has teamed with CEM to produce a joint Comparative & Experimental Medicine and Public Health Research Symposium hosting a large group of scientists.

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 networking and collaboration that will enhance health-related research at the UT Knoxville campuses. The joint sponsorship of the symposium by the College of Veterinary Medicine, the Department of Public Health, Tennessee AgResearch, 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

Table of Contents

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

College of Veterinary Medicine

Department of Public Health

Tennessee AgResearch

UT Office of Research & Engagement

UT Graduate School of Medicine

Misty Bailey
Kristen Bass
Michael Cunningham
Paul Campbell Erwin
Stephen Kania
Michael McEntee
Kim Rutherford
Phil Snow

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**, 2015 Center of Excellence/Merial Summer Student Research Program participants, and our sponsors and exhibitors.

Jimmy Cheek, *Chancellor*
UT Knoxville

Larry Arrington, *Chancellor*
UT Institute of Agriculture

James Thompson, *Dean*
College of Veterinary Medicine

James Neutens, *Dean*
UT Graduate School of Medicine

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William F. Brown, *Dean*
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Robert Rider, *Dean*
College of Education, Health & Human Sciences

Carolyn Hodges, *Dean*
UT Graduate School

Schedule at a Glance

Monday, May 18

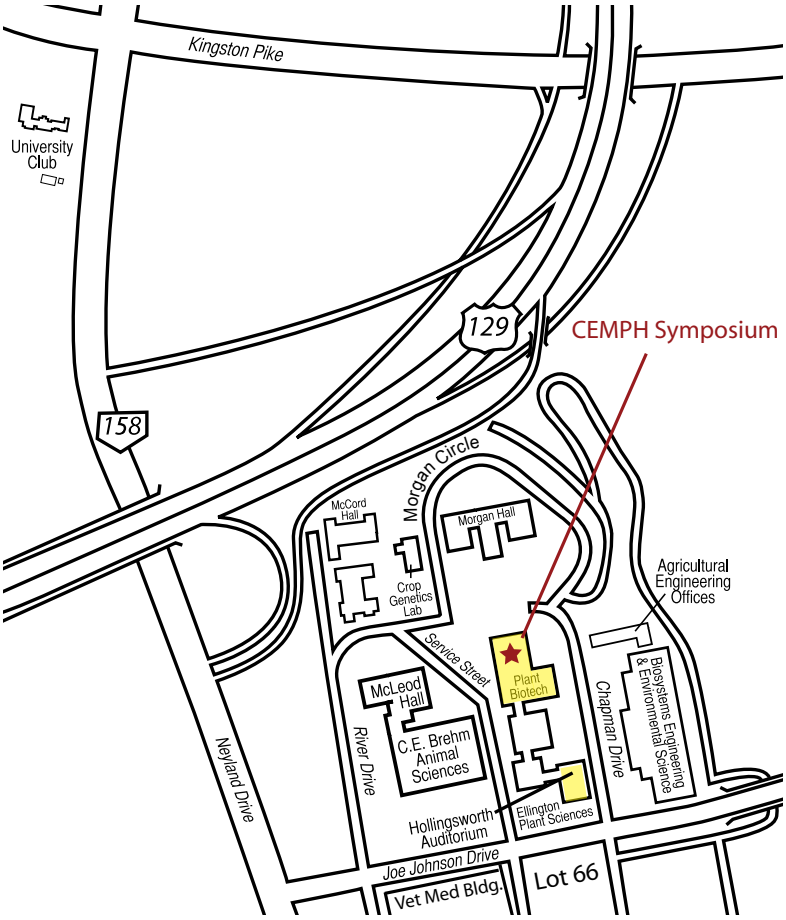
	Room	Event
8:30-9:00	PBB*	Morning refreshments
9:00-10:00	PBB* 156/157	*Keynote address:* **Carolyn J. Henry,** DVM, MS, DACVIM, "One Health: Clear Vision and Blurred Lines"
10:30-12:00	See session matrix (p. 6)	New investigator presentations
12:00-12:30	E.J. Chapman Drive	Patronize food trucks for lunch
12:30-1:30	PBB 160	*Plenary address:* **James Mazzouccolo**, MA, "Introduction to SciVal Funding"
1:45-3:30	See session matrix (p. 7)	New investigator presentations

Tuesday, May 19

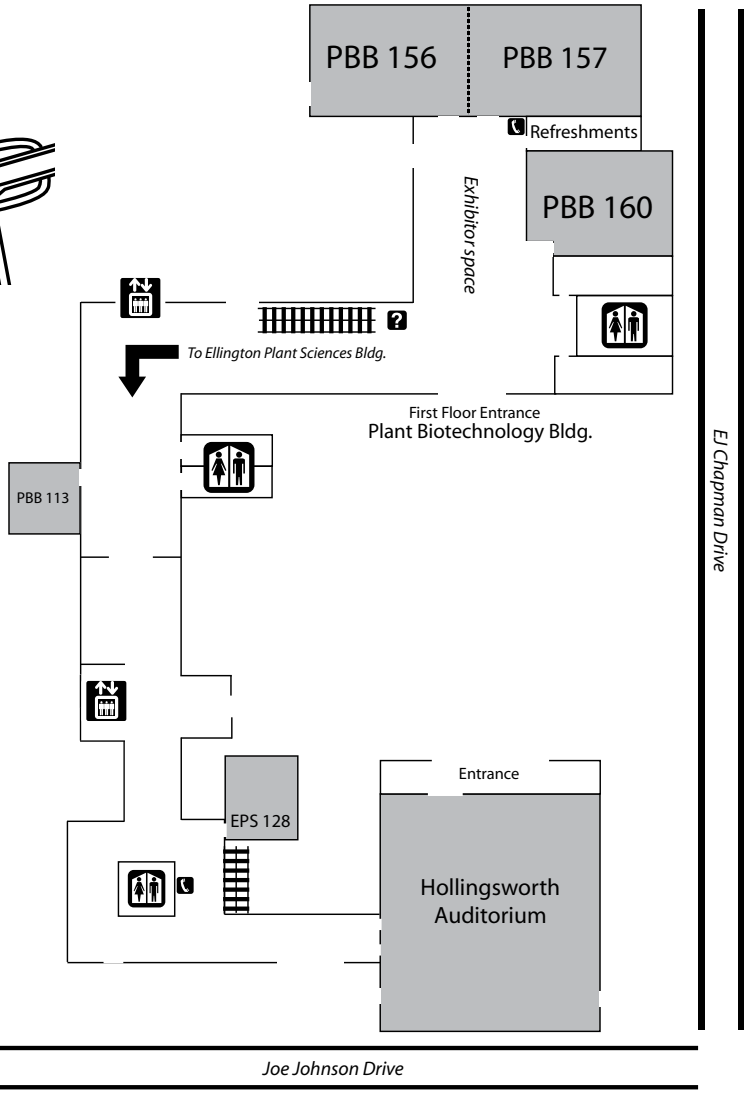
	Room	Event
8:30-9:00	PBB*	Morning refreshments
9:00-10:00	PBB* 156/157	*Keynote address:* **Dale S. Bond,** PhD, "Are We All Sitting Ducks? The Health Risks of Sedentary Behavior and Strategies to Increase Participation in the Stand Up Movement"
10:30-11:45	See session matrix (p. 8)	New investigator presentations
11:45-1:15	E.J. Chapman Drive	Patronize food trucks for lunch and network
1:15-3:15	See session matrix (p. 9)	New investigator presentations
5:30	Hollingsworth Auditorium	Awards Reception–heavy hors d'oeuvres

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

Location Information



Plant Biotechnology Building



University of Tennessee Institute of Agriculture

Symposium Parking

A valid student, faculty, or staff parking permit is required to park in university lots. Faculty/staff with a valid hang tag may park in the UTIA Campus student lots CFN1 and C4. 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

(Abstracts on pp. 15-33)

Monday, May 18

9:00	Keynote address: **Carolyn J. Henry,** DVM, MS, DACVIM "*One Health: Clear Vision and Blurred Lines*" PBB* 156/157	
	Infectious Diseases	**Clinical Sciences**
	Rm. PBB* 160	Rm. PBB* 156/157
10:30	1. Genome-wide association study identifies loci associated with mastitis phenotypes following experimental challenge with *Streptococcus uberis* (**Siebert**)	15. Prospective, randomized blinded pilot study of the effect of oral glutamine supplementation on the severity of radiation-induced oral mucositis (**Chetney**)
10:45	2. *Streptococcus uberis* vaccine: Current and future directions (**Kerro-Dego**)	16. Magnetic resonance imaging findings of lymphoma affecting the canine spine and paraspinal soft tissues (**Allett**)
11:00	3. Detection and identification of horizontally transferred genetic elements in Staphylococci: Implications for drug resistance and disinfectant use in veterinary medicine (**Riley**)	17. Analysis of variables associated with diabetic foot ulcer healing (**Jagadish**)
11:15	4. Role of retinoic acid in herpes stromal keratitis (**Jaggi**)	18. Expression of cyclooxygenase enzymes in normal and inflammatory skin and muscle tissues in ball pythons (*Python regius*) (**Sadler**)
11:30	5. The persistence of *Trichomonas gallinae* isolates in simulated bird baths under various reproduced environmental conditions (**Purple**)	19. Prevalence of *Tritrichomonas foetus* in Tennessee beef bulls (**Jones**)
11:45	6. Genetic characterization of the meningeal worm *Parelaphostrongylus tenuis* from multiple host species and across spatial scales (**Grunenwald**)	
12:00	Patronize food trucks for lunch – E.J. Chapman Drive	

*PBB, Plant Biotechnology Building

12:30 Plenary address: **James Mazzouccolo,** MA
"*Introduction to SciVal Funding*"
PBB 160

	Infectious Diseases	Clinical Sciences
	Rm. PBB* 160	Rm. PBB* 156/157
1:45	8. Trichlorocarbanilide exposure during early life induces the overgrowth of *Clostridium difficile* in rat offspring cecum contents (**Kennedy**)	20. The effect of ketamine on the minimum infusion rate of propofol preventing motor movement in dogs (**Reed**)
2:00	9. Transduction of hematopoietic stem cells to stimulate RNA interference for treatment of feline infectious peritonitis (**Anis**)	21. Pharmacokinetics of single dose rectal zonisamide in healthy dogs (**Michaels**)
2:15	10. Pulmonary surfactant protein D as a biomarker for bronchopneumonia in calves (**Storer**)	22. Osmolality and electrolyte composition of the tear film in normal horses (**Best**)
2:30	11. Discovery and characterization of fungal phosphatidylserine synthase inhibitors (**Cassilly**)	23. Maintenance energy requirements of odor detection, explosive detection, and human detection working dogs (**Mullis**)
2:45	12. Detection and genotyping of circulating bovine viral diarrhea virus in dairy cattle and buffalo farms in Ismailia Province, Egypt (**Soltan**)	24. The effect of laser therapy on first-intention incisional wound healing in ball pythons (*Python regius*) (**Cole**)
3:00	13. Molecular explanation of protein A deficiency in the *Staphylococcus aureus* strain Wood 46 (**Balachandran**)	25. Use of cardiac troponin I (cTnI) as a biomarker of myocyte injury in an avian model following administration of the cardiotoxin doxorubicin: A pilot study (**McCleery**)
3:15	14. Emergence of peste des petits ruminants virus lineage IV in Ismailia Province, Egypt (**Soltan**)	

*PBB, Plant Biotechnology Building

Tuesday, May 19

9:00	Keynote address: **Dale S. Bond,** PhD *"Are We All Sitting Ducks? The Health Risks of Sedentary Behavior and Strategies to Increase Participation in the Stand Up Movement"* PBB* 156/157	
	Food Safety	**Innovative Biomedical Technologies**
	Rm. PBB* 160	Rm. PBB* 156/157
10:30	26. Grape seed extract against human noroviral surrogates in model food systems and simulated gastric conditions (**Joshi**)	36. Computer cursor control using imagined body kinematics (**Abiri**)
10:45	27. Reduction of Aichi virus by sodium metasilicate and calcium hypochlorite in suspension (**Arreaza**)	37. Synovial fluid-derived mesenchymal stem cells as a cell source for cartilage tissue (**Zayed**)
11:00	28. The impact of culture-independent diagnostic testing on foodborne illness surveillance (**Tandy**)	38. Canine mesenchymal stem cells cultured on novel 3D nanofiber scaffolds have increased proliferation and differentiation efficiency, while maintaining cell multipotency (**Conway**)
11:15		39. Graphene and stem cell combination for bone tissue regeneration: In vitro to in vivo (**Elkhenany**)
11:30		40. Characterization of ligand-induction of influenza hemagglutinin (**Valverde**)
11:45	Network and patronize food trucks for lunch – E.J. Chapman Drive	

*PBB, Plant Biotechnology Building

Tuesday, May 19

	Metabolism & Nutrition	Oncology & Cancer Cell Biology
	Rm. PBB* 156/157	Rm. PBB* 160
1:15	29. Potential new drug compounds for Z-Alpha1 antitrypsin deficiency (**Estenson**)	41. Pharmacokinetics of orally administered low-dose rapamycin in healthy dogs: A pilot study (**Larson**)
1:30	30. Pro-inflammatory cytokine IL-1B diminishes butyrate oxidation in colorectal cancer cells (**Johnstone**)	42. Effects of doxorubicin and its derivative, AD198, in oral squamous cell carcinoma cells in vitro (**Smolensky**)
1:45	33. Heat stress reduces phosphorylation activity of the mTOR signaling cascade in bovine mammary cells (**Kaufman**)	43. A novel derivative of doxorubicin, AD198, inhibits cell growth of canine transitional cell carcinoma and osteosarcoma cells in vitro (**Rathore**)
2:00	48. 3,4,4'-Trichlorocarbanilide exposure induces gut microbial dysbiosis in weaned rats (Fling)	44. Effects of novel receptor tyrosine kinase inhibitors and non-steroidal anti-inflammatory drugs in bladder cancer (**Bourn**)
2:15	32. "I do it for her": Mothers' experiences pumping breastmilk for their preterm infants (**Bower**)	45. Effects of chronic exposure to benzo[a] pyrene, a lipophilic mammary carcinogen, on stromal fibroblasts: Role in breast carcinogenesis (**Heal**)
2:30	35. Effects of television viewing on enjoyment of exercise in college students (**Wilkerson**)	46. Reactive oxygen species-mediated breast cell carcinogenesis enhanced by multiple carcinogens and intervened by dietary ergosterol and mimosine (**Pluchino**)
2:45	34. Vitamin A status affects hepatic expression of key genes for fuel metabolism (**Kuang**)	47. Carnitine is a critical contributor for butyrate oxidation in colon cancer cells (**Han**)
3:00	31. Insulin and heat stress alters the activity of the mTOR signaling cascade in bovine mammary cells (**Kassube**)	
5:30	Awards Reception (Hollingsworth Auditorium)	

*PBB, Plant Biotechnology Building

Featured Speakers

Carolyn J. Henry, DVM, MS, DACVIM

Professor of Oncology
Associate Dean for Research and Graduate Studies
College of Veterinary Medicine
University of Missouri

"One Health: Clear Vision and Blurred Lines"

Monday Keynote Address
9:00 am, PBB 156/157

Dr. Henry is a graduate of Auburn University College of Veterinary Medicine. She practiced small animal and emergency medicine in Georgia and Alabama before returning to Auburn to complete an MS and an oncology residency. Dr. Henry became board certified in Oncology by the American College of Veterinary Internal Medicine (ACVIM) in 1994 and served on the faculty at Washington State University for 3 years before accepting a faculty position at the University of Missouri's College of Veterinary Medicine in 1997. She also serves as the Associate Director of Research at the Ellis Fischel [human] Cancer Center at the University of Missouri, Faculty Facilitator for the One Health/One Medicine Initiative of the Mizzou Advantage Program within the Provost's Office, and Associate Dean for Research and Graduate Studies at the College of Veterinary Medicine. She is past president of both the Veterinary Cancer Society and the ACVIM Specialty of Oncology and is a member of the European Society of Veterinary Oncology and Fellow in the National Academies of Practice. Dr. Henry's textbook, Cancer Management in Small Animal Practice, was published in 2010, and she has authored or co-authored over 90 peer-reviewed manuscripts. Dr. Henry's research interests center on translational models of human disease, with bladder cancer and novel drug therapies being areas of emphasis.

Featured Speakers

James Mazzouccolo, MA

Coordinator
Office of Research and Engagement
University of Tennessee

"Introduction to SciVal Funding"

Monday Plenary Address
12:30 pm, PBB 160

Jim Mazzouccolo joined the staff of the Office of Research and Engagement in 2008 and previously assisted with identifying foundation, corporate, and federal funding opportunities for faculty research initiatives as well as matching federal and corporate research needs and opportunities with faculty expertise. He joined the Faculty Development Team in 2014. Jim conducts workshops for the Office of Research and Engagement and assists with proposal development, identifying possible research collaborators at UT, and consulting with faculty regarding Department of Defense Young Investigator programs. His educational background includes a dual B.A. in Political Science and Philosophy from St. Peter's University, and a B.A. in History and an M.A. in Philosophy from the University of Tennessee.

Featured Speakers

Dale S. Bond, PhD

Associate Professor of Psychiatry and Human Behavior
The Miriam Hospital & Alpert Medical School
Brown University
Providence, RI

"Are We All Sitting Ducks? The Health Risks of Sedentary Behavior and Strategies to Increase Participation in the Stand Up Movement"

Tuesday Keynote Address
9:00 am, PBB 156/157

Dr. Bond's research focuses on assessing and intervening on physical activity and sedentary behavior within the context of obesity and bariatric surgery. Dr. Bond has been awarded grants from the National Institutes of Health to test in-person and mobile health approaches to increasing physical activity and/or decreasing sedentary behaviors in obese populations. Additionally, he is currently testing the efficacy of a behavioral weight loss intervention combining physical activity and dietary components for treating migraine pain in obese women.

Food Trucks - Lunch Options

Food trucks will be parked on E.J. Chapman Drive alongside the Plant Biotechnology Building on both days of the symposium. Please patronize our vendors by purchasing lunch from them.

POUTINE MOBILE

POUTINES

All our poutines are made with homemade fresh cut French fries, our original sauces and fresh Wisconsin cheese curds.

- **The Authentic** 6.00 — Fries, Cheese Curds, Poutine Sauce
- **Hogtown Poutine** 9.00 — Fries, Cheese Curds, Hot dog, Bacon, Poutine Sauce
- **Pulled Pork Poutine** 10.00 — Fries, Cheese Curds, Pulled Pork, Yellow Corn, BBQ Sauce
- **Grandma's Poutine** 7.00 — Fries, Cheese Curds, Homemade Tomato Meat Sauce
- **Hot Chicken Poutine** 9.00 — Fries, Cheese Curds, Diced Chicken, Baby Green Peas, Poutine Sauce
- **Veggie Authentic** 8.00 — Fries, Cheese Curds, Sliced Mushrooms, Baby Green Peas, Yellow Corn, Veggie BBQ Sauce
- **French Twist Poutine** 7.00 — Fries, Cheese Curds, Sliced Corn Dog, Poutine Sauce
- **Breakfast Poutine** 9.00 — Fries, Cheese Curds, Sunny Side up Egg, Bacon, Poutine Sauce

Our menu can vary from day to day, according to availabilities and inspiration.

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BEVERAGE

Soft Drink	2.00
Iced Tea	2.00
Water Bottle	2.00

SIDES

Corn-Dog	1 for 3.00 / 2 for 5.00
Fries	4.00
Fries+Sauce	5.00

EXTRA

Cheese	3.00
Sauce	2.00
Meat	3.00

Our menu can vary from day to day, according to availabilities and inspiration.

The Breezy Weenie

Today's Menu

The Classic Weenie $3.50
1/4# weenie, mayo, mustard, ketchup

The Single Wide Weenie $5.50
1/4# weenie, housemade chili, shredded mild cheddar cheese

Pullin' My Weenie $8.00
1/4# weenie, topped with smoked BBQ pulled pork and creamy cole slaw

BBQ Pulled PorkTacos (2) $8.50
A southern favorite, smoked BBQ pulled pork, topped with red onions and creamy cole slaw

Caesar Salald Wrap $6.00
12" spinach tortilla filled with romaine lettuce and topped with house made Caesar dressing.
ADD CHICKEN BREAST FOR $2.00

Beverages $2.00
Coke, Diet Coke, Dr Pepper,Water

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Abstracts

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
- 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

UTCVM INSTITUTE OF AGRICULTURE THE UNIVERSITY OF TENNESSEE

THE UNIVERSITY OF TENNESSEE KNOXVILLE
DEPARTMENT OF PUBLIC HEALTH

1. Genome-wide association study identifies loci associated with mastitis phenotypes following experimental challenge with *Streptococcus uberis*

Lydia Siebert, Meg Staton, Stephen Oliver, Gina Pighetti

Animal Science (Siebert, Oliver, Pighetti), Entomology and Plant Pathology (Staton), AgResearch (Oliver)

Mastitis is a detrimental disease in the dairy industry that costs upwards of $2 billion annually and decreases milk quality. Often, mastitis results from bacteria entering the gland through the teat end. A common mastitis-causing pathogen is *Streptococcus uberis*, which is responsible for 14–26% of subclinical and clinical mastitis cases. Following an intramammary experimental challenge with *S. uberis* on Holstein cows (n = 40), aseptic milk samples were collected, and viable plate counts were used to determine the number of *S. uberis* colony forming units (CFU) present. Traditional genome-wide association studies have used somatic cell counts and/or sire pedigrees to identify loci of interest for mastitis. We propose a novel approach, using *S. uberis* CFU data to create three quantitative phenotypes: days until *S. uberis* was cleared, number of *S. uberis* re-infections, and peak area for *S. uberis* CFU for 7 days post-challenge. To identify loci of interest, a 50K SNP chip analysis was performed using the BovineSNP50 v2 DNA Analysis BeadChip from Illumina. Associations were tested using Plink software. Preliminary analyses revealed 37 highly significant ($P < 1.0 \times 10\text{-}5$) SNPs across the three phenotypes. Of these, 16 are part of significant SNP clusters on BTAs 2, 3, 10, 18, and 22 and an additional two SNPs are located directly in immune-related genes. The identified loci should be further investigated to potentially identify causation behind the observed phenotypes. Such investigations could lead to novel treatment and prevention compounds/protocols for mastitis or genetic selection methods for cows with greater potential to resist infection.

2. *Streptococcus uberis* vaccine: Current and future directions

Oudessa Kerro-Dego, Raul Almeida, Susan Ivey Headrick, Stephen Oliver

Animal Science

Streptococcus uberis mastitis has become increasingly prevalent in well-managed dairy herds. The increasing concern about the prophylactic use of antibiotics has been causing changes in the use of classical prevention mastitis measures. A noticeable changing trend is the shifting from blanket dry cow treatment (DCT) to selective DCT, thus reducing the beneficial effect of the former on controlling new *S. uberis* infections. Thus, development of effective *S. uberis* vaccines is not only a logical approach but also a feasible control tool for this important disease of dairy cows. Our approach for development of a *S. uberis* vaccine is a multi-step process including identification of relevant antigens and evaluation of the protective effect of specific antibodies under in vitro conditions. These are followed by animal studies including development of vaccination protocols aiming to afford protection at times when *S. uberis* mastitis is highly prevalent such as the peripartum period. The final aspect of the study is the challenge of vaccinated cows by intramammary infusion of heterologous strains around calving. We have conducted all these steps successfully; however protection effect was not as good as expected. A detailed evaluation of results suggested that antibody dilution by sudden milk production, calving related immunosuppression, and overwhelming challenge dose were among the factors responsible for the obtained results. We are currently redesigning the challenge model focusing on the challenge timing and experimental infection model. In conclusion, classical concepts on vaccine testing protocols should be adapted to real physiological conditions of the target animal species.

3. Detection and identification of horizontally transferred genetic elements in Staphylococci: Implications for drug resistance and disinfectant use in veterinary medicine

Matthew Riley, David Bemis, Stephen Kania

Comparative and Experimental Medicine (Riley, Bemis, Kania), United States Army, Medical Service Corps (Riley), Biomedical and Diagnostic Sciences (Bemis, Kania)

The spread of multidrug-resistant bacteria in both human and animal medicine is intrinsically linked to use of antibiotics for treatment and prevention of disease, yet is a cause of great concern as the mechanism of acquisition of the underlying genetic components are not well understood. Resistance to disinfectant products, specifically those containing quaternary ammonium compounds, is established in human medicine but is an emerging problem in veterinary medicine. Mobile genetic elements such as small plasmids are known to carry genes conveying both drug and disinfectant resistance, but are thought to be infrequently carried in animal pathogens, specifically *Staphylococcus pseudintermedius*. However, using novel

biomolecular techniques to identify these elements, we have shown that they may be more common and widespread than previously believed simply due to inability to detect low copy number plasmids using traditional techniques. We also have uncovered novel genes carried on these plasmids that have not been reported in animal medicine to date that potentially confer resistance to drugs and disinfectants. In addition, we have shown that standard antibiotic resistance screening may not be able to identify some plasmid-borne resistances and new diagnostic tests have been developed to help bridge this gap. Finally, a screen of *Staphylococcus* species both in-house an in a collection of North American isolates for identical plasmids has shown that these genes are not unique to the specific isolates in which we initially identified them. Discovery and monitoring of these emerging or overlooked elements is essential for effective preventive medicine efforts.

4. Role of retinoic acid in herpes stromal keratitis

Ujjaldeep Jaggi, Sachin Mulik, Naveen Rajasagi, Sid Bhela, Fernanda Giminez, Karthik Varanasi, Barry T. Rouse

Comparative and Experimental Medicine (Jaggi, Mulik, Bhela, Giminez, Rouse), Biomedical and Diagnostic Sciences (Rajasagi, Varanasi, Rouse)

Ocular infection with herpes simplex virus (HSV) can result in a chronic immune-inflammatory lesion that is a significant cause of human blindness. A key to controlling SK lesion severity is to identify cellular and molecular events responsible for tissue damage. Retinoic Acid (RA) has been identified as a critical regulator of host inflammatory responses. However, its role in Immuno-pathological diseases remains unclear. Here, we show that RA targets the T-effector cell population and thereby reduces the severity of HSK. It targets mainly the TH1 cells, which are the main orchestrators of the disease. Also, it is known to stabilize TREGs, which help in suppressing the immune response and hence, controls the progression of HSK. It proves to be a promising curing agent as it plays an important role in both preventive and therapeutic stages of HSK. Administration of RA effectively reduces HSK lesions and can prove to be a very reliable therapeutic agent.

5. The persistence of *Trichomonas gallinae* isolates in simulated bird baths under various reproduced environmental conditions

Kathryn Purple, Richard Gerhold

Comparative and Experimental Medicine (Purple, Gerhold), Biomedical and Diagnostic Sciences (Gerhold)

Bird baths are often implicated in the dissemination of the protozoan parasite *Trichomonas gallinae*, the causative agent of the potentially fatal disease trichomonosis, to naïve avian hosts. These implications, however, are based upon speculation rather than scientifically established findings of the behavior of *T. gallinae* in aquatic environments. We used a set of laboratory experiments to document the persistence of *T. gallinae* in differing water conditions in rigid plastic containers to simulate bird baths. Each simulated bird bath contained 500 mL distilled water with various treatments, including addition of 15 g organic material, increased temperature, and common disinfectants. We inoculated into each container one of multiple isolates of *T. gallinae* from wild avian hosts. We discovered that a *T. gallinae* isolate from a Cooper's hawk persisted in distilled water with the addition of organic material for at least 16 hr, far exceeding the previously published persistence of 20 min. We also characterized higher temperature, 33˚C, as a beneficial condition for the persistence of a broad-winged hawk *T. gallinae* isolate in the organic material treatment yielding a persistence of 20 hr. Finally, we have found certain concentrations of common disinfectants inhibit persistence by up to 32 fold. These experimental results provide scientific evidence that bird baths have the potential to serve as a nidus of infection in trichomonosis outbreaks and could serve as a practical target for managing future epidemics.

6. Genetic characterization of the meningeal worm *Parelaphostrongylus tenuis* from multiple host species and across spatial scales

Caroline M. Grunenwald, Richard W. Gerhold, Lisa Muller, Chunlei Su

Microbiology (Grunenwald, Su), Biomedical and Diagnostic Sciences (Gerhold), Forestry, Wildlife, and Fisheries (Muller)

Parelaphostrongylus tenuis is a metastrongylid nematode harbored by white-tailed deer and transmitted by ingesting infected gastropod intermediate hosts while grazing. In

atypical hosts such as moose, *P. tenuis* infection causes severe neurological disease and death due to nematode migration within the central nervous system. Although *P. tenuis* is commonly distributed throughout northeastern and select parts of southeastern North America, nothing is known about the genetic diversity of this parasite. To better understand the genetic population structure, 36 adult *P. tenuis* were collected from five different host species in seven different states, including 27 white-tailed deer and nine clinically diseased animals. The second internal transcribed spacer region and the cytochrome oxidase I and II genes were amplified, cloned, sequenced, and compared against known *P. tenuis* sequences in GenBank. Bioinformatic and phylogenetic analysis of the sequences revealed limited variation between isolates, with the majority of *P. tenuis* samples (68%) clustering into a single genotype. No geographic or host patterning associated with genotype was observed. The lack of intraspecies diversity and the absence of geographical and host patterning suggests *P. tenuis* may have undergone a recent genetic bottleneck event. One explanation could be the near extermination of deer in North America during the early 1900s. Significant loss in deer host numbers likely resulted in a significant decrease in environmental parasite load and thus parasite diversity. This study represents the first attempt to genetically characterize the *P. tenuis* parasite and suggests past anthropogenic activities may have significantly shaped the parasite's current population genetic structure.

8. Trichlorocarbanilide exposure during early life induces the overgrowth of *Clostridium difficile* in rat offspring cecum contents

Rebekah Kennedy, Russell Fling, David Bemis, Elizabeth McPherson, Ling Zhao, Paul D. Terry, Jiangang Chen

Comparative and Experimental Medicine (Kennedy), Public Health (Chen, Terry), Microbiology (Fling, McPherson), Biomedical and Diagnostic Sciences (Bemis), Nutrition (Zhao)

Clostridium difficile is an important endospore-forming, nosocomial pathogen associated with substantial morbidity and mortality. *C. difficile* infection (CDI) is a common source of antibiotic-associated diarrhea (AAD). Most cases of CDI result from exposure to antibiotics along with toxin-producing strains of *C. difficile*. Widely used as an antimicrobial in personal care products, triclocarban (3,4,4′-trichlorocarbanilide; TCC) is effective against gram-positive bacteria, but to date has not been tested against endospore-forming bacteria. The effect of exposure to antimicrobials found in personal care products to the overgrowth of *C. difficile* is elusive. Previously, we have shown that TCC exposure from 0.2–0.5% w/w during lactation substantially reduced offspring survival in rats. In the current study, we investigated whether early life TCC exposure resulted in a gastrointestinal change that would favor the overgrowth of *C. difficile*. Pregnant SD rats were randomized and provided either control chow or chow supplemented with 0.1% w/w TCC (a dose non-lethal to offspring) from gestational day 5 (GD5) to postnatal day 22 (PND 22). On PND 22, offspring cecum contents were removed and pooled, and *C. difficile* was inoculated into cecum content pools and incubated anaerobically for 24 or 48 hr. Half the cecum slurries were plated on *C. difficile*-selective agar, and total CFU/mL was enumerated. Vegetative *C. difficile*–like CFU/mL counts were significantly increased in cecum content pools collected from TCC-exposed compared with unexposed offspring and compared to unexposed offspring after inoculation. Our results suggest that the integrity of gut microbiota provides protection against *C. difficile* growth, and that disturbances in the composition of the gut microbiota by TCC may increase susceptibility to CDI.

9. Transduction of hematopoietic stem cells to stimulate RNA interference for treatment of feline infectious peritonitis

Eman Anis, Madhu Dhar, Rebecca P. Wilkes

Biomedical and Diagnostic Sciences (Anis, Wilkes), Large Animal Clinical Sciences (Dhar), Virology-Sadat University, Egypt (Anis)

Feline infectious peritonitis (FIP) is a highly fatal disease caused by virulent feline coronavirus (FCoV) that has the ability to infect monocytes/macrophages. In a previous study, we proved that RNA interference (RNAi) can be used to inhibit FCoV replication in vitro. RNAi, mediated by small interfering RNA (siRNA), has therapeutic potential if the siRNA can be delivered in sufficient quantity to monocytes/macrophages. The goal of the current study is to assess the feasibility of transducing hematopoietic stem cells with FCoV-specific, siRNA-coding DNA (miRNA) by ex-vivo introduction of a non-replicating lentivirus vector. To assess the effectiveness of the designed miRNAs to inhibit viral replication, stably transduced CrFK cells were prepared and infected with FCoV. Inhibition of coronavirus replication was determined by quantitative real-time RT-PCR. The amount

of virus production (viral mRNA) was compared between infected cells expressing coronavirus-specific miRNA and infected cells expressing scrambled miRNA (negative control). Three miRNAs – microRNA-L1, L2, and microRNA-N – that target the leader sequence and the nucleocapsid gene exhibited variable inhibitory effects on viral replication in vitro, resulting in more than a 50% reduction in the mRNA expression of FCoV, when compared with negative control. These preliminary results suggest that genetic modification of hematopoietic stem cells for constitutive production of these anti-coronavirus siRNA will reduce FCoV replication. Hematopoietic stem cells have been obtained from feline bone marrow and cultured in vitro. Stably transduced cells will be produced and evaluated for toxicity and ability to replicate. This proof of concept work will potentially lead to in vivo introduction of these genetically modified cells for the treatment of FIP.

10. Pulmonary surfactant protein D as a biomarker for bronchopneumonia in calves

Jennifer Storer, Marc Caldwell, Robert Donnell, David Anderson

College of Veterinary Medicine (Storer), Large Animal Clinical Sciences (Caldwell, Anderson), Biomedical and Diagnostic Sciences (Donnell)

Bronchopneumonia in cattle is a costly disease caused by multiple pathogens. *Mannheimia haemolytica* is the most frequently isolated etiologic agent and induces tremendous inflammation through the production of a leukotoxin. Surfactant protein D is produced by type II pneumocytes and is tissue specific for the lungs. During alveolar inflammation, these proteins are up-regulated and may be released into the blood. We hypothesized that these proteins could be detected in the serum and serve as biomarkers for alveolar membrane damage and overall pulmonary inflammation. This study used bronchoselective endoscopic inoculation of *M. haemolytica* or sterile saline of the right apical lung lobe of twelve 4-month-old Holstein calves. Six principal calves received 3-5 x 109 colony forming units of *M. haemolytica* in a 5-mL suspension of phosphate-buffered saline (PBS), while six control calves received an equivalent volume of sterile PBS. Serum and bronchoalveolar lavage (BAL) samples were collected to analyze surfactant protein D with a bovine-specific ELISA. Blood serum samples were collected daily for 7 days, while BAL samples were collected on days 0, 1, 3, 5, and 7. Calves were additionally assigned a clinical illness score twice daily, and a data logger outfitted with accelerometers was placed on the left rear fetlock of each calf to assess behavioral changes. This study showed that pulmonary-specific protein can be detected in the serum and may prove to be important still in naturally occurring or more diffusely infected calves with bronchopneumonia. Developing a field-side test that can quantify surfactant D protein could prove to be beneficial for the cattle industry by allowing feedlot operators the ability to use antibiotic stewardship of their herds.

11. Discovery and characterization of fungal phosphatidylserine synthase inhibitors

Chelsi Cassilly, Martin Cheramie, Robin Lee, Abigail Tester, Shahrina Alam, Michael Best, Shawn Campagna, Richard Lee, Todd Reynolds

Microbiology (Cassilly, Reynolds), St. Jude Children's Research Hospital, Chemical Biology and Therapeutics (Cheramie, Lee, Lee), Chemistry (Tester, Alam, Best, Campagna)

The pathogenic fungus *Candida albicans* is the leading cause of hospital-acquired fungal infections in immunocompromised individuals. Invasive bloodstream infections have a 30% mortality rate. Three antifungal classes are used to treat invasive fungal infections, including azoles, echinocandins, and polyenes. Rising drug resistance and drug toxicity have made these compounds less effective, and new drugs are needed. The fungal phosphatidylserine (PS) synthase (Cho1p) has been suggested as a drug target because it is 1) required for *C. albicans* virulence, 2) conserved among fungi, and 3) absent from mammals. Thus, Cho1p inhibitors could be broad-range antifungals with few detrimental side effects. To identify Cho1p inhibitors, we have taken two approaches. The first approach is a novel compound screen. Cells lacking Cho1p cannot survive without supplemented ethanolamine because de novo biosynthesis of phosphatidylethanolamine (PE), a vital phospholipid, is downstream of PS production. Thus, these cells survive only by making PE from imported ethanolamine by an alternative pathway. We screened compounds for their ability to inhibit growth of wildtype *C. albicans* in media lacking ethanolamine, but not in media containing ethanolamine, thus indicating ethanolamine auxotrophy. A preliminary screen yielded one positive hit, and further screens are still ongoing. Positive hits will be characterized using biochemical and molecular methods. Our second approach to find Cho1p inhibitors is through synthesized

serine analogs. N-alkynyl L-serine decreases Cho1p activity in an in vitro PS synthase assay. Further compound modifications and testing are underway. This research is the first step in identifying specific and effective next generation drugs.

12. Detection and genotyping of circulating bovine viral diarrhea virus in dairy cattle and buffalo farms in Ismailia Province, Egypt

Mohamed A. Soltan, Rebecca P. Wilkes, Mohamed N. Elsheery, Mahmoud M. Elhaig, Matthew C. Riley, Melissa A. Kennedy

Suez Canal University, Egypt (Soltan, Esheery, Elhaig), Biomedical and Diagnostic Sciences (Wilkes, Kennedy), Comparative and Experimental Medicine (Riley)

Bovine viral diarrhea (BVD) is one of the most economically significant diseases in the bovine industry, causing losses due to diarrhea, reproductive disorders, increase susceptibility to other diseases, and mortalities. The aim of our investigation was to detect and genotype BVD virus from calves on two dairy cattle and two buffalo farms in Ismailia province, Egypt, as an indicator for BVD virus infection status. A total of 298 blood samples were collected and tested using an optimized one-step, real-time multiplex Taqman-based RT-PCR. Forty-six (15.4%) of the samples were positive for BVDV1. For subgenotyping, all positive samples by multiplex real-time RT-PCR were further tested using RT-PCR to amplify multiple areas of the genome. Only three samples, all from a single dairy cattle farm, had enough viral RNA to be amplified by RT-PCR. The PCR products were sequenced, and phylogenetic analysis revealed circulation of the same strain, which clustered within BVDV subgenotype 1b. The detected BVDV strain is closely related to worldwide BVDV 1b strains, making it difficult to trace its origin. Nucleotide and amino acid alignments of the E2 glycoprotein of the detected strain with other BVDV 1b strains showed high divergence, with homology ranging from 81.3 to 93.6% and 85.3 to 93.6%, respectively. Using the E2 glycoprotein in phylogenetic analysis, in comparison with the 5´ UTR and Npro region, resulted in clearer differentiation between BVDV subgenotypes. To our knowledge, this is the first report to document circulation of BVDV1b in Egyptian dairy cattle populations.

13. Molecular explanation of protein A deficiency in the *Staphylococcus aureus* strain Wood 46

Manasi Balachandran, David Bemis, Stephen Kania

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

Protein A is encoded by the spa gene and binds to immunoglobulin G. The *Staphylococcus aureus* strain Wood 46 (ATCC 10832) has been considered protein A-deficient and/or spa negative for decades. For this reason, it has been used as a negative control in studies involving *S. aureus* virulence. The reason for low surface expression of protein A has not previously been investigated. Sortase A (SrtA) is a transpeptidase commonly produced by gram-positive bacteria. It has specificity for proteins that harbor the LPXTG motif, such as protein A, and covalently anchors them on to the peptidoglycan cell well. Many of these sortase-anchored proteins are potent virulence factors. The absence of sortase A would result in decreased presence of surface proteins harboring the LPXTG motif. In this study, we investigated the expression of sortase A in Wood 46. Our results suggest that Wood 46 has very low expression of sortase A compared to wild-type *S. aureus*. Further, we found a mutation in the TATA box which could result in lower binding of RNA polymerase, and hence a lower rate of DNA transcription and, consequently, less RNA translated from the srtA gene. This could explain why Wood 46 displays very low amounts of protein A on its surface. Future studies will focus on sortase A inhibition, which may result in the global reduction of *S. aureus* virulence factors. Therefore, inhibition of sortase A is a potential therapy against *S. aureus*.

14. Emergence of peste des petits ruminants virus lineage IV in Ismailia Province, Egypt

Mohamed Soltan, Mohamed Abd-Eldaim

Suez Canal University, Egypt (Soltan, Abd-Eldaim)

Peste des petits ruminants (PPR, also known as ovine rinderpest) is an acute, highly contagious fatal disease of small ruminants characterized by high fever, ocular and nasal discharge, pneumonia, erosive stomatitis, and severe enteritis that ultimately results in high mortalities. Peste des petits ruminants virus (PPRV) is widely distributed and endemic in several African, middle eastern, and south Asian countries, and it poses a threat to European countries. Egyptian veterinary medical authorities stated that Egypt is

free from PPRV, and the only measures for disease control are test and slaughter of an infected population to maintain the free status. The aim of our investigation was to detect PPRV in Ismailia province as an indicator of the infection status in Egypt and perform molecular characterization of the emerging virus to gain insight into the origin of circulating virus. A total of 40 representative clinical samples, from a single goat case and goat flock in 2010 and sheep flock in 2012, were tested for PPRV by RT-PCR. About 21 (52.5%) samples were positive. The phylogenetic analysis of the detected viruses revealed circulation of PPRV lineage IV. The circulating viruses are closely related to Sudanese and Saudi Arabian strains with nucleotide identity ranging from 99.2% to 99.6%, respectively. Also, it is closely related to the Moroccan 2008 viruses with identities ranged from 97.6% to 98%. Epidemiological investigation at the national level is recommended for monitoring PPRV spread and implementing an appropriate control program.

15. Prospective, randomized blinded pilot study of the effect of oral glutamine supplementation on the severity of radiation-induced oral mucositis

Richard A. Chetney, Jr., Nathan D. Lee, Jamie N. Pawlik

Small Animal Clinical Sciences

Previous studies in human and rat models suggest oral glutamine decreases acute radiation effects on the gastrointestinal mucosa. Currently, there is a lack of information regarding lessening of radiation-induced oral mucositis with similar supplementation. The goal of this study was to analyze whether orally supplemented L-glutamine decreases the severity of radiation-induced oral mucositis in dogs treated for head and neck tumors. Nine dogs received either 32Gy or 48Gy of fractionated radiation for head and neck tumors. Dogs were assigned at random to receive OraBlend suspension alone, or suspension containing 40 mg/kg L-glutamine, every 12 hr. Radiation-induced oral mucositis was graded weekly by two blinded clinicians using the VRTOG acute radiation toxicity scheme. Data were analyzed using the repeated measures ANOVA, with a *P* value of less than .05 representing statistical significance. Three dogs received 48 Gy radiation therapy, and six dogs received 32 Gy radiation therapy. Most adverse radiation effects occurred within week 3 and 4 after initiation of radiation therapy. The repeated measures ANOVA value was 0.3665, meaning L-glutamine supplementation had no effect on the severity of oral mucositis. Lack of evidence of lessened radiation-induced oral mucositis due to L-glutamine supplementation may be due to small sample size and disproportionate subject number and supplementation between the 32 Gy and 48 Gy groups. Review of the literature supports a higher dose of L-glutamine, which will be used in further study. L-glutamine was well-tolerated by the dogs in the study at the dosage prescribed.

16. Magnetic resonance imaging findings of lymphoma affecting the canine spine and paraspinal soft tissues

Brian Allett, Silke Hecht

Small Animal Clinical Sciences

Lymphoma is one of the most commonly occurring neoplasms in the dog. Despite its prevalence and the increasing use of magnetic resonance (MR) imaging, reports of lymphoma affecting the spine using MR imaging have been limited. The purpose of this study was to describe the magnetic resonance imaging findings in dogs with lymphoma affecting the spine and/or paraspinal soft tissues. Medical records were searched for patients that had MR imaging of the spine and had a diagnosis of lymphoma of the spine or paraspinal tissues during the period of 2009–2013. The imaging studies were evaluated for the presence of focal or multifocal disease; identification of the structures involved including the spinal cord, meninges, spinal canal, vertebrae, and paraspinal soft tissues; and the signal characteristics on T2-W, STIR, and T1-W sequences prior to and following intravenous contrast medium administration. Six dogs met the inclusion criteria. Common MR imaging findings included multifocal disease (4/6), vertebral involvement (5/6), spinal cord compression (4/6), and involvement of the paraspinal soft tissues (5/6). Vertebral changes were confined to the medullary cavity without evidence of osteolysis. There was questionable involvement of the spinal cord in only one case. All spinal and paraspinal lesions identified were T2-W isointense to hyperintense, STIR hyperintense, T1-W hypointense to isointense, and showed consistent but variable moderate to strong contrast enhancement. Additional lesions identified included enlarged intra-abdominal lymph nodes, splenomegaly, and a splenic nodule. The STIR and T1-W post-contrast sequences were subjectively the most useful in identification of the lesions.

17. Analysis of variables associated with diabetic foot ulcer healing

Mayuri Jagadish, Eric Heidel, Susan Teffeteller, Mitchell Goldman

Microbiology (Jagadish), Graduate School of Medicine Department of Surgery (Heidel, Teffeteller, Goldman)

The estimated cost for care of diabetic foot ulcers (DFU) is approximately $1.5 billion annually. This retrospective study was designed to discover what factors affect the healing of DFU in a wound care center, so treatment methods based on contributing factors could be optimized. Data from a total of 114 patients from ages 18 to 98 were studied. Variables included age, gender, ethnicity, body mass index (BMI), A1C, ankle brachial index (ABI), neuropathy, and smoking history. Duration and family history of diabetes, past vascular events, renal complications, and deep vein thrombosis (DVT) were also assessed for possible relationships to wound healing. The properties of the wound itself, and its characteristics were analyzed. Different wound treatment methods were studied to see if any of them promoted healing. Statistical analyses included independent sample *t* tests, χ^2 tests, and unadjusted odds ratios with 95% confidence intervals with a significance value α of 0.05. The results showed that smoking and history of DVT significantly decreased the likelihood of wound healing while total contact casting (TCC) was associated with increased healing. Other factors did not significantly affect wound healing. There were no significant differences between healing groups in age, BMI, A1C, ABI, duration of diabetes, and wound characteristics. To conclude, patients treated in a wound care center where weekly debridement occurred for diabetic foot ulcers benefited from TCC. A previous history of DVT made healing 87% less likely. Patients who smoked were 81% less likely to heal. Patients with a past vascular event also had decreased healing.

18. Expression of cyclooxygenase enzymes in normal and inflammatory skin and muscle tissues in ball pythons (*Python regius*)

Ryan Sadler, Juergen Schumacher, Kusum Rathore, Kim Newkirk, Grayson Cole, Rachel Seibert, Maria Cekanova

Small Animal Clinical Sciences (Sadler, Schumacher, Cole, Seibert, Cekanova, Rathore), Biomedical and Diagnostic Sciences (Newkirk)

Despite the common use of non-steroidal anti-inflammatory drugs (NSAIDs) in reptiles, the expression and role of reptilian cyclooxygenase (COX) enzymes are still unknown. This study evaluated COX-1, COX-2, and other inflammatory mediators (p-ERK, p-AKT, NFKB) in normal (day 0) and laser-induced inflamed (day 7) skin and muscle biopsies from six healthy male ball pythons (*Python regius*) using histologic grading and Western blot. All day-0 tissues were found to be free of underlying dermatologic disease in both skin and muscle samples, and all day 7 samples had elevated histologic inflammation scores compared with day 0. Expression of COX-1 and p-ERK proteins was significantly increased on day 7 in inflamed skin samples. COX-1 expression was significantly decreased on day 7 in muscle samples, despite significantly increased p-ERK and p-AKT levels. No significant increases were noted in COX-2 expression in either skin or muscle samples after laser-induced inflammation. The lack of significant increases in COX-2 expression despite increased COX-1 and inflammatory mediator expression in these inflamed tissues indicates further research of reptilian inflammatory mechanisms is needed to help direct the use of selective versus non-selective COX inhibitors.

19. Prevalence of *Tritrichomonas foetus* in Tennessee beef bulls

Brittni M. Jones, Brian K. Whitlock, Lew G. Strickland, Stephen Kania

Large Animal Clinical Sciences (Jones, Whitlock, Strickland), Animal Science (Strickland), Biomedical and Diagnostic Sciences (Kania)

Tritrichomonas foetus is a venereal transmitted protozoan of cattle in which infected bulls are asymptomatic carriers, and infected cows and heifers may experience embryonic and fetal loss, thus costing the U.S. beef industry over $100 million annually. While the southeastern region of the United States has an estimated prevalence rate reported for the disease, Tennessee has yet to estimate the prevalence within the state. The objective of this study was to estimate the prevalence of *T. foetus* infections in Tennessee beef bulls through prospective and retrospective surveys. The prospective survey included 380 Tennessee beef bulls that were sampled between March 2013 and January 2014. Preputial smegma was collected from the 380 bulls with a bull rasper (Tricamper) sampling device and cultured in an InPouch *T. foetus* culture pouch. The samples were evaluated microscopically every other day for 7 days for growth, and an aliquot of the culture media from each sample was used for DNA extraction and subsequent real-time quantitative reverse transcription polymerase chain reaction. Of the

380 bulls cultured in the prospective survey, two (0.53%) cultures were considered suspect on microscopic evaluation; however, all real-time PCR-based assays were negative for *T. foetus*, suggesting contamination with fecal trichomonads. The retrospective analysis included 659 *T. foetus* tests (culture and/or real-time PCR) performed at the Kord Animal Laboratory in Nashville, TN, and the UT College of Veterinary Medicine between November 2013 and January 2015. Of the 659 samples, *T. foetus* was observed and subsequently confirmed by real-time PCR in samples from two (0.30%) of the bulls.

20. The effect of ketamine on the minimum infusion rate of propofol preventing motor movement in dogs

Rachel A. Reed, Reza Seddighi, Agricola Odoi, Sherry K. Cox, Christine M. Egger, Thomas J. Doherty

Small Animal Clinical Sciences (Egger), Large Animal Clinical Sciences (Seddighi, Doherty, Reed), Biomedical and Diagnostic Sciences (Odoi, Cox)

The objective of this study was to determine the minimum infusion rate (MIR-NM) of propofol required to prevent movement in response to a noxious stimulus in dogs anesthetized with either propofol alone or propofol in combination with one of two constant rate infusions (CRI) of ketamine. Six male beagles were anesthetized on three occasions, at weekly intervals, and were given one of three different treatments on each occasion. Treatments were administered as a loading dose (LD) and an initial propofol CRI as follows: treatment P, propofol 6 mg/kg LD and 0.45 mg/kg/min CRI; treatment PLDK, propofol 5 mg/kg LD and 0.35 mg/kg/min CRI combined with ketamine 2mg/kg LD and 25 µg/kg/min CRI; treatment PHDK, propofol 4 mg/kg LD and 0.3 mg/kg/min CRI combined with ketamine 3 mg/kg LD and 50 µg/kg/min CRI. After 60 min, MIR-NM determination was initiated using a noxious stimulus (50V, 50Hz, 10 msec). If the response to stimulation was positive, the propofol CRI was increased by 0.025 mg/kg/min. Conversely, if the response to stimulation was negative, the propofol CRI was decreased by 0.025 mg/kg/min. MIR-NM was determined in duplicate. The propofol MIR-NM was 0.76 ± 0.1, 0.60 ± 0.1, and 0.41 ± 0.1 mg/kg/min for treatments P, PLDK, and PHDK, respectively. Treatments PLDK and PHDK resulted in significant ($P = 0.045$ and $P = 0.032$, respectively) decreases in propofol MIR-NM of 27 ± 10 % and 30 ± 10%, respectively.

21. Pharmacokinetics of single dose rectal zonisamide in healthy dogs

Jennifer R. Michaels, Dawn Boothe, William Thomas, Amy Hodshon, Lindsay Williams

Small Animal Clinical Sciences (Michaels, Thomas, Hodshon, Williams); Auburn University College of Veterinary Medicine, Department of Anatomy, Physiology and Pharmacology (Boothe)

The purpose of the study was to evaluate the pharmacokinetics of zonisamide administered per rectum via a commercially available encapsulated powder mixed with either sterile water or polyethylene glycol and to determine whether a dose of 20 mg/kg or 30 mg/kg would result in target plasma concentrations (10–40 mcg/mL). Eight healthy, mixed-breed dogs were randomly assigned to four groups of two dogs each in a crossover-design study. Zonisamide was administered rectally in a suspension with sterile water or polyethylene glycol at a dose of 20 mg/kg or 30 mg/kg, and blood samples were collected at predetermined time points. After a 7-day washout period, each group received an alternate treatment until all groups had received all treatments. Plasma concentrations of zonisamide were analyzed via high-performance liquid chromatography. Plasma zonisamide concentrations were within the target range at > 1 time point after rectal administration in only two dogs. These concentrations were reached after 3 h (n = 1) and 6 h (n = 1). There was a significant difference in area under the time-concentration curve between the groups receiving 20 mg/kg in water and 30 mg/kg in water and between groups receiving 30 mg/kg in water and 20 mg/kg in PEG. There were no other statistically significant differences in pharmacokinetic parameters between the treatment groups. On the basis of these results, rectal administration of 20 mg/kg or 30 mg/kg of zonisamide via a sterile water or polyethylene glycol suspension as used in the present study cannot be recommended.

22. Osmolality and electrolyte composition of the tear film in normal horses

Lori Best, Diane Hendrix, Daniel Ward

Small Animal Clinical Sciences

Our objective was to establish normal parameters for osmolality and electrolyte composition of the equine tear film. Fifteen adult teaching horses were restrained for ophthalmic examinations, including slit-lamp biomicroscopy, indirect ophthalmoscopy, and Schirmer's tear test

evaluation. Tear samples were collected using microcapillary tubes from both eyes of ophthalmologically normal horses three times at 5-min intervals. The collected tear volume was pooled from each horse, and ionic composition (including Na+, K+, Mg2+, and Ca2+) and osmolality were measured. Means and standard deviations for each parameter were established in order to obtain a normal reference range for horses. The mean (SD) osmolality was 283.58 (9.30) mmol/kg. The mean (SD) electrolyte concentrations were Na+: 134.75 (10) mmol/L, K+: 16.3 (5.77) mmol/L, Mg2+: 3.48 (1.97) mmol/L, and Ca2+: 1.06 (0.42) mmol/L. The osmolality of the equine tear film was similar to that of the human tear film. The sodium concentration of the equine tear film was similar to serum concentrations. Similar to other species, the tear potassium concentration was approximately 4.75 times greater than the normal serum concentration. The tear film concentration of divalent cations was greater than expected and was higher than divalent cation concentrations in the rabbit and the human tear film.

23. Maintenance energy requirements of odor detection, explosive detection, and human detection working dogs

Rebecca A. Mullis, Angela L. Witzel, Joshua Price

Small Animal Clinical Sciences (Mullis, Witzel), Office of Information Technology (Price)

Despite their important role in security, little is known about the energy requirements of working dogs, such as odor, explosive, and human detection dogs. Previous researchers have evaluated the energy requirements of individual canine breeds as well as dogs in exercise roles such as sprint racing. This study is the first to evaluate the energy requirements of working dogs trained in odor, explosive, and human detection. This retrospective study evaluated 20 adult dogs who maintained consistent body weights over a 6-month period. During this time, the average energy consumption was 135.65 ± 37.97 kcal•BWkg 0.75 or two times the calculated resting energy requirement (RER = 70•BWkg0.75). No statistical differences were found between breeds, age, or sex, but a statistically significant association (P = 0.0033, r^2 = 0.0854) was seen between the number of searches a dog performs and its energy requirement. Based on this study's population, it appears that working dogs have energy requirements similar to the 1974 National Research Council's (NRC) suggested maintenance energy requirements of 132 kcal•BWkg 0.75 (NRC, 1974) and the 139 ± 42 kcal•BWkg 0.75 reported for young laboratory beagles (Rainbird & Kienzle, 1990). Additional research is needed to determine if these data can be applied to all odor, explosive, and human detection dogs and to determine if other types of working dogs (tracking, search and rescue etc.) have similar energy requirements.

24. The effect of laser therapy on first-intention incisional wound healing in ball pythons (*Python regius*)

Grayson Cole, Cassie Lux, Juergen P. Schumacher, Rachel Seibert, Ryan Sadler, Andrea Henderson, Agricola Odoi, Kim M. Newkirk

Small Animal Clinical Sciences (Cole, Lux, Schumacher, Henderson, Seibert, Sadler) Biomedical and Diagnostic Sciences (Newkirk, Odoi)

Our objective was to evaluate the effects of therapeutic laser therapy on incisional wound healing in ball pythons (*Python regius*). Six healthy, adult ball pythons had skin biopsies collected on day 0 for histopathologic examination, and eight (four control and four treatment) 2-cm skin incisions were made in each sedated snake and closed with staples. All incision sites were grossly evaluated daily for 30 days, and a wound score was assigned using a previously-applied scoring system. Starting on day 1, four incisions of each snake were treated daily with a class IV solid state laser for 7 consecutive days at a fluence of 5 J/cm^2 and a wavelength of 980 nm on a continuous wave sequence. Two skin biopsies (control and treatment) were taken from each snake on days 2, 7, 14, and 30 and subsequently evaluated microscopically and scored for total inflammation, degree of fibrosis, and collagen maturity. Generalized linear models were used to investigate the effect of treatment on each gross and histologic measure. On gross examination, wound scores of laser-treated incisions were significantly improved over control incisions on day 2, but at no other time points. Histologically, no significant differences in necrosis, fibroplasia, inflammation, granuloma formation, or bacterial contamination were seen between control and treatment groups. Collagen maturity was significantly improved in the laser-treated incisions on day 14 only. The protocol for biomodulation used in this study did not significantly improve healing of skin incisions in ball pythons, with the exception of an increase in collagen maturity at day 14.

25. Use of cardiac troponin I (cTnI) as a biomarker of myocyte injury in an avian model following administration of the cardiotoxin doxorubicin: A pilot study

Brynn McCleery, Michael Jones, Sophy Jesty, Sara Johns, Agricola Odoi

Small Animal Clinical Sciences (McCleery, Jones, Jesty, Johns), Biomedical and Diagnostic Sciences (Odoi)

The purpose of this pilot study was to provide preliminary evaluation of the clinical use of cardiac troponin I (cTnI) as a cardiac biomarker in an avian model. Baseline cTnI levels were measured using an iSTAT point-of-care analyzer and a high-sensitivity assay in eight 4-week-old turkey poults. Doxorubicin, an anthracycline antibiotic with known cardiotoxic effects to turkey poult myocytes, was administered intravenously to four poults three times weekly for 2 weeks. Control poults received an equivalent volume of sterile saline intravenously. Troponin levels were monitored using serial iSTAT assays, and a high-sensitivity cTnI assay was repeated after the final doxorubicin or saline treatment. Baseline echocardiograms were performed on all birds prior to treatment and repeated after each week of treatment to monitor for signs of cardiac disease. The statistical significance of the results will be presented.

26. Grape seed extract against human noroviral surrogates in model food systems and simulated gastric conditions

Snehal S. Joshi, Doris H. D'Souza

Food Science and Technology

Grape seed extract (GSE) is reported to have antiviral activities against human norovirus surrogates (feline calicivirus [FCV-F9] and murine norovirus [MNV-1]) in vitro. The objectives of this study were to understand the antiviral effects of GSE in (1) apple juice (AJ) and 2% milk as model food systems and (2) under simulated gastrointestinal conditions at 37°C. FCV-F9 and MNV-1 at ~5 log PFU/mL were treated with GSE at 2, 4, or 8 mg/mL prepared in 2% milk, AJ (pH 3.6), simulated gastric fluid (SGF; pH 1.5) or simulated intestinal fluid (SIF; pH 7.5), and AJ, 2% milk, SGF, SIF, malic acid (pH 1.5 and 3.0) and phosphate buffered saline (pH 7.2) over 24 hr at 37°C. Virus infectivity of triplicate treatments was evaluated using plaque assays in duplicate. GSE at 1 mg/ml in AJ reduced FCV-F9 to undetectable levels after 5 min, while 4 mg/ml GSE in milk reduced FCV-F9 by 1.07 ± 0.03 log PFU/mL after 24 hr. GSE at 1 mg/mL in AJ reduced MNV-1 to undetectable levels after 1 hr, while 1, 2, and 4 mg/mL GSE in milk reduced MNV-1 by ~0.3 log PFU/mL after 24 h. The tested viruses did not survive in SGF after 1 hr, while GSE at 1 mg/mL in SIF caused reduction of FCV-F9 to undetectable levels after 1 hr, and reduced MNV-1 by 1.03 ± 0.04 PFU/mL log after 1 hr. Results indicate that time-released GSE encapsulation may be needed for use or application in food systems to cause optimal foodborne viral reduction.

27. Reduction of Aichi virus by sodium metasilicate and calcium hypochlorite in suspension

Andres Arreaza, Doris D'Souza

Food Science and Technology

Aichi virus (AiV) is a newly emerging virus responsible for gastroenteritis outbreaks worldwide. There is limited data in literature on effective methods to control AiV spread. Therefore, improved disinfectants are being researched for AiV inactivation. Sodium metasilicate (SMS) and calcium hypochlorite (Ca[ClO]2) are known for their antimicrobial properties and used in industry. Hence, their effects against AiV need to be explored. The objective of this research was to determine the ability of SMS and Ca(ClO)2to inactivate AiV in suspension at room temperature. AiV at ~ 5 log PFU/mL was treated with equal volumes of SMS at 5% and 10%, Ca(ClO)2 at 0.02% and 0.2%, or phosphate buffered saline (pH 7.2 as control) at room temperature for 0.5, 1, and 5 min. At each time-point, treated viruses were initially serially diluted in cell culture media containing fetal bovine serum, and plaque assayed in duplicate using Vero host cells. Data obtained from three replicates were statistically analyzed. AiV at 5 log PFU/mL was reduced to non-detectable levels after 5 min with both 5% and 10% SMS, but showed insignificant reduction after 0.5 min with both 5% and 10% SMS, and 0.59 ± 0.37 log PFU/mL reduction after 1 min with only 10% SMS ($P > .05$). However, Ca(ClO)2 at both tested concentrations of 0.2% and 0.02% reduced AiV to non-detectable levels after 15 sec. This study showed that Ca(ClO)2 at 0.02% could more rapidly reduce AiV than 5% or 10% SMS. Thus, Ca(ClO)2 shows potential to control AiV spread.

28. The impact of culture-independent diagnostic testing on foodborne illness surveillance

Corinne Tandy, Amy Woron, Sheri Roberts, Cara Williams

Comparative and Experimental Medicine (Tandy), Tennessee Department of Health (Woron, Roberts, Williams)

Culture independent diagnostic testing (CIDT) panels for gastrointestinal illness have changed the face of foodborne illness surveillance. Until recently, isolate-based, pulsed-field gel electrophoresis (PFGE) was the gold standard in laboratory surveillance methods for foodborne illnesses, but with the increased use of CIDT, public health surveillance must find a way to adapt. The purpose of this study was to evaluate the impact of CIDT in clinical and reference laboratories on public health laboratory surveillance. One year of submissions from the Tennessee Public Health Enteric Bacteriology Laboratory, comprising samples of *Salmonella*, *Shigella*, Shiga-toxin-producing *E. coli* (STEC), and *Campylobacter* (n = 2443), were evaluated for submission type, organism requested, recovery rate, and workload. This study found that CIDT comprised 25–45% of all *Salmonella*, *Shigella*, STEC, and *Campylobacter* testing in all but the first month of the study period. Recovery rates from CIDT submissions were 63% for *Salmonella*, 73% for *Shigella*, 54% for STEC, and 40% for *Campylobacter*. Excess cost of workups of CIDT to isolate-based testing ranged from approximately $9–$29 per submission, demonstrating a diminishing return for continued workups when considering technician time and pay and cost of necessary media for these workups. These data suggest the wide variety of recovery rates and workups required to identify *Salmonella*, *Shigella*, STEC, and *Campylobacter* from a CIDT specimen indicates a need for public health laboratory recommendations. This guidance will be valuable until public health labs can implement an isolate-free surveillance system for foodborne pathogens.

29. Potential new drug compounds for Z-Alpha1 antitrypsin deficiency

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Polymerization of the Z variant alpha1 antitrypsin (ZA1AT) results in the most common and severe form of Alpha1 antitrypsin deficiency (a1ATD), a debilitating genetic disorder with clinical manifestations ranging from being asymptomatic to fatal liver and/or lung disease. There is no cure for a1ATD, and current therapeutics are expensive and labor intensive, leaving patients with disability and a short life expectancy. As the altered conformation of Z-a1AT and its attendant aggregation are responsible for pathogenesis, the polymerization process per se has become a major target for the development of therapeutics. In order to search and identify small molecules as inhibitors of ZA1AT polymer growth, a coupled in silico and in vitro strategy was undertaken. From molecular docking performed using the NIH/NCI compounds library, we found 16 hits, some of them sharing chemical homologies. We will present our results for B9, for which in vitro characterization studies have been completed.

30. Pro-inflammatory cytokine IL-1B diminishes butyrate oxidation in colorectal cancer cells

Megan Johnstone, Dallas Donohoe

Nutrition

Most colorectal cancers undergo the Warburg effect. This shifts cell energetics toward increased glycolytic metabolism and away from oxidative metabolism and butyrate oxidation. Butyrate, derived from the fermentation of dietary fiber, is the preferred energetic substrate of the non-cancerous colonocyte. Butyrate, at physiologically relevant levels in the colon, inhibits histone deacetylases to regulate gene expression and subsequently increase apoptosis in colorectal cancer cells. Accordingly, we sought to determine the effects of the pro-inflammatory cytokine interleukin-1 β (IL-1β) on butyrate oxidation in real-time cellular respiration using a Seahorse Analyzer. We used two colorectal cancer cell lines (HCT116 and HT-29) in these studies. Cells were placed in fatty acid oxidation buffer supplemented with or without IL-1B (1 ng/mL) treatment for 1 hr prior to Seahorse assay. Butyrate and 2-deoxyglucose (2DG) were injected into wells. Baseline measurements reveal injected butyrate (final concentration 5 mM) to significantly increase oxidative consumption rate (OCR) as compared with control ($P < .05$, n = 5). Concurrently, cells injected with butyrate and treated with IL-1B showed significantly increased OCR as compared to control ($P < .05$, n = 5), yet significantly decreased butyrate oxidation as compared with butyrate

injected wells ($P < .05$, n = 5). Upon injection of 2DG (final concentration 5 mM), which blocks glucose oxidation leaving only butyrate as the sole energetic substrate, only butyrate-treated cells showed significant increases in OCR ($P < .05$, n = 5). Thus, IL-1β appears to diminish butyrate oxidation in colorectal cancer cells, which may affect subsequent genomic acetylation and all downstream targets including proliferation and apoptotic pathways.

31. Insulin and heat stress alters the activity of the mTOR signaling cascade in bovine mammary cells

Kimberly Kassube, Jeffery Kaufman, Agustin Ríus

Animal Science

The objective of this study was to determine the effects of insulin and heat stress on phosphorylating activity in the mammalian target of rapamycin (mTOR) in protein kinase B (Akt), P70 S6 kinase (S6K1), ribosomal protein S6 (rpS6), and eukaryotic elongation factor 2 (eEF2) in immortalized bovine mammary cell line (MAC-T). Cells were cultured in 15 mL Dulbecco's Modified Eagle Medium with 10% fetal bovine serum and 1 µg/mL insulin at 37°C and 5% CO_2 before treatments were imposed. The experimental design consisted of a 2 × 2 factorial arrangement of treatments with temperature environments 37°C thermoneutral and 41°C HS, and two insulin concentrations, 0 µg/mL and 1 µg/mL for 12 hr. Cell lysates were used in Western blotting to identify total and site-specific phosphorylated forms of Akt (Thr308/Ser473), S6K1 (Thr389), rpS6 (Ser235/236), and eEF2 (Thr56). The relative densities for phosphorylated and total forms of Akt, S6K1, rpS6, and eEF2 were quantified and expressed as phosphorylated to total ratio. Preliminary results indicate a significant HS by insulin interaction for rpS6 ($P < .05$). There was an increase in phosphorylated to total ratio from 0.265 ± 0.09 to 0.6 ± 0.09 response to insulin when cells were exposed to heat stress. However, there was a reduction of this ratio from 0.38 ± 0.09 to 0.2±0.09 in response to insulin when cells were exposed to thermoneutral conditions. The remaining protein factors were not affected by treatments. These results would indicate that the response of mTOR signaling cascade to insulin was altered in MAC-T cells exposed to heat stress.

32. "I do it for her": Mothers' experiences pumping breastmilk for their preterm infants

Katherine Bower, Tara Burnette, Daniel Lewis, Courtney Wright, Katherine Kavanagh

Nutrition (Bower, Lewis, Wright, Kavanagh), Obstetrics and Gynecology, Graduate School of Medicine (Burnette)

Regardless of infant gestational age and/or birthweight, breastfeeding is the recommended mode of infant feeding. When an infant is born preterm, breastfeeding might not be an immediate option. Consequently, many mothers initiate pumping. Despite high initiation rates, continuation of pumping and/or breastfeeding until infant discharge is a concern. Using phenomenological methodology, our aim was to gain an understanding of mothers' experiences pumping for their preterm infant in a Level III Neonatal Intensive Care Unit (NICU). Following purposive sampling, 17 mothers of preterm, very-low-birthweight (VLBW) infants, who had initiated pumping were interviewed. In-depth interviews were conducted in person at the NICU or over the phone, per the mother's preference. All interviews were audio-recorded, transcribed verbatim, and analyzed using Colaizzi's seven steps for data analysis. Five global themes emerged and are presented in the language of participating mothers: 1) "I had one job and that was to make milk," 2) "I learned to cope," 3) "You think of your situation as different and unique," 4) "It's not your baby, it's cold plastic," and 5) "In the end, pumping was worth it." The pumping experience of mothers of preterm infants is unlike the experience of mothers of healthy, term infants. To provide more effective support, it is important to understand the unique challenges these mothers encounter when pumping. Using findings from the global themes, resources are being developed that are specifically tailored toward the needs of mothers of preterm infants to support their pumping and/or breastfeeding efforts.

33. Heat stress reduces phosphorylation activity of the mTOR signaling cascade in bovine mammary cells

Jeffrey Kaufman, Kimberly Kassube, Agustin Rius

Animal Science

Heat stress (HS) alters the metabolism of amino acids and reduces synthesis of caseins in bovine mammary glands. The mammalian target of the rapamycin (mTOR) signaling pathway regulates protein synthesis, and is mediated by protein factors that are activated or inhibited upon

phosphorylation. Our objective was to determine the effect of HS in the phosphorylation activity of mTOR protein factors in a bovine mammary cell line (MAC-T). Cells were cultured in 15 mL Dulbecco's Modified Eagle Medium with 10% fetal bovine serum at 37°C and 5% CO_2. Cells were subjected to one of two treatments: 1) 37°C (control) and 2) 41.5°C (HS) for 12 hr. Cell proteins were harvested and separated by gel electrophoresis and transferred to a polyvinylidene fluoride membrane. Western blotting was conducted to identify total and site-specific phosphorylated forms of protein kinase B (Akt; Thr308/Ser473), P70 S6 kinase (S6K1; Thr389), ribosomal protein S6 (rpS6; Ser235/236), and eukaryotic elongation factor 2 (eEF2; Thr56). The relative densities for phosphorylated and total forms of Akt, S6K1, rpS6, and eEF2 were quantified and expressed as phosphorylated to total ratio. Analysis of variance was conducted using a mixed model. Compared with control, cells exposed to HS decreased phosphorylation to total ratio of Akt (0.41 vs. 0.29; $P < .001$), S6K1 (1.65 vs. 0.97; $P = .042$), and rpS6 (1.45 vs. 1.07; $P < .001$). However, preliminary results indicated that HS did not affect the ratio of eEF2. These results indicate that HS impaired the translation of protein synthesis by altering the activity of mTOR signaling factors in MAC-T cells.

34. Vitamin A status affects hepatic expression of key genes for fuel metabolism

Heqian Kuang

Nutrition

In an effort to understand how VA influences metabolic diseases, we collected hypatocytes from Zucker rats that were fed a vitamin A-deficient (VAD) or a vitamin A-sufficient (VAS) diet. Then the expression level of fuel metabolism genes was measured by Western blot. We found that genes involved in fatty acid synthesis, phospho-acetyl-CoA carboxylase (P-ACC), ATP citrate lyase (ACL), and fatty acid synthesis (FAS), had a higher expression level in VAD rats. VAD rats had higher cluster of differentiation 36 (CD36) and AMP activated protein kinase (AMPKβ) than VAS rats, which were involved in fatty acid oxidation. On the other hand, VAS rats had higher retinoic acid receptor α (RARα), retinaldehyde dehydrogenase family 1 (RALDH1), and glucokinase (GK) than VAD rats. GK is the key enzyme for glycolysis. We conclude that VAD status could increase both fatty acid synthesis and fatty acid metabolism, and that VAS could increase glycolysis and proteins involved in VA metabolism and gene regulation. The mechanisms will help us to understand how VA influences metabolic diseases.

35. Effects of television viewing on enjoyment of exercise in college students

Brittany S. Wilkerson, Brian C. Rider, Kelley Strohacker, Scott E. Crouter, Cary M. Springer, Deborah Baldwin, David R. Bassett, Jr,

Kinesiology, Recreation and Sports Studies (Wilkerson, Rider, Strohacker, Crouter, Bassett), Psychology (Baldwin), Information Technology (Springer)

The purpose of the study was to determine if television viewing increases enjoyment of exercise in college students. Forty-three students (mean ± SD; age = 19 ± 2 years, body mass index = 23.7 ± 3.2 kg/m2) completed two 30-min exercise sessions on a cycle ergometer, in randomized order. During one session, participants viewed the British Broadcasting Corporation's TV program Life (TV), while in the other they did not (No-TV). Heart rate (HR), rating of perceived exertion (RPE), and felt arousal (FAS) were measured at 10, 20, and 30 min of exercise. The physical activity enjoyment scale (PACES) was used to measure enjoyment following each session. Enjoyment of exercise was analyzed with a paired samples *t* test (for PACES). Correlations were used to determine relationship of baseline trait measurements of exercise motivation, preference of exercise intensity, and stress with enjoyment of exercise between conditions. Baseline measures significantly correlated with enjoyment of exercise were divided into high and low groups using a median split, then repeated measures ANOVAs (group × condition) were used to determine if interactions existed. Repeated measures ANOVAs (condition × time) were also performed on HR, RPE, and FAS. Exercise enjoyment during the TV condition was significantly higher than during No-TV ($P = .016$). Motivation was positively correlated with NO-TV ($P = .027$). Specifically, the subcategories of amotivation, identified, and intrinsic motivation were significantly correlated with NO-TV ($P < .05$). No significant interactions between motivation subcategories and exercise conditions existed. HR was significantly lower during TV ($P = .019$), but there were no significant differences in RPE ($P = .127$) or FAS ($P = .215$) between sessions. Television viewing increased enjoyment of exercise, and enjoyment was not influenced by exercise motivation, preference of exercise intensity, or stress.

36. Computer cursor control using imagined body kinematics

Reza Abiri, Griffin Heise, Fernando Schwartz, Xiaopeng Zhao

Mechanical, Aerospace, and Biomedical Engineering (Abiri, Heise, Zhao), Mathematics (Schwartz)

In recent years, the brain-computer interface (BCI) has become one of the most popular research areas in health care and rehabilitation devices. BCI systems create communication between the information of recorded brain activities and computers in order to manipulate the environment based on a patient's/subject's intentions. One interesting neuro-prosthetic device in BCI, a computer cursor controlled by brain activity, was first offered to patients with spinal cord injury via an invasive device; later, its application was employed in games and controlled by external, noninvasive devices. The most popular noninvasive approach, electroencephalography (EEG), has been investigated to control a cursor on a monitor based on various EEG paradigms such as stimuli. Here, we propose to deal with the activation and control of a computer cursor in two dimensions by engaging brain activity signals collected using EEG devices through a novel paradigm called "imagined body kinematics." In contrast to previous noninvasive studies, we will discuss how quickly (min) the subject/patient can be trained based on the proposed paradigm and then for verification, will show the success rate of patient ability to hit random targets on different edges of a monitor in two dimensions. Our goal is to use noninvasive EEG signals to develop a new approach to control a cursor in a fast, efficient, and more affordable manner than existing invasive and noninvasive devices.

37. Synovial fluid-derived mesenchymal stem cells as a cell source for cartilage tissue engineering

Mohammed Zayed, Christopher Caniglia, Nabil Misk, Madhu Dhar

Large Animal Clinical Sciences

To date, no drugs or therapies are available to regenerate affected tissues in cases of osteoarthritis because of low regenerative capacity of articular cartilage. Adult mesenchymal stem cells (MSCs) have been suggested as an alternative solution for cartilage tissue engineering due to their proliferation and chondrogenic capacity. Since MSCs derived from bone marrow have been shown to undergo hypertrophy during chondrogenesis, it is important to identify an alternate source of MSCs that do not undergo hypertrophy while maintaining chondrogenic potential. We hypothesize that synovial fluid-derived MSCs (SFMSCs) may be superior to bone marrow-derived MSCs. Prior to their application in clinical cases, however, their biological properties should be evaluated. To test our hypothesis, synovial fluid was collected aseptically from normal joints and MSCs were isolated in the Regenerative Medicine Laboratory in the Department of Large Animal Clinical Sciences at the University of Tennessee. Cell proliferation was assessed using the CellTiter 96 Aqueous Non-Radioactive (MTS) assay. Nuclear/cytoplasmic staining with wheat germ agglutinin and TO-PRO-3 iodide were used for evaluating cellular morphology and viability throughout the expansion process. Adipogenesis, osteogenesis, and chondrogenesis were monitored microscopically and confirmed by cell-specific staining. To further understand the mechanism of chondrogenesis, we used indirect immunofluorescence and immunoblot analyses to investigate the expression of key chondrocyte progenitor proteins. All data show that SFMSCs adhere to the tissue culture plastic, proliferate, and have higher chondrogenic potential. The results suggest that synovial fluid represents a potentially attractive source of MSCs, which may have utility for cartilage repair therapies in trauma.

38. Canine mesenchymal stem cells cultured on novel 3D nanofiber scaffolds have increased proliferation and differentiation efficiency, while maintaining cell multipotency

Michael K. Conway, Kusum Rathore, Maria Cekanova

Department of Small Animal Clinical Sciences

Novel methods to improve culture and directed differentiation of canine adipose-derived mesenchymal stem cells (cADMSC) are important for advancing in vitro studies and cell-based therapies. The extracellular matrix (ECM) microenvironment, in which cells are cultured in vitro, has a strong influence on the biological activity of MSCs. Novel substrates and scaffolds that enable MSCs to recapitulate complex in vivo biological activities are highly desirable. With the immediate aim of improving cADMSC culture efficiency and long-term goals of developing improved cADMSC-based clinical therapies, we characterized cADMSC growth on aligned or randomly oriented polycaprolactone nanofibers integrated into standard culture dishes sold under the

trade names NanoAligned and NanoECM 3D cell culture scaffolds (Nanofiber Solutions). Proliferation of cADMSCs was increased on both aligned and random nanofiber substrates compared with a standard treated tissue culture plate. Despite an increased proliferation rate, Nanog, Sox2, and Oct4 gene expression in cADMSCs cultured on the nanofiber substrates was similar to a control plate. Directed differentiation efficiency of cADMSCs into osteo-, chondro- and adipogenic lineages was also enhanced when cultured on the nanofiber substrates as compared with control plates. Together, our preliminary data suggest cADMSCs cultured on aligned or randomly deposited 3D polycaprolactone substrates have increased proliferation and differentiation efficiency, while maintaining cell multipotency.

39. Graphene and stem cell combination for bone tissue regeneration: In vitro to in vivo

Hoda Elkhenany, Silke Hecht, Alexandru Biris, David Gerard, David Anderson, Ramadan Abdelwahed, Madhu Dhar

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When tissue engineering is applied to create bone substitutes or enhance osseous healing, various cell types, scaffolding materials, and growth factors are considered. In the current study, we tested the in vitro proliferation and osteogenic differentiation of caprine bone marrow derived MSCs (BMMSCs) on graphene films. To test the in vivo biocompatibility and the bone-forming potential, 3-month-old Sprague Dawley rats were divided into three groups: graphene only, BMMSCs only, and graphene+ BMMSCs. Cells and/or graphene was implanted into a 2–4-mm diameter unicortical defect in the tibia. In each rat, the right tibia was used as the control limb, and the contralateral tibia served as the treated limb. Bone response was assessed with computed tomography at days 0 (pre) and 45 (post) and with histomorphometry at the end of the study. Results demonstrated that in vitro, the BMMSCs could not undergo osteogenic differentiation in the presence of osteogenic enhancing factors but were able to differentiate in the presence of regular growth media. To measure bone healing in vivo, the regions of interest were marked, and the area was calculated using CT scans and compared amongst different groups. The BMMSC-treated limb showed a significant increase in healing compared with the control limb, while there was no significant difference in the graphene-alone group and the graphene+ BMMSCs. In conclusion, BMMSCs are a suitable cell type to be included in regenerative medicine for bone tissue regeneration, in particular. More research is needed to understand the use of BMMSCs and graphene in bone healing in vivo.

40. Characterization of ligand-induction of influenza hemagglutinin

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Chemical and Biomolecular Engineering

Fusion proteins facilitate the combining of two membranes in processes such as viral infection, secretion, and phagocytosis. Hemagglutinin (HA) is viral fusion protein that undergoes an irreversible conformational change upon acidification to catalyze the fusion of endosomal and viral membranes during influenza infection. This rearrangement, which is essential for fusion activity, exposes a 20 amino acid sequence, the fusion peptide, which is previously buried within the protein's core. The conformational change of HA to the fusion active state has been associated with a cell-wide autocatalytic activation. Other viral fusion proteins go through similar rearrangements in order to deliver viral DNA to the host cell. Understanding and engineering the mechanism of viral fusion protein activity will lend insight toward the design of efficient delivery systems for transport of drugs or genes across cell membranes. Here, we probed the kinetics of autocatalytic activation and aggregation via fluorescence assays. Kinetic data demonstrate a delay of approximately 7 min prior to activation. We also demonstrated the creation of an alternative pathway to HA activation; by engineering the fusion peptide region of HA to contain a ligand binding peptide, HA is induced to refold in response to ligand exposure, as assessed by flow cytometry of cells expressing engineered proteins. Thus, extrusion of the fusion peptide region appears sufficient to induce the HA conformational change. Furthermore, transfected cells co-expressing HA engineered for ligand induction with non-ligand-induced protein show activation of all HA via intermolecular communication, consistent with the autocatalytic induction model.

41. Pharmacokinetics of orally administered low-dose rapamycin in healthy dogs: A pilot study

Jeanne Larson, Sara Allstadt, Tim Fan, Chand Khanna, Paul Lunghofer, Ryan Hansen, Dan Gustafson, Alfred Legendre, Gina Galyon, Amy LeBlanc, Tomas Martin-Jimenez

Small Animal Clinical Sciences (Larson, Allstadt, Legendre, Galyon); Cancer Care Clinic, University of Illinois at Urbana-Champaign (Fan); National Institutes of Health, National Cancer Institute, Center for Cancer Research, Comparative Oncology Program (Khanna, LeBlanc); Flint Animal Cancer Center, Colorado State University (Lunghofer, Hansen, Gustafson); Biomedical and Diagnostic Sciences (Martin-Jimenez)

Rapamycin is an antifungal antibiotic agent that has immunosuppressant and anti-cancer properties. By inhibiting mTOR, rapamycin acts as an anti-proliferative and anti-angiogenic agent. The purpose of this pilot study was to determine the blood concentrations of rapamycin achieved following a single dose and multiple oral doses in the dog. Five healthy, purpose-bred hound dogs were enrolled. In experiment 1, dogs received a single 0.1 mg/kg dose orally. Blood was collected at 0, 0.5, 1, 2, 4, 6, 12, 24, 48, and 72 hr. In experiment 2, dogs received 0.1 mg/kg daily for 5 days. Blood was collected at 0, 3, 6, 24, 27, 30, 48, 51, 54, 72, 75, 78, 96, 96.5, 97, 98, 100, 102, 108, 120, 144, and 168 hr. Samples were analyzed using a validated LC/MS/MS assay and evaluated via compartmental and non-compartmental analysis. Variable blood concentrations between single and consecutive dosing were noted. The mean pharmacokinetic values for experiment 1 were half-life (t1/2) = 38.7 ± 12.7 hr, area under the curve (AUC) = 140 ± 23.9 ng*h/mL, and maximum concentration (Cmax) = 8.39 ± 1.73 ng/mL. The mean pharmacokinetic values on day 5 for experiment 2 were t1/2 = 99.3 ± 89.5 hr, AUC = 126 ± 27.1 ng*h/mL, and Cmax = 5.49 ± 1.99 ng/mL. Rapamycin administered orally at 0.1 mg/kg achieves measurable blood concentrations. Goals of ongoing studies in tumor-bearing dogs are to determine the optimal dose and frequency to achieve clinically relevant concentrations and to determine toxicity.

42. Effects of doxorubicin and its derivative, AD198, in oral squamous cell carcinoma cells in vitro

Dmitriy Smolensky, Kusum Rathore, Maria Cekanova

Small Animal Clinical Sciences (Smolensky, Rathore, Cekanova), Graduate School of Genome Science and Technology (Smolensky, Cekanova)

Over 40,000 new cases of oral squamous cell carcinoma (OSCC) will be diagnosed in 2015 in the United States. Most OSCC cancers are extremely hard to treat surgically due to the aggressiveness and location of cancer. The 5-year survival rate of OSCC is approximately 57%. In order to improve survival, new chemotherapy agents and combinations need to be researched. Doxorubicin (Dox) is a widely used chemotherapeutic agent for several types of cancers, but has been shown to have severe adverse events with low efficiency for treatment of OSCC. A novel derivative of Dox, AD198, has shown in previous studies similar effectiveness as Dox on treatment of lymphoma in rodent models in vitro and in vivo but without developing drug resistance and with less adverse events. In this study, we evaluated the effects of Dox and AD198 on human OSCC cell lines in vitro and explored their molecular mechanisms on OSCC cell growth inhibition. Our preliminary results suggest that AD198 is more effective that Dox in inhibiting OSCC viability. Dox and AD198 induce apoptosis through activation of the MAPK p38 signaling pathway; however, Dox and AD198 also activate the pro-survival PI3K/AKT signaling pathway. Inhibition of the PI3K/AKT pathway by LY294002 further enhanced the activation of MAPK p38 pathway and increased downstream apoptosis induced by Dox and AD109. Taken together, our preliminary results suggest that AD198 may be an effective treatment option for OSCC resistant to Dox, and co-treatment of AD198 or Dox with PI3K/AKT inhibitors may increase anti-proliferative effects in OSCC.

43. A novel derivative of doxorubicin, AD198, inhibits cell growth of canine transitional cell carcinoma and osteosarcoma cells in vitro

Kusum Rathore, Maria Cekanova

Small Animal Clinical Sciences

Doxorubicin (DOX) is one of the most commonly used chemotherapies for a wide range of cancers. Because of the adverse effects of DOX, including hair loss, nausea, vomiting, liver dysfunction, and cardiotoxicity, novel derivatives

of DOX have been synthesized and characterized. In this study, we evaluated and compared the effects of DOX and its derivative, N-benzyladriamycin-14-valerate (AD198). AD198 is a lipophilic anthracycline that has shown to target protein kinase C delta (PKC-δ) in the cytoplasm of cells. We evaluated the effects of DOX and AD198 in three canine transitional cell carcinoma (K9TCC-Dakota, K9TCC-Lillie, and K9TCC-Molly) and three canine osteosarcoma (K9OSA-Zoe, K9OSA-Nashville, and K9OSA-JJ) primary cell lines. AD198 significantly inhibited cell proliferation in all tested K9TCC and K9OSA primary cell lines in a dose-dependent manner. AD198 increased inhibition of cell viability of K9TCC and K9OSA compared with DOX after 48-hr treatments using MTS assay. AD198 had lower IC50 values compared with DOX for all tested K9TCC and K9OSA cell lines. In addition, AD198 increased apoptosis in all tested K9TCC and K9OSA primary cell lines through increased caspase activity and poly ADP ribose polymerase (PARP) cleavage. Subsequently, the AD198-activated PKC-δ activated p38 signaling pathway resulted in enhanced apoptosis of tested canine primary cancer cell lines. AD198-induced apoptosis through activation of the PKC-δ and p38 pathway in K9TCC and K9OSA cells in vitro suggests that AD198 might be considered as a new treatment option for TCC and OSA cancers in vivo.

44. Effects of novel receptor tyrosine kinase inhibitors and non-steroidal anti-inflammatory drugs in bladder cancer

Jennifer Bourn, Kusum Rathore, Maria Cekanova

Genome Science and Technology (Bourn), Small Animal Clinical Sciences (Rathore, Cekanova)

A variety of carcinomas, such as transitional cell carcinoma (TCC), overexpress several receptor tyrosine kinases (RTK), such as the platelet-derived growth factor receptor, c-kit receptor, epidermal growth factor receptor, as well vascular endothelial growth factor receptor. RTK inhibitors (RTKIs), such as AB1010 and Axitinib, are used as targeted treatment options for patients diagnosed with cancer that have high expression of RTKs. Cyclo-oxygenase-2 (COX-2) is highly over-expressed in TCC and other types of cancers and is a key protein in tumorigenesis. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used for treatment of not only inflammation, but also for prevention and treatment of cancers. COX-2 selective inhibitors, such celecoxib, have been shown to prevent and inhibit cancers not only in vitro, but also in vivo. In this study, we validated the effects of RTKIs in combination with NSAIDs to inhibit TCC cell proliferation. Our preliminary results have shown that both RTKIs (AB1010 and Axitinib) and novel NSAIDs inhibit cell proliferation in both canine and human bladder cancer cell lines (UMUC-3, T24, K9TCCLillie, and K9TCCLilly) in a dose-dependent manner in vitro by MTS assay. The RTKI Axitinib, at a 10-µM concentration, was one of the most effective treatments in human and canine TCC cells in vitro. Interestingly, RTKIs (AB1010 and Axinitib) increased expression of COX-2 levels in the tested human and canine TCC cell lines. In a future study, we plan to evaluate the effects of combination therapy of tyrosine kinase inhibitors and NSAIDs in bladder cancers in vitro and in vivo.

45. Effects of chronic exposure to benzo[a]pyrene, a lipophilic mammary carcinogen, on stromal fibroblasts: Role in breast carcinogenesis

Emily Heal, Hwa-Chain Robert Wang, Ling Zhao

Nutrition (Heal, Zhao), Biomedical and Diagnostic Sciences (Wang)

Breast cancer is the most common cancer in women and the second leading cause of cancer-related deaths in women worldwide. More than 85% of breast cancers are sporadic and attributed to chronic exposure to carcinogens derived from diet, smoking, and environmental pollution. It has been recognized that the development and progression of breast cancer results from complex interactions between cancerous epithelial cells and the surrounding stromal environment. While effects of carcinogens on cellular carcinogenesis of breast epithelial cells are well documented, the effects of carcinogens on stromal cells, stromal fibroblasts in particular, have not been studied. A better understanding of the role of carcinogen-exposed stromal fibroblasts in breast carcinogenesis may lead to new and effective strategies for breast cancer prevention and treatment. Using benzo[a] pyrene (B[a]P), a known lipophilic mammary carcinogen, we aim to characterize the role of B[a]P-exposed fibroblasts in breast carcinogenesis. 3T3-L1 fibroblasts were exposed to B[a]P at an environmentally relevant dose of 100 pM for 5, 10, and 20 cycles. The effects of B[a]P exposure on fibroblast gene expression, growth, and survival were examined. Our preliminary results show B[a]P exposure decreased fibroblast survival and induced characteristic changes in gene expression, some changes that are synonymous to cancer-associated fibroblasts known to contribute to tumor growth and chemoresistance.

46. Reactive oxygen species-mediated breast cell carcinogenesis enhanced by multiple carcinogens and intervened by dietary ergosterol and mimosine

Lenora Pluchino, Hwa-Chain Robert Wang

Biomedical and Diagnostic Sciences

Most breast cancer is sporadic and attributable to long-term exposure to small quantities of carcinogens present in our environment and diet, specifically in smoke, polluted air, and high-temperature cooked meats. To understand how multiple carcinogens act together to induce breast cell carcinogenesis, we investigated the activity of the tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), the environmental carcinogen benzo[α]pyrene (B[α]P), and the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). We demonstrated that co-exposure to physiologically achievable doses of combined NNK, B[α]P, and PhIP (NBP) holistically enhanced initiation and progression of breast cell carcinogenesis. Reactive oxygen species (ROS) and ERK pathway activation were transiently induced by each NBP exposure, and crosstalk between reinforced ROS elevation and ERK activation played an essential role in increased DNA oxidation and damage. After cumulative NBP exposures, this crosstalk contributed to enhanced initiation of cellular carcinogenesis and led to enhanced acquisition of cancer-associated properties. Using NBP-induced transient changes, including ROS elevation and ERK pathway activation, and cancer-associated properties as targeted endpoints, we revealed, for the first time, that two less-studied dietary compounds, ergosterol and mimosine, at physiologically achievable non-cytotoxic levels, were highly effective in intervention of NBP-induced cellular carcinogenesis. Combined ergosterol and mimosine was more effective than individual agents in blocking NBP-induced transient endpoints, including ROS-mediated DNA oxidation, which accounted for their preventive ability to suppress progression of NBP-induced cellular carcinogenesis. Thus, dietary components, such as mushrooms containing ergosterol and legumes containing mimosine, should be considered for affordable prevention of sporadic breast cancer associated with long-term exposure to environmental and dietary carcinogens.

47. Carnitine is a critical contributor for butyrate oxidation in colon cancer cells

Anna Han, Donohoe Dallas

Nutrition

Butyrate, derived from colonic microbial fermentation of dietary fiber, is the primary energy source of the colonocyte. Butyrate also regulates gene expression in the colonocyte by acting as an inhibitor of histone deacetylases (HDACs). The main objective of this study was to identify mechanisms that influence butyrate oxidation in cancerous colonocytes (HCT116). We hypothesized that carnitine, which is used to mainly shuttle long-chain fatty acids into the mitochondria via CPT1, would be required to achieve full butyrate oxidation in the cancerous colonocyte. The Seahorse XF24 Analyzer was used to measure butyrate oxidation in HCT116 cells with and without carnitine. The butyrate oxidation in HCT 116 cells incubated with carnitine was significantly higher than those without carnitine ($P < .05$). Next, we predicted that if carnitine increased butyrate oxidation, it would lower intracellular butyrate, suppress HDAC inhibition, and diminish butyrate induced histone acetylation. This was indeed the case as the relative H3 acetylation induced by butyrate was significantly decreased in HCT116 cells treated with carnitine ($P < .05$). Furthermore, we found that only butyrate oxidation was shown to be carnitine dependent when compared with other short-chain fatty acids ($P < .05$). This suggests that carnitine is a pivotal contributor toward maximum butyrate oxidation and butyrate-induced histone acetylation in colorectal cancer cells.

48. 3,4,4′-Trichlorocarbanilide exposure induces gut microbial dysbiosis in weaned rats

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Widely used as an antimicrobial in bar soaps, triclocarban (3,4,4′-trichlorocarbanilide; TCC) shows greater efficacy against gram-positive bacteria than gram-negative bacteria. This dichotomy may lead to overgrowth of bacterial populations less susceptible to the action of TCC. Sprague-Dawley (SD) rats were exposed to TCC (at 0.2% or 0.5% w/w) through chow for 4 weeks starting on postnatal day (PND) 22, followed by a washout period of 4 weeks without exposure. Same age SD rats served as sham controls without TCC exposure during the whole study period. Baseline samples were collected on PND 21 prior to TCC exposure and collected weekly. Genomic DNA was extracted, followed by PCR with barcode-labeled primers targeting the v4 region of 16S rDNA. The barcode-labeled PCR products were sequenced on the MiSeq platform. TCC exposure significantly altered GI tract microbiota composition, which was revealed by a dose-dependent overall bacterial community richness (ADONIS; $P \leq .001$). This perturbation was noticeable as early as 2 days post-treatment with approximately 16.5–18.6% of OTUs significantly enriched ($P \leq .05$) and continued throughout study period. At the community level, TCC withdrawal produced a gradual return of microbial diversity to a pre-treatment state in the 0.2% w/w group, but not the 0.5% w/w group as assessed by weighted Unifrac distance metric. Collectively, these data highlight the present and long-term impacts of early life TCC exposure on gut microbial ecology and imply the potential niche for opportunistic pathogen growth due to alteration of microbiota composition by antimicrobials.

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