34th ANNUAL RESEARCH SYMPOSIUM

Research Without Boundaries

VIRGINIA-MARYLAND COLLEGE OF VETERINARY MEDICINE

May 9, 2025 • The Inn at Virginia Tech • 7:00 AM - 8:00 PM

Welcome to the 34th Annual Research Symposium

Welcome to the 34th Annual Research Symposium of the Virginia-Maryland College of Veterinary Medicine. This year's theme, "Research Without Boundaries," reflects not only the breadth and depth of the scholarship across our college, but also the essential role of research in advancing science and improving lives—particularly at a time when the value of scientific inquiry is being questioned in some sectors of society. We believe that now, more than ever, it is important to reaffirm our commitment to the pursuit of knowledge and to celebrate the researchers who make that possible.

This annual symposium is a joint effort between the Biomedical and Veterinary Sciences (BMVS) graduate program and the VMCVM Research Committee, chaired by Dr. Coy Allen. The event showcases the incredible diversity of research within our college—diversity that enhances our intellectual community and strengthens our collective impact.

We are honored to host Dr. Michael D. L. Johnson as our keynote speaker. Dr. Johnson is an Associate Professor in the Department of Immunobiology at the University of Arizona, where he studies metal homeostasis in bacteria. His leadership in science communication and mentoring—through programs such as the National Summer Undergraduate Research Project and The BIO5 Postdoctoral Fellowship—exemplifies the spirit of boundary-crossing research and outreach that this year's theme celebrates.

This year's symposium features eight faculty research talks representing all four departments within the college, and eight graduate student oral presentations, selected through a highly competitive abstract review process. These student speakers represent the top submissions from among more than 70 abstracts received. We commend all of our student researchers for their hard work and scholarly contributions. Every student who submitted an abstract is presenting a poster today, and we encourage all attendees to engage with them and learn more about their work.

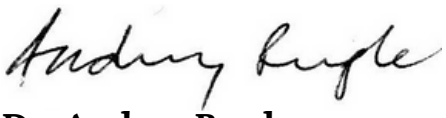
This event would not be possible without the dedication and support of many individuals. Special thanks go to the faculty and staff of the Office of Research and Graduate Studies, including Dr. Jessica Crawford, Dr. Shannon Johnson, Monica Taylor, Liz Bowman, and Alejandro Saiden Gonzalez, as well as our Graduate Teaching Assistant, Brittany Heath.

I am also grateful to the members of the VMCVM Research Committee:

- From Biomedical Sciences and Pathobiology (BSP): Drs. Coy Allen, Erin Gloag, James Weger, and Kylene Kehn-Hall
- From Small Animal Clinical Sciences (SACS): Drs. Go Togawa and Orsi Balogh
- From Large Animal Clinical Sciences (LACS): Drs. Sophie Bogers and Becky Funk
- From Population Health Sciences (PHS): Dr. Ryan Calder

Thank you to the graduate students from the BMVS, Master of Public Health (MPH), and Translational Biology, Medicine, and Health (TBMH) programs who helped organize this event, reviewed abstracts, and participated in the symposium. And finally, thank you to our many dedicated faculty mentors for your continued support of student scholarship and discovery.

Together, we reaffirm our belief that science is a shared endeavor that knows no boundaries.



Dr. Audrey Ruple
Program Director, Biomedical and Veterinary Sciences Graduate Program

BIOMEDICAL AND VETERINARY SCIENCES
VIRGINIA TECH

PUBLIC HEALTH
VIRGINIA TECH

GRADUATE SCHOOL
VIRGINIA TECH

TRANSLATIONAL BIOLOGY, MEDICINE, & HEALTH

PROGRAM

Research Without Boundaries

In a time when science and expertise are increasingly questioned, "Research Without Boundaries" reaffirms our dedication to open, rigorous, and inclusive discovery. It underscores our belief that addressing today's most pressing challenges requires not only scientific excellence, but also collaboration that reaches across disciplines, cultures, and geographies.

7:00 am	**Registration and Continental Breakfast**
7:45 am	**Welcome**
8:00 am	**Population Health Sciences** *Faculty Speaker: Dr. Sophie Wenzel* *Faculty Speaker: Dr. Alasdair Cohen* *Graduate Student Speaker: Syeda Fatema* *Graduate Student Speaker: Janice O'Brien*
9:15 am	**Break/Networking**
9:30 am	**Biomedical Sciences and Pathobiology** *Faculty Speaker: Dr. Kirsten Nielsen* *Faculty Speaker: Dr. Erin Gloag* *Graduate Student Speaker: Katie Tiller* *Graduate Student Speaker: Kaylee Petraccione*
10:45 am	**Break/Networking**
11:00 am	**Keynote Speaker Dr. Michael D. L. Johnson** **"Using Copper Toxicity to Exploit a Microbial Achilles Heel"**

PROGRAM

Research Without Boundaries

12:00 pm **Lunch**

1:00 pm **Poster Session**
1:00PM to 2:00PM Even numbered posters
2:00PM to 3:00PM Odd numbered posters

3:00 pm **Large Animal Clinical Sciences**
Faculty Speaker: Dr. Sophie Bogers
Faculty Speaker: Dr. Vitor Mercadente
Graduate Student Speaker: Katherine Gottleib
Graduate Student Speaker: Emilee Lacey

4:15 pm **Break/Networking**

4:30 pm **Small Animal Clinical Sciences**
Faculty Speaker: Dr. Michele Borgarelli
Faculty Speaker: Dr. Richard Shinn
Graduate Student Speaker: Ny Luong
Graduate Student Speaker: Samantha McCarter

5:45 pm **Reception**

6:00 pm **Dinner and Awards**

KEYNOTE SPEAKER

Dr. Michael D. L. Johnson

“Using Copper Toxicity to Exploit a Microbial Achilles Heel”

Associate Professor
Department of Immunobiology
College of Medicine - Tucson
The University of Arizona

Dr. Michael D. L. Johnson received an A.B. in Music from Duke University and his Ph.D. in Biochemistry and Biophysics at the University of North Carolina at Chapel Hill. After completing his dissertation in bacterial motility and attachment, he went to St. Jude Children’s Research Hospital in the Department of Infectious Disease to study how bacteria process nutrients, specifically metals, during infections. Currently, Dr. Johnson is an Associate Professor at the University of Arizona in the Department of Immunobiology where his main field of study is copper homeostasis in Streptococcus species. He is active in science outreach through developing and directing the National Summer Undergraduate Research Project and The BIO5 Postdoctoral Fellowship Program.

ORAL PRESENTATIONS

Population Health Sciences

FACULTY SPEAKERS

Dr. Sophie Wenzel

Interpersonal violence and substance use as precursors to incarceration: interviews with women at the New River Regional Jail

Dr. Alasdair Cohen

Advancing Methods for Wastewater-based Surveillance and Epidemiology at Sub-sewershed and Rural Community Scales

GRADUATE STUDENT SPEAKERS

Syeda Fatema
(Mentor - Dr. Nick Ruktanonchai)

Analyzing Healthcare Accessibility Patterns in Virginia's New River Valley during the COVID-19 Crisis

Dr. Janice O'Brien
(Mentor - Dr. Audrey Ruple)

Home-prepared diets for companion dogs feature diverse ingredients and few are nutritionally complete

ORAL PRESENTATIONS

Biomedical Sciences and Pathobiology

FACULTY SPEAKERS

Dr. Kirsten Nielsen

Development of Anthelmentic Benzamidazoles as Antifungals

Dr. Erin Gloag

Early Kinetics of Chronic Infection

GRADUATE STUDENT SPEAKERS

Katie Tiller
(Mentor - Dr. James Weger)

Auranofin Demonstrates Antiviral Effects Against Hepatitis E Virus Via Reactive Oxygen Species

Kaylee Petraccione
(Mentor - Dr. Kylene Kehn-Hall)

Discovering host-viral protein interactions in autophagy:Identifying LC3-interacting region (LIR) motifs in hemorrhagic fever viruses for therapeutic targeting

ORAL PRESENTATIONS

Large Animal Clinical Sciences

FACULTY SPEAKERS

Dr. Sophie Bogers

Leading the Field: An Equine-Centered Approach to One-Health Research

Dr. Vitor Mercadente

Going to prison for the good of science!

GRADUATE STUDENT SPEAKERS

Dr. Katherine Gottleib
(Mentor - Dr. Christopher Byron)

Comparison of gait characteristics for horses without shoes, with steel shoes, and with aluminum shoes

Dr. Emilee Lacey
(Mentor - Dr. Katie Wilson)

Pharmacokinetics of Oral Mirtazapine in Healthy Adult Alpacas

ORAL PRESENTATIONS

Small Animal Clinical Science Block

FACULTY SPEAKERS

Dr. Michele Borgarelli

The LOOK-mitral study: relevance of registry studies in the clinical research

Dr. Richard Shinn

Technology in Veterinary Neurology - Both Old & New

GRADUATE STUDENT SPEAKERS

Ny Luong
(Mentor - Dr. Joanne Tuohy)

Immunomodulatory effects of histotripsy and N-dihydrogalactochitosan in preclinical murine and comparative canine models of osteosarcoma

Dr. Samantha McCarter
(Mentor - Dr. Orsolya Balogh)

Examining milk composition and neonatal puppy growth between overweight and lean canine dams

O1/P50
Population Health Sciences
Analyzing Healthcare Accessibility Patterns in Virginiaís New River Valley during the COVID-19 Crisis

Syeda Nahid Fatema, Dr. Nick Ruktanonchai
[1]*Virginia Tech, USA*

Introduction:
Healthcare accessibility varies across space and time, particularly for vulnerable populations. The COVID-19 pandemic exacerbated disparities, especially in rural areas where travel distance significantly impacts healthcare-seeking behavior. Geographic inequalities in healthcare access contribute to negative health outcomes, as longer travel times often deter individuals from seeking timely care. This study examines healthcare utilization patterns across Virginiaís New River Valley (NRV), focusing on Montgomery, Pulaski, Radford, Floyd, and Giles counties during the pandemic. Understanding these disparities is crucial for developing targeted interventions.

Methodology and Approach:
We used SafeGraph cell phone mobility data to analyze healthcare access trends. This dataset allowed us to track visits to healthcare points of interest (POIs) from census block groups (CBGs) and assess travel distances from residential areas to healthcare facilities. We measured distances between CBG centroids and POIs, weighting them by visitor numbers to quantify travel burdens. GIS-based mapping visualized spatial disparities, while temporal analysis examined shifts in healthcare-seeking behavior across different pandemic phases.

Results:
Findings reveal significant disparities in healthcare access. Residents in certain CBGs, particularly in rural areas, experienced longer travel distances to healthcare facilities. Temporal analysis showed fluctuating healthcare utilization, with mobility patterns shifting across different pandemic stages. Travel burdens were highest in remote areas, exacerbating existing inequities. The combination of spatial and qualitative data underscores systemic barriers affecting healthcare-seeking behaviors.

Conclusion:
This study reveals how geographic disparities impact healthcare accessibility, especially during public health crises, enriching the understanding of how long travel distances disproportionately burden rural populations, leading to potential delays in care and poorer health outcomes. Findings emphasize the need for data-driven policies to improve accessibility in underserved areas, ensuring healthcare reaches those most in need. This study will guide future research by identifying critical gaps in accessibility and highlighting strategies to build more resilient and equitable healthcare systems.

O2/P18
Population Health Sciences
Home-prepared diets for companion dogs feature diverse ingredients and few are nutritionally complete

Janice O'Brien, Ellyott Lawson, Katie Tolbert, Audrey Ruple

[1]*Population Health Sciences Department, Virginia-Maryland College of Veterinary Medicine at Virginia Tech*
[2]*Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX*

Introduction: Home-prepared diets for pet dogs have gained popularity over recent decades. Owners often cite concerns about ingredient sourcing and perceived health benefits as reasons for this dietary shift. However, many owners may unknowingly provide nutritionally inadequate meals. The Association of American Feed Control Officials (AAFCO) provides nutritional guidelines for commercial pet foods, but most recipes for home-prepared diets that are published online or in books do not follow these guidelines. Moreover, studies focusing on diets intended to be fed for specific health conditions have shown that none of the analyzed recipes were appropriate for managing diseases like kidney disease and cancer. However, no studies have previously evaluated home-prepared diets as fed by owners.

Methodology and approach: Survey responses from dog owners with dogs enrolled in the Dog Aging Project included free-text dietary information between January and December 2023 for owners who self-identified as feeding an entirely home-prepared diet. Two independent coders reviewed the responses to categorize dietary ingredients, while five additional coders input the ingredients into a specialized website designed by veterinary nutritionists (Balance.it) to evaluate dietary completeness, categorizing diets as ìcompleteî (no deficiencies), ìpartially deficientî (deficient in 10 nutrients) or ìcannot codeî if there was not enough information to code. A board-certified veterinary nutritionist assessed a subset of the raw meat diets to assess whether the coding scheme adequately handled raw meat diets.

Results: 1765 home-prepared diets consisted of various ingredients, primarily meat (90%) and vegetables (65%). Within ingredient categories, there was considerable variability of individual ingredients: owners are not just feeding chicken and rice. Nearly half (45%) of dog owners supplemented their home-prepared meals with some form of commercially prepared food. Alarmingly, only 6% of the analyzed diets were nutritionally complete.

Conclusion: While home-prepared diets can include a wide range of ingredients, most of the diets in this population were not nutritionally complete. Veterinarians should thoroughly evaluate home-prepared diets reported by owners, utilizing various resources, including consultation with veterinary nutritionists, to guide owners in selecting appropriate maintenance diets.

O3/P15
Biomedical Sciences and Pathobiology
Auranofin Demonstrates Antiviral Effects Against Hepatitis E Virus Via Reactive Oxygen Species

Tiller, K., Williams, S.T., Wang, B., Meng, X., Weger-Lucarelli, J.

[1]*Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA*
[2]*Center for Emerging, Zoonotic and Arthropod-borne Pathogens, Virginia Tech, Blacksburg, VA†*

Hepatitis E virus (HEV) is a globally distributed hepatotropic virus that can cause severe disease and death in immunocompromised individuals and pregnant women. While there are currently no approved antiviral therapeutics for treating HEV, ribavirin and pegylated interferon are used off-label and represent the current standard of care for treating severe HEV infections. However, the identification of ribavirin resistant HEV strains highlights the urgent need to discover or repurpose additional drugs that are safe and effective for treating HEV infections. Auranofin, an FDA-approved antirheumatic drug, has been implicated to be repurposed for treating a wide range of ailments, including viral, bacterial, and parasitic infections as well as inflammatory diseases and cancer. Thus, auranofin was examined for its antiviral potential against HEV. At non-toxic concentrations, auranofin displays dose-dependent antiviral activity against multiple HEV genotypes in a human hepatocyte cell line using replicon and infectious virus systems. Auranofin also displays antiviral activity against a ribavirin resistant HEV mutant in vitro. Investigations into the antiviral mechanism of action reveal that auranofin treatment results in an intracellular accumulation of reactive oxygen species (ROS). When common ROS inhibitors are applied in conjunction with auranofin, a reversal of antiviral activity and ROS accumulation is observed, suggesting that ROS mediates auranofinís antiviral activity. Other ROS promoting compounds also display antiviral activity that can be reversed by ROS inhibitors. Together this data suggest that auranofin mediates antiviral effects against HEV through ROS. Lastly, a combined treatment of auranofin and ribavirin display synergistic antiviral activity in vitro, highlighting the potential for combined auranofin and ribavirin treatments in vivo to increase antiviral potential and prevent ribavirin resistance since these drugs act via different pathways and do not display synergistic toxicity in vitro. Altogether, our data supports the repurposing of auranofin as an antiviral against several HEV genotypes that could be administered in combination with the current standard of care to increase antiviral efficacy and prevent drug resistance.

O4/P28

Biomedical Sciences and Pathobiology

Discovering host-viral protein interactions in autophagy: Identifying LC3-interacting region (LIR) motifs in hemorrhagic fever viruses for therapeutic targeting.

Kaylee Petraccione[1,2], Mohamed G. H. Ali[3,4], Normand Cyr[3], Haytham M. Wahba[3,4], Timothy Stocker[1], Ivan Akhrymuk[1,2], Nicole Bracci[1,2], Yossira Swese[3], Danuta Sastre[5], Andrew Silberfarb[6], Paul OíMaille[5], James G. Omichinski[3], Kylene Kehn-Hall[1,2]

[1]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA
[2]Center for Emerging, Zoonotic, and Arthropod-borne Pathogens, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA
[3]Department of Biochemistry and Molecular Medicine, UniversitÈ de MontrÈal, MontrÈal, QC, Canada
[4]Department of Biochemistry, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt
[5]Biosciences Division, SRI International, Menlo Park, CA, USA
[6]Artificial Intelligence Center, SRI International, Menlo Park, CA, USA

Hemorrhagic fever viruses (HFVs) are highly infectious, causing severe disease with bleeding, organ failure, and high mortality. HFVs represent a major global health and economic concern, particularly in areas with limited access to healthcare. Despite their devastating impact, there are limited FDA-approved treatments available. To address this, we developed an AI and machine learning (ML)-driven pipeline to identify and analyze LC3-Interacting Region (LIR) motifs in HFV proteins. These motifs are critical for HFV interaction with host LC3 proteins, which play a key role in autophagy, either combating infections by enhancing immune responses and promoting viral degradation or, in rare cases, supporting viral replication. We hypothesize that HFV proteins interact with LC3-family members via LIR motifs to modulate the host autophagy pathway which represents a target for therapeutic development. Our LIR discovery pipeline identified 38 putative LIR motifs in 166 proteins from 22 HFVs using the iLIR and ELM databases. The structure of viral proteins containing a putative LIR motif was determined using AlphaFold3 to determine if the LIR motif was in a predicted unstructured region of the protein. LIR motifs found within an unstructured region were further prioritized for analysis. 16-mer peptides from the viral proteins were then modeled in complex with LC3 using AlphaFold3and the complexes were refined and ranked using FoldX. Focusing on one high-ranking interaction, we explored the NSs protein of Rift Valley fever virus (RVFV), a key virulence factor and antiviral target. Validation through isothermal titration calorimetry, X-ray crystallography, co-immunoprecipitation, and co-localization confirmed that the C-terminal LIR motif (NSs4) interacts with all six human LC3 proteins. We identified phenylalanine 261 (F261) in NSs4 as essential for LC3 interaction, nuclear retention, and autophagy inhibition in RVFV-infected cells, highlighting how RVFV inhibits autophagy via the NSs4 LIR motif. Our LIR discovery pipeline also identified highly favorable interactions between LC3 and the Marburg virus nucleoprotein, Nipah virus phosphoprotein, and dengue virus capsid . Ongoing studies are using this pipeline to investigate LIR interactions in HFV proteins and their impact on autophagy. Given the threat of reemerging HFVs, this research is crucial for public health, exploring LIR motifs as therapeutic targets to disrupt viral replication and prevent future outbreaks.

O5/P7
Large Animal Clinical Sciences
Comparison of gait characteristics for horses without shoes, with steel shoes, and with aluminum shoes

Gottleib K, Trager-Burns L, Santonastaso A, Bogers S, Burns T, Werre S, Byron

Objective information regarding effects of shoeing on gait characteristics of performance horses is scant. Such information is relevant to competition regulation and animal welfare. This studyís objective was to determine differences in gait characteristics for horses under various shoeing conditions (barefoot, aluminum, steel). We hypothesized shoeing condition would not affect symmetry of head and pelvic movements but would affect hoof arc height, stride length, and duration of stride swing, stance, and breakover times. Twelve healthy, adult, client-owned horses without musculoskeletal abnormalities or lameness were included in a prospective crossover study design. Horses were evaluated with body- and hoof-mounted inertial sensors at a trot on firm and soft surfaces under barefoot, aluminum shoe and steel shoe conditions. Data collected included Q score, hoof arc height and lateral deviation, stride length, and mid-stance, breakover, swing, and landing stride phase times. Data were compared among shoeing conditions and surfaces. Significant differences in arc height were detected between aluminum and steel shoes in right and left forelimbs ($P<0.0001$) and remained consistent on soft footing ($P<0.0001$) and asphalt ($P=0.0009$). Differences in hoof arc height were also significant between barefoot and steel shoes on soft footing in right ($P=0.0297$) and left ($P=0.0006$) forefeet. Limitations included small sample size. Our findings of significant differences in hoof arc height of horses shod with steel shoes versus aluminum or barefoot conditions have potential implications on competition regulation and animal welfare. Further investigation is warranted to determine the impact this has on competition fairness and animal welfare.

O6/P8
Large Animal Clinical Sciences
Pharmacokinetics of Oral Mirtazapine in Healthy Adult Alpacas

Lacey EK, Wilson KE, Council-Troche M, Davis JL
[1]*Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA*

Introduction:
Inappetence is a common clinical problem in critically ill alpacas. Mirtazapine (MRZ) is a noradrenergic and specific serotonergic antidepressant used in small animals for appetite stimulation and weight gain. The pharmacokinetics of MRZ show species-specific differences but have not been investigated in alpacas. The aim of this study was to investigate the plasma concentrations and effect of sample site on the pharmacokinetics of MRZ in healthy adult alpacas.

Methods:
A single dose of 2 mg/kg MRZ was administered orally via syringe to seven healthy adult alpacas. Blood was collected from cephalic vein (CV; n=5) and jugular vein (JV; n=7) catheters immediately prior to and at pre-determined times for 24 hours after administration. Plasma MRZ concentrations were determined by ultra-pressure liquid chromatography/tandem mass spectrometry. Statistical analysis was performed by paired t-test in animals where both JV and CV samples were obtained (significance at P?0.05).

Results:
Mean maximum plasma concentrations (Cmax) from JV samples (183±95.2ng/mL) occurred at 15 minutes and were significantly higher than CV samples (4.84±2.37ng/mL) which occurred at 30 minutes (P = 0.027). Mean JV Cmax in alpacas reached the mean JV Cmax published in cats (73.1ng/mL) but the mean CV Cmax failed to reach mean CV Cmax reported in the dog (164ng/mL).

Conclusions:
Sample site had a significant effect on plasma concentrations of MRZ in alpacas, suggesting possible oral transmucosal absorption. Additional information is needed before using oral MRZ in alpacas, including studies of different doses, formulations, or routes of administration.

07/P41
Small Animal Clinical Sciences
Immunomodulatory effects of histotripsy and N-dihydrogalactochitosan in preclinical murine and comparative canine models of osteosarcoma.

Ny Luong, Alayna Hay, Elliana Vickers, Minsung Kim, Samuel S. K. Lam, Wei R. Chen, Eli Vlaisavljevich, Joanne Tuohy.

[1]*Virginia-Maryland College of Veterinary Medicine, Roanoke, VA*
[2]*Virginia Tech, Roanoke, VA*
[3]*Immunophotonics, Inc., St. Louis, MO*
[4]*University of Oklahoma, Norman, OK*

Background:
Osteosarcoma (OS) is the most common primary malignant bone tumor, known for its high potential for metastasis. Histotripsy, a non-thermal, non-invasive high-intensity focused ultrasound modality, mechanically disrupts tumor cells, releasing neoantigens and damage-associated molecular patterns, potentially promote an anti-tumor immune response. N-dihydrogalactochitosan (GC) is a novel immunostimulant previously reported to enhance systemic immune responses when administered intratumorally following local thermal tumor ablation. However, the combination of GC with histotripsy remains unexplored. This study aimed to investigate the safety and feasibility of combining histotripsy and GC as a novel treatment modality in both murine orthotopic and canine spontaneous OS models.
Methods:
Pet dogs with spontaneous OS underwent 1ñ5 fractionated histotripsy treatments, ablating most of the tumor, followed by intratumoral GC injection (10 mg/mL) with doses adjusted for tumor size. Peripheral blood was collected at baseline and at 2-, 4-, and 8-weeks post-treatment to assess immune activation, and lung metastases were monitored via thoracic radiographs. In the murine syngeneic C3H/HeN orthotopic OS model, DLM8-tumor bearing mice underwent histotripsy ablation followed by intratumoral GC injection. Mice were euthanized at 5- or 14-days post-treatment to assess tumor necrosis, immune infiltration, and pulmonary metastases via histopathology. Tumor and splenic immune cells were analyzed by flow cytometry.
Results:
In dogs, the combination of histotripsy and GC was tolerated, with swelling at the treatment site resolving within one week. At 2-weeks post-treatment, circulating monocytes (CD11b+CD14+) increased with upregulation of CD80 and CD62L expression compared to baseline level. CD80 expression in circulating monocytes continued to increase at 8-weeks post-treatment, suggesting sustained monocyte activation. Two of three patients remained free of lung metastases at 10 weeks post treatment. In mice, at 5 days post-treatment, the combination therapy increased splenic neutrophils (CD11b?Ly6G?) and activated NK cells (CD49b?Nkp46?). By 14 days, CD4? T cells were increased compared to controls. Immune infiltration and metastasis analyses are ongoing.
Conclusion:
These preliminary findings suggest that histotripsy combined with GC may induce immune activation and holds promise as a therapeutic strategy for targeting primary tumors and metastases in OS.

O8/P14
Small Animal Clinical Sciences
Examining neonatal puppy growth between overweight and lean canine dams

Samantha McCarter[1], Stephen Werre[2], Julie Cecere[3], Orsolya Balogh[3]

[1]Department of Biomedical and Veterinary Sciences
[2]Department of Population Health Sciences
[3]Department of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA

Lactation is the final pathway for maternal metabolism to influence a neonate. In women, metabolic hormones positively correlate with maternal body mass index and pass through breastmilk, which affected infant weight gain through the first 6 months of life1. While studies have examined the effect of many variables on neonatal puppy growth rates2, body condition of the dam has never been examined. We hypothesized that puppies born and nursed by overweight dams would have increased growth rates compared to those from lean bitches. Thirteen litters from 12 healthy medium to large breed client-owned bitches were enrolled after whelping. Dams were classified based on initial postpartum body condition into lean (LE, Body Condition Score (BCS):4-5/9, n=6) and overweight (OW, BCS: 6-7/9, n=7) groups. A total of 96 puppies were born alive to LE (n=48) and OW (n=48) mothers. Birth weights and daily weights of all puppies were recorded by owners until 21 days of age, before weaning. Puppies were nursed without supplemental feeding. Birth weight, daily weights, average daily weight gain (ADG, %), and average weight gain since birth (AGB, %) of all puppies were analyzed using mixed model ANOVA; significance was set at $P < 0.05$. Puppy birth weights were lower in larger litters but unaffected by the damís BCS. Puppy growth curves, based on day-to-day weight changes, were significantly different between the two maternal groups, despite no difference in puppy weights on any given day between OW and LE mothers. ADG on days 2 and 4 was 6% and 3.1% higher, respectively, in puppies of OW dams compared to LE, while puppies of LE dams gained 2.2% more on day 13. AGB did not differ significantly on any given day between BCS groups, however, tripling and quadrupling of birth weights occurred approximately a day later in puppies of OW bitches. A larger litter size accounted for significantly lower AGB. Puppies with higher birth weights had generally higher weights throughout the neonatal period, although they gained (ADG, AGB) at a slightly slower rate. In conclusion, despite the similar day-to-day neonatal body weights between maternal groups, puppies from OW dams grew differently in the first 21 days of life compared to puppies from LE dams. Litter size or birth weight are also significant determinants of puppy weight and growth. Milk composition may explain some of the differences seen in growth pattern between puppies from OW and LE bitches and is currently being investigated.

POSTER PRESENTERS

Session 1

1:00 - 2:00pm

Even numbered posters

2. Elliana Vickers
4. Gabriela Carneiro de Sousa
6. Tian Xu
8. Emilee Lacey (ORAL)
10. Christina Vezza
12. Ama Amoakoma Agyei
14. Samantha McCarter (ORAL)
16. Rachel Persinger
18. Janice O'Brien (ORAL)
20. Suzanne Pinar
22. Samar Elsaadawy
24. Sai Navya Vadlamudi
26. Manali Powar
28. Kaylee Petraccione (ORAL)
30. Sierrah Travis
32. Ahmed Abouelkhair
34. Padmaja Mandadi
36. Chelsea Cereghino
38. Matthew Irwin
40. Brice Stolz
42. Claire Read
44. Nour Alkashef
46. Rafaela Flor
48. Carley Elliott
50. Syeda Nahid Fatema (ORAL)
52. Morgen VanderGiessen
54. Rana Estaleen
56. Chen-I Hsu
58. Laura Victoria Quishpe Contreras
60. Marlie Nightengale
62. Leanne Jankelunas
64. Amanda Moore
66. Fabian J. Roa
68. Andrianna Krippaehne
70. Caitlin Armstrong

POSTER PRESENTERS

Session 2

2:00 - 3:00pm

Odd numbered posters

1. Minsung Kim
3. Mitchell Caudill
5. Mahfuzul Islam
7. Katherine Gottleib (ORAL)
9. Brittany Heath
11. Vikram Kahlon
13. Amir Mortazavigazar
15. Katie Tiller (ORAL)
17. Lezith Chavez
19. Cassandra Poole
21. Tamalika Paul
23. Emma Hare
25. Abraham Adeyemo
27. Mikhala Stafford
29. Kimberly Martinez
31. Dima Hajj Ali
33. Somaia Abdelmegeed
35. Kirsten Masters
37. Md Mahabub Arefin Chowdhury
39. Ammar Khan
41. Ny Luong (ORAL)
43. Chad Artman
45. Abdallah Abdelsattar
47. Ian Taylor
49. Jillian Green
51. Taylor Nolen
53. Abdullahi Jamiu
55. Aida Shakeri
57. Hilary Montano
59. Shannon Carney
61. Annie Showers
63. James May
65. Elizabeth Harris
67. Nadine Altmann
69. Catie Burgess

ABSTRACTS

P1
Characterization of immune cell infiltration in response to histotripsy tumor ablation in combination with anti-CTLA4 therapy

Ny Luong[1,2], Minsung Kim[1,2], Elli Vickers[1,2], Alayna Hay[1,2], Joanne Tuohy[1,2]*

[1]Department of Small Animal Clinical Sciences, VA-MD College of Veterinary Medicine, Blacksburg, VA, USA
[2]Virginia Tech Animal Cancer Care and Research Center, Virginia-Maryland College of Veterinary Medicine, Roanoke, VA, USA
**Presenting author*

Introduction
Osteosarcoma is the most common primary bone cancer with high metastatic potential to the lungs dropping five-year overall survival rates from 70% to 10-30% with metastatic disease. Histotripsy is a non-invasive mechanical ablation method utilizing high-intensity focused ultrasound waves. Anti-CTLA4 prevents binding with B7 of antigen presenting cells allowing CD28 binding leading to active state of T cell. By combining histotripsy and anti-CTLA4 treatments, we hypothesize an increase in intratumoral immune infiltration and activation profiles. Our study aims to 1) determine efficacy of combination treatment of histotripsy and anti-CTLA4 treatment and 2) characterize and compare the immune cell population between treatments.

Methods
C3H/HeN were given subcutaneous injections of 4x10^6 DLM8 cells to the right flank. Mice were treated with anti-CTLA4 alone, histotripsy alone, or histotripsy + anti-CTLA4 (combination) Anti-CTLA4 monoclonal antibody injections were administered intraperitoneally at day 8, 12 and 15. Histotripsy treatments were performed using 1 MHz histotripsy delivery system. Mice were monitored thrice weekly, and tumor volumes were estimated by measuring the length and width using calipers. Mice were sacrificed 6 days after treatment or following IACUC criteria for euthanasia. Flow cytometry was used to quantify CD4 T cells, CD8 T cells and Treg cells in spleen samples while myeloid and lymphoid cells were quantified in blood samples.

Results
Two-way ANOVA between pre-treatment and post-treatment estimates of tumor volumes revealed significant interaction between untreated with anti-CTLA4 ($p=0.0057$), histotripsy ($p=0.0376$) and combination ($p=0.0134$) treated tumors. No significant difference was found between treatment and control groups in splenic T cell populations in blood and spleen samples. In blood lymphoid populations, combination treatment had significantly higher B cell compared to histotripsy and untreated. In blood myeloid populations, combination treatment group had significantly higher neutrophil levels compared to histotripsy group.

Conclusion
Our results indicate that all treatment groups had reduced tumor volumes suggesting successful treatments. However, there were no differences in T cell populations in either spleen or blood samples for any treatment groups. Longer post-treatment observations may be required to observe further tumor regression and T cell populations.

P2
Focused ultrasound for the treatment of osteosarcoma in a canine comparative oncology model: Findings from veterinary clinical trials

Elliana Vickers[1,2,3], Lauren Ruger[1], Alayna Hay[2], Ny Luong[2,4], John Kett[5], Summer Vander Kooi[6], Sheryl Coutermarsh-Ott[7], Gregory Daniel[8], Timothy Ziemlewicz[9], Steven Soliman[10], Gunjan Malhotra[10], Adam Maxwell[1], Eli Vlaisavljevich[1], Joanne Tuohy[2,8]

[1]Department of Biomedical Engineering and Mechanics, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA
[2]Virginia Tech Animal Cancer Care and Research Center, Virginia-Maryland College of Veterinary Medicine, Roanoke, VA, USA
[3]Graduate Program in Translational Biology, Medicine and Health, Virginia Polytechnic Institute and State University, Roanoke, VA, USA
[4]Graduate Program in Biomedical and Veterinary Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA
[5]Doctor of Veterinary Medicine Program, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA
[6]School of Animal Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA
[7]Department of Biomedical Sciences and Pathobiology, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA
[8]Department of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA
[9]Department of Radiology, University of Wisconsin, Madison, WI, USA
[10]Division of Musculoskeletal Radiology, Department of Radiology, University of Michigan/Michigan Medicine, Ann Arbor, MI, USA

Osteosarcoma (OS) is the most common primary bone tumor in dogs and humans, and the similarities between human and canine OS make the dog a valuable comparative oncology model. Histotripsy, a non-invasive ablation modality using pulsed focused ultrasound to mechanically disintegrate tissue, shows promise for the treatment of bone tumors. Previous canine clinical trials have shown the safety and feasibility of ablating canine OS tumors, followed by limb amputation. The present study is the first use of histotripsy without standard-of-care tumor resection in OS, allowing for evaluation of long-term clinical outcomes. Pet dogs with spontaneous bone tumors were assessed at baseline and over time with MRI, gait analysis, pain & quality of life surveys, and lameness assessments. Histotrips treatments were fractionated (separate treatments applied on different days) with a plan to ablate >50% of the tumor in 1-5 treatments. Additional treatments were applied if residual tumor was remaining or if untreated portions of tumor continued to grow on contrast-enhanced T1-weighted MRI. Histotripsy was delivered by a 700 kHz transducer designed for targeting musculoskeletal tumors, with coaxial ultrasound imaging to guide treatment planning and execution. 9 dogs with primary bone tumors were treated, and 6 dogs received long-term follow-up. Dogs generally tolerated histotripsy treatment well and were able to return home within 1–3 hours post-treatment. The maximum volume targeted in a single session was 29.2 cm3. The mean total ablation volume was 35.6 cm3, which equated to a mean percent ablation of 50.2% when compared to the pre-treatment tumor size on MRI. For each treatment, an area closely matching the targeted region was observed as a non-enhancing area on MRI. Whole tumor signal intensity as well as enhancing tumor volume decreased immediately after treatment but tended to return to baseline or increase by 6 weeks after the initial treatment. Considering clinical outcomes, 4/6 dogs with follow-up (all patients with >50% ablation coverage of their tumor) showed a clinically significant reduction in pain at the end of their follow-up period (mean 8 weeks) as compared to baseline. Across those 6 dogs, there was a significant increase in peak pressure on the tumorbearing limb at the end of follow-up as compared to baseline. These results highlight the promise of histotripsy as a non-invasive limb salvage treatment for OS.

P3
Reanalysis of publicly available RNA-seq datasets to determine transcriptional regulators of bacterial small RNAs

Mitchell T. Caudill, Jillian R. Marshall, Clayton C. Caswell

[1]*Center for One Health Research*

Regulatory small RNAs (sRNAs) are important mediators of bacterial protein translation and allow for a rapid and controlledresponse by a bacterium to changing metabolic or environmental conditions. Despite the growingnumber of discovered and characterized sRNAs with physiological importance, sRNAs are oftennot annotated within reference genomes and thus not examined in transcriptomic studies. This lackof analysis obscures potentially important insights into transcriptional control and translationdynamics. Given this situation, we sought to reanalyze publicly available RNA-seq datasets from the bacterial genus Brucella using a custom reference “genome” consisting exclusively of known andvalidated sRNAs. This tailored sRNA reference file allowed for targeted analysis of changing sRNAtranscript levels in a variety of environmental and regulatory conditions, and ultimately for theprediction of transcriptional regulators that control specific sRNA transcript expression. Predictedrelationships were validated via northern blot analysis, with transcript levels detected via the blotlargely correlating to the RNA-seq result. The procedures for these analyses may be of use tothe greater field of bacteriology and allow for greater data yield from transcriptomic studies.

ABSTRACTS

P4
Changes in biological processes in the uterus throughout parturition in the bitch: a transcriptomic approach

Gabriela Carneiro de Sousa[1], Fernando Biase[1], Bruce Christensen[2], Alyssa Shelby[3], Orsolya Balogha[3]

[1]Dept. of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA
[2]School of Animal and Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA
[3]Kokopelli Veterinary Center, Sacramento, CA

We hypothesized that global gene expression analysis will pinpoint crucial biological functions, e.g. inflammatory and immune response, steroid hormone-mediated and contractility-associated processes, that will elucidate how the uterus prepares for and progresses through labor. The aim of this study was to perform RNA sequencing (RNAseq) on canine uterine samples to determine differentially expressed genes and functional pathways from term prepartum through 2nd stage labor. Full-thickness uterine biopsies were collected during Cesarean section (C-section) from bitches divided into three groups based on serum progesterone and clinical presentation: planned c-section (PCS) at term pregnancy without 1st stage labor signs (n=7, P4 ?3.4 ng/mL); elective c-section (ECS) at term pregnancy after temperature drop and/or 1st stage labor signs (n=6, P4 <1.5ng/mL); obstructive dystocia (OD) at 2nd stage of labor presenting with strong spontaneous abdominal contractions (n=5). RNA isolation was performed as previously described1. Samples included in the study had RIN between 7.4-9.7. Differential transcript abundance for contrasts PCS vs. OD, PCS vs. ECS, ECS vs. OD were determined by employing the quasi-likelihood negative binomial generalized log-linear model from the R package ìedgeRî and the Wald test from the R package ìDESeq2î. False Discovery Rate threshold was set < 0.05 in both tests for a gene to be differentially expressed (DEG). A total of 541 DEGs were identified for PCS vs ECS, 3443 DEGs for PCS vs OD, and 10 DEGs for ECS vs OD. After filtering gene ontology terms by FDR < 0.1 and at least 6 genes per category, preliminary analysis for PCS vs ECS contrast highlighted changes in biological and cellular processes such as angiogenesis, positive regulation of cell migration and cell population proliferation, cellular response to hypoxia, and negative regulation of apoptotic process. In the PCS vs OD contrast, we found DEGs involved in functions that were overlapping with the PCS vs ECS contrast, and other processes such as actin cytoskeleton organization, protein-coupled receptor signaling, inflammatory response and immune system processes, and carbohydrate transport, which appear to be more characteristic of the actively contracting uterus. These preliminary results provide insights into a broad range of biological and molecular processes that regulate uterine function during the parturition process in the dog.

P5

The role of EGR2 in B cell development in Systemic lupus erythematosus (SLE)

Mahfuzul Islam, Rujuan Dai, B Heid, Ran Lu, S. Ansar Ahmed

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with an unclear pathogenesis and no effective cure. It is characterized by B cell hyperactivity, which produces pathogenic autoantibodies that contribute to inflammation and tissue damage in multiple organs. Therapeutic depletion of B cells in human lupus patients had mixed success due to the complex heterogeneity of B cell subsets, differences in autoantibody production, and the persistence of antibody-secreting plasma cells (PCs). Our laboratory reported that the transcription factor Early Growth Response 2 (EGR2), a key regulator of immune cell function, was markedly upregulated in the lymphocytes of murine and human lupus. Further, we reported that conditional deleting Egr2 in lymphocytes in lupus-prone B6/lpr mice suppressed pathogenic anti-dsDNA autoantibodies. Notably, Egr2-/-B6/lpr mice exhibit an increased germinal center B (GCB) cell population but fail to differentiate progress into fully differentiated PCs. These data suggest a novel and unexpected regulatory role of EGR2 in the transition from GCB cells to antibody-secreting PCs. These findings provide compelling evidence that EGR2 functions aberrantly in lupus, contributing to disease pathology by facilitating the generation of autoreactive PCs. To further investigate the novel role of EGR2 on B cells, we are conducting extensive experiments using newly developed CD2Cre-Egr2-/- MRL/lpr mice, which have Egr2 deletion in both T and B lymphocytes and CD19Cre-Egr2-/-MRL/lpr mice, which have Egr2 deletion specifically in B lymphocytes to fully understand the role of EGR2 on B cell development. Throughout this experiment we are using flow cytometry and molecular analysis), functions (antibody production, protein expression), and immune complex deposition in kidneys. We aim to identify the molecular pathways by which EGR2 influences GCB differentiation and PC function. Understanding these pathways will provide critical insights into the signaling mechanisms that promote autoreactive PC development in lupus. This study will contribute novel knowledge regarding EGR2ís role in B cell differentiation and autoantibody production, offering a new era for precision-targeted lupus therapies. As there are still no effective treatments, it is urgent to explore. Our findings may lay the foundation for developing therapeutic strategies aimed at selectively inhibiting EGR2-mediated PC differentiation while preserving normal immune function.

P6
Establishment of a double humanized lupus mouse model with human immune system and SLE patient microbiota

Tian Xu[1], Ran Lu[1], David N. Oakland[1], Rana Estaleen[1], Amelia Rawlings[1], Hilary Montano[1], Christopher M. Reilly[2], Xin M. Luo[1]

[1]*Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA, USA*
[2]*Department of Biomedical Sciences, Edward Via College of Osteopathic Medicine, Blacksburg, VA, USA*

Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disease influenced by complex interactions between the immune system and microbiota. To investigate these interactions, we established a Double humanized SLE (DhuSLE) mouse model by engrafting NSG mice with human CD34+ hematopoietic stem and progenitor cells (NSG-hu mice) and fecal microbiota transplantation from SLE patients (SLE-FMT). FMT transiently suppressed the development of human T cells in NSG-hu mice, indicating a modulatory effect of the transplanted microbiota on immune reconstitution. SLE-FMT alone did not induce most lupus clinical signs but promoted skin lesions, highlighting a microbiota-driven cutaneous response. Contrary to a previous report, pristane injection alone failed to drive lupus-like disease in NSG-hu mice. However, the combination of SLE-FMT and pristane induced lupus nephritis in DhuSLE mice. Interestingly, however, clinical signs observed in DhuSLE mice did not fully reflect those of the microbiota donors. Together, these results suggest that the combination of SLE-FMT and pristane induces lupus nephritis in NSG-hu mice. This indicates the successful establishment of a DhuSLE humanized lupus mouse model. However, limitations exist, and the model may benefit from estrogen conditioning and a different immunodeficient background, as shown in the recently published THX mouse. Upon further development, the DhuSLE mouse can be used to elucidate the pathogenetic mechanisms of human SLE.

P9
New World alphavirus antagonizes the cGAS-STING pathway

Brittany Heath[1,2], Caitlin Woodson[1,2], Maryna Akhrymuk[1,2], Alicia Pickrell[3], Kylene Kehn-Hall[1,2]

[1]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA
[2]Center for Emerging, Zoonotic, and Arthropod-borne Pathogens, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA
[3]School of Neuroscience, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

Venezuelan equine encephalitis virus (VEEV) is a mosquito-borne pathogen causing high morbidity in humans, with 4-14% of cases exhibiting neurological sequelae. The cyclic GMP-AMP synthaseñstimulator of interferon genes (cGASñSTING) pathway is canonically associated with double-stranded (dsDNA) detection; however, it can also respond to RNA viruses and subsequently limit viral pathogenesis. Several viruses have been shown to antagonize this signaling cascade underlying the importance cGASñSTING plays in host immunity. Previous studies regarding single-stranded RNA viruses revealed cGAS-STING limits viral replication in Old World alphavirus chikungunya virus infections, but little is known about New World alphaviruses such as VEEV. We hypothesize STING plays a critical role in immune modulation as a protective function against VEEV infection. To test this hypothesis, human microglia cells were pre-treated with dsDNA for six hours to activate STING, followed by VEEV infection at increasing multiplicity of infection (MOI) for 17 hours. Pre-treatment led to STING activation by phosphorylation and reduced infectious virus, with the greatest effect at a low MOI (0.1) as determined via western blot and plaque assay. However, VEEV infection alone did not trigger STING phosphorylation, suggesting that VEEV infection may suppress STING activation. We then sought to determine if post-treatment with dsDNA after VEEV infection impacted viral replication and STING phosphorylation. Microglial cells were infected with VEEV for six hours at increasing MOIs, and post-treated with dsDNA for 17 hours. STING activation occurred in an MOI-dependent manner with the greatest phosphorylation of STING seen at MOI 0.1, but did not reduce infectious virus. We expanded our testing to include mouse embryonic fibroblasts in which we pretreated cells with STING agonist DMXAA at increasing concentrations (25, 50, 100 ?M) prior to infection with VEEV. DMXAA reduced VEEV viral titers in a dose-dependent manner. These results suggest VEEV antagonizes the cGAS-STING pathway, and priming this pathway can reduce viral infection.

ABSTRACTS

P10
First-In-Dog Histotripsy for Intracranial Tumors Safety Trial: The FIDOhist study

Christina Vezza[1], DVM, Lauren Ruger, PhD[2], Maya Langman, MD[3], Elliana Vickers[4], Francesco Prada, MD[5,6], Jonathan Sukovich, PhD[7], Timothy Hall, PhD[7], Zhen Xu, PhD[7], Rell L. Parker, DVM, PhD[1], Eli Vlaisavljevich, PhD[2], and John H. Rossmeisl, DVM[1]

[1]Department of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA
[2]Department of Biomedical Engineering and Mechanics, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA
[3]Department of Neurosurgery, Medical College of Wisconsin, Milwuakee, WI, USA
[4]Graduate Program in Translational Biology, Medicine, and Health, Virginia Polytechnic Institute and State University, Roanoke, VA, USA
[5]Department of Neurosurgery, Fondazione IRCCS Istituto Neurologico C. Besta, Milano, Italy
[6]Department of Neurological Surgery, University of Virginia, Charlottesville, VA, USA
[7]Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA

Objective: Brain tumors represent some of the most treatment refractory cancers, and there is a clinical need for additional treatments for these tumors. Domesticated dogs are the only other mammalian species which commonly develop spontaneous brain tumors, making them an ideal model for investigating novel therapies. Histotripsy is a non-thermal ultrasonic ablation method that emulsifies tissue through acoustic cavitation. The primary objectives of this prospective study were to assess the feasibility and safety of histotripsy to ablate naturally occurring canine brain tumors. Secondary endpoints included characterization of magnetic resonance imaging (MRI) responses to histotripsy treatment, and exploratory immunogenomic tumor response analyses.

Methods: The study design utilized a treat and resect paradigm, where tumors were approached using craniotomy, partially ablated with histotripsy delivered through the cranial defect, imaged with MRI, and then resected. Dogs were evaluated with clinical, brain MRI, immunopathologic, and genomic examinations before treatment, intraoperatively, and 1, 14, and 42 days post-treatment. Here we report the results of the three dogs with meningiomas, all of which were treated with a custom eight element 1 MHz histotripsy transducer at a pulse repetition frequency of 100 Hz and a treatment dosage of 400 pulses/point.

Results: Histotripsy was successfully delivered to all dogs, resulting in histopathologic evidence of ablations that were sharply demarcated from untreated tumor, with measured treatments approximating planned volumes in 2/3 dogs. One dog experienced an adverse event consisting of transient cerebral edema that was possibly attributable to histotripsy. Histotripsy ablations could be grossly visualized and identified on MRI, with features consistent with hemorrhage and necrosis. Significant expression or upregulation of the damage associated molecular pattern HMGB1, cytokine-cytokine receptor interaction, and NF-?b signaling pathways were observed in histotripsy treated tumors. Conclusion: Ablation of canine meningiomas with histotripsy through an open cranial window was feasible and clinically well tolerated.

P11
Thromboelastography in dogs with suspected primary brain tumors

Vikram Kahlon, DVM, Audrey Keebaugh, DVM, MS, DACVIM, Priscila Serpa, DVM, MSc, DSc, DACVPñClinical Pathology, Richard Shinn, DVM, MS, DACVIMñNeurology & Ashley Wilkinson, DVM, MS, DACVIM

[1]*Virginia Tech Graduate School*
[2]*Virginia-Maryland College of Veterinary Medicine*

Background: Dogs with primary brain tumors typically have chronic progressive clinical signs, but some dogs will decline suddenly and unexpectedly. A portion of these dogs experience strokes in addition to their brain tumor. Humans with brain tumors are often hypercoagulable and predisposed to thromboembolic disease. It is currently unknown if dogs with primary brain tumors experience hemostatic derangements. Dogs can be screened for hypercoagulability using a blood test called thromboelastography (TEG).

Hypothesis: Dogs with primary brain tumors will have one or more of the following TEG 6s alterations suggestive of hypercoagulability compared to healthy dogs: Decreased R, decreased K, increased alpha angle, and increased MA.

Animals: One dog has been enrolled. Six dogs were excluded after initial enrollment due to lack of a primary brain tumor on advanced imaging. The control group is comprised of 40 healthy adult dogs that were enrolled in a separate study.

Methods: Prospective observational study. Thromboelastography will be performed on all dogs with the Haemonetics TEG 6s system. Dogs will be diagnosed with a primary brain tumor if they have clinical signs, exam findings suggestive of intracranial disease and brain MRI results consistent with a primary brain tumor. Dogs must have a chemistry, CBC, and urinalysis within 1 week of brain MRI to exclude concurrent disease that would affect hemostasis. Dogs receiving medications known to alter hemostasis within 2 weeks of enrollment will be excluded. Healthy dogs were enrolled in a separate study. Power analysis with an ? value of 0.05 and power of 80% revealed that nine dogs in both the control group and study group would be needed. Statistical analysis will compare the mean or median of TEG parameters of affected dogs to healthy control dogs using a 2-sample t-test if data are normally distributed. A Wilcoxon rank sum test will be performed if data are not normally distributed. The level of significance will be set at $P < 0.05$.

Results: The study is currently ongoing. Preliminary results reveal that the dog with a primary brain tumor has the following TEG parameters: R 1.7 minutes, K 1.3 minutes, alpha angle 74.5 degrees, and MA 57 mm. Healthy dogs (n = 40) have the following median (range) TEG parameters: R 2.55 minutes (1.3-3.6), K 1.8 minutes (0.8-4.2), alpha angle 70.2 degrees (53.6-77.2), and MA 53.45 mm (40-65.2).

Conclusions: Further conclusions will be made once additional dogs are enrolled.

P12
Responsiveness of the viral latency associated transcript to epinephrine during HSV-1 infections

Agyei, Ama Amoakoma[1,3]; Bertke, Andrea[1,2,3]

[1]Biomedical and Veterinary Sciences, Virginia Tech, Blacksburg, VA
[2]Department of Population Health Sciences, Virginia Tech, Blacksburg, VA
[3]Center for Emerging, Zoonotic and Arthropod-borne Pathogens, Virginia Tech, Blacksburg, VA

Herpes simplex virus (HSV) causes lifelong acute and latent infections in about 67% (HSV-1) and 13% (HSV-2) of humans globally. Disease presentation and recurrences take the form of oral, ocular, or genital skin and mucosal lesions. While acute HSV disease symptoms in humans and animal models are exacerbated by stress, stress has also been found to be involved in reactivation of the virus from latency. Epinephrine, a stress hormone involved in the ìfight or flightî response, has been shown to enhance productive HSV-1 infections and induce HSV-1 reactivation. Epinephrine-induced reactivation of HSV-1 in animal models is significantly reduced when the viral Latency Associated Transcript (LAT) gene is deleted. To begin identifying the specific mechanism through which epinephrine acts on HSV-1, we used HSV-1 viruses with various deletions in the LAT gene to map the region of LAT that may be involved in the action of epinephrine on the virus. Following infection of primary sensory dorsal root ganglion (DRGs) and sympathetic superior cervical ganglion (SCGs) neurons with wildtype and deletion viruses in vitro, we used qPCR to assess viral DNA load and virus plaque assays to determine the infectious particles with or without treatment with epinephrine. We have been able to show that epinephrine does not seem to show significant biological effects on HSV-1 or the LAT deletion viruses in DRGs and SCGs during acute infections. Also, while the LAT promoter region may contribute to an epinephrine-induced increase of DNA synthesis in sympathetic neurons (SCGs) it does not seem to affect the production of infectious virus particles. Although LAT does not appear to be involved in epinephrine-induced exacerbation of productive infection, determining the region of LAT that responds to epinephrine during latency will give more insight on how HSV-1 reactivates in response to physiological stress.

P13
Estimating human attitudes on benefits and impacts of clean energy sources using large language models

Amir M. Gazar[1,2], Chloe Jackson[3], Georgia Mavrommati[3], Rich B. Howarth4, Ryan S.D. Calder[1,2,5,6,7]

[1]Department of Population Health Sciences, Virginia Tech, Blacksburg, VA, 24061, USA
[2]Global Change Center, Virginia Tech, Blacksburg, VA, 24061, USA
[3]School for the Environment, University of Massachusetts Boston, Boston, MA 02125, USA
[4]Environmental Program, Dartmouth College, HB 6182, 113 Steele Hall, Hanover, NH, 03755, USA
[5]Department of Civil and Environmental Engineering, Duke University, Durham, NC, 27708, USA
[6]Faculty of Health Sciences, Virginia Tech, Roanoke, VA, 24016, USA
[7]Department of Civil and Environmental Engineering, Virginia Tech, Blacksburg, VA, 24061, USA

A growing number of researchers are examining how large language models (LLMs) can estimate human attitudes, aiding in survey development, bias detection, and related tasks. In this study, we aim to understand public attitudes toward the benefits and impacts of clean energy alternatives by conducting an online survey and compare human-collected responses to LLM-generated responses (referred to as silicone samples).

First, we administered an online survey in New England to gauge the perceived importance of ecological, economic, and environmental health benefits and impacts of clean energy sources, alongside demographic questions such as age, income, education, and political lean. Next, we collected Pew Research Centerís National Public Opinion Reference Survey (NPORS) demographic data for the entire United States. We then ìprimedî multiple LLM models to generate survey responses reflecting specified demographic profiles, with and without covariates (e.g., level of knowledge on decarbonization). Neutral LLMs were subsequently used to review these generated responses, evaluating them against the state of their knowledge and a specific rubric for coherence and consistency. We compared results among these different approaches.

Our results suggest that LLM-generated silicon samples can capture broad patterns found in human responses, particularly with respect to economic and environmental health attributes at a very low cost (approximately $15 for 6,000 silicone samples produced in two hours). For example, for environmental health attributes, models conditioned on both demographics and issue-related covariates achieved an aggregated accuracy of about 88.4% (with KullbackñLeibler divergence (KLD) = 0.024 and JensenñShannon divergence (JSD) = 0.011), while for economic attributes, aggregated accuracy reached roughly 81.1% with similarly low divergence. In contrast, ecological attributes showed lower fidelity (about 55ñ65% accuracy and KLD up to 1.31), likely influenced by online survey selection biases

We contribute to the literature by evaluating LLM fidelity for ordinal and open-ended questions, examining impacts of covariates on fidelity, using LLMs to review survey responses, and examining online survey selection biases in the context of clean energy decision-making. Furthermore, we show how LLMs can supplement traditional surveys and offer a rapid tool for estimating public opinion on clean energy.

P16
North American Culex pipiens mosquitoes require a high Usutu virus dose for infection

Persinger R.[1,2], Duggal N.[1,2]

[1]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA
[2]Center for Emerging, Zoonotic, and Arthropod-borne Pathogens, Virginia Tech, Blacksburg, VA

Usutu virus (USUV) is an emerging mosquito-borne virus belonging to the Flaviviridae family and a close relative of West Nile virus (WNV). It is maintained in an enzootic cycle primarily between passerine birds and Culex spp. mosquitoes. USUV was first isolated in South Africa in 1959 and has since spread throughout Africa and Europe, causing mass mortality of the Eurasian blackbird (Turdus merula). In addition, there has been an increase in human cases ranging from asymptomatic to neuroinvasive disease. Previously, we found that North American Cx. pipiens were competent vectors for USUV. To further understand enzootic transmission of USUV, we sought to investigate the minimum threshold of virus needed to infect Cx. pipiens mosquitoes. To do so, we utilized both an artificial infectious blood meal and a live avian host. First, we exposed mosquitoes to a blood meal spiked with a range of doses, 3-8 log10 PFU/mL, of USUV strain Netherlands 2016. We found the minimum threshold for infecting Cx. pipiens to be ~6 log10 PFU/mL. We wanted to further assess the minimum threshold for Cx. pipiens through a bird-to-mosquito enzootic transmission model. We inoculated domestic canaries (Serinus canaria forma domestica) with USUV strain Netherlands 2016. Canaries were susceptible to USUV, reaching viremias predicted to be transmissible to mosquitoes. Next, we fed Cx. pipiens on USUV infected canaries at the mean peak of viremia. We found a similar minimum threshold for infection of Cx. pipiens as the artificial feeds. Surprisingly, the percentage of infected mosquitoes fed on canaries at the highest doses was significantly lower than the artificial feeds. Together, the results from these studies suggest that Cx. pipiens have the potential to be primary vectors of USUV in North America. Understanding USUV enzootic transmission dynamics can better predict its emergence potential and maintenance.

P17
Real-time three dimensional evaluation of the feline heart; feasibility and reference intervals

Lezith Chavez, Giulio Menciotti, Michele Borgarelli, Alessandra Franchini, Sunshine Lahmers

[1]*Virginia Tech*

Two-dimensional (2D) echocardiography is the gold-standard noninvasive diagnostic tool to assess cardiac disease in veterinary medicine. Transthoracic real-time three-dimensional echocardiography (RT3DE), is also noninvasive, and is better equipped to assess the complex conformational and functional aspects of a beating heart. Little is known about RT3DE use in cats demonstrated by limited published literature on the topic.

The objectives of this ongoing study are to assess the feasibility of RT3DE for a comprehensive cardiac evaluation in cats and to propose 3D reference intervals for the cardiac chambers in cats. We hypothesized this imaging modality would prove to be feasible and would successfully generate reference intervals in the healthy feline. This study requires 120 healthy client-owned cats between the ages of one and five years-old. Each cat is auscultated, screened for systemic hypertension via noninvasive blood pressure (Doppler) and, if normotensive, undergoes standardized 2D and 3D echocardiography. Gentle restraint is utilized. Mild sedation is utilized as needed.

For RT3DE feasibility, a convenience sample of the first 50 enrolled cats will be assessed. The number of patients that has good quality images allowing chamber analysis will be expressed as a percentage out of the total 50 cats. After feasibility is assessed, we will enroll the remining cats and analyze the images to derive reference echocardiographic values.

We are presenting preliminary results as at this time, 59 cats have undergone 2D and 3D echocardiographic assessments, of which 48 were enrolled (81%). The most common reason for exclusion of cats was left ventricular hypertrophy. Enrolled breeds include Domestic Shorthair (n=38), Domestic Longhair (n=7), Persian (n=1), Sphynx (n=1), British Shorthair (n=1). The median age was 2 years old. The mean blood pressure was 125 mmHg ±14.5. The mean time to complete the 2D, 3D, and entire (2D+3D) echocardiograms were 14.6 minutes ±8.6, 2.7 minutes ±1.8, and 17.4 minutes ±9.0 respectively.

P19
Investigating the immunogenic effects of High-Frequency Irreversible Electroporation (H-FIRE) in the treatment of breast cancer

Cassandra Poole, Carley Elliott, Tamalika Paul, Manali Powar, Mackenzie Woolls, and Irving Coy Allen

[1]BMVS Program
[2]MCB Program
[3]Coy Allen Lab

Breast cancer is the second most frequent cancer type found in US women, excluding skin cancer, and worldwide, is the single most common cause of death in women. Breast cancer is most deadly when it metastasizes from the primary tumor to other organs of the body. Metastatic breast cancer is currently incurable, and treatment methods focus on slowing tumor growth and shrinking tumor sizes. Standards of care for metastatic cancer can include surgery, hormone therapy, chemotherapy, and radiation. Many of these treatments have unpleasant side effects, such as nausea, hair loss, heartburn, cardiotoxicity, suppression of the immune system, and osteoporosis. The 5-year survival rate for metastatic breast cancer is just 31%, an unacceptably low number indicative of the ineffectiveness of current treatment techniques and the need for new interventions. High-frequency irreversible electroporation (H-FIRE) is a novel, minimally-invasive, non-thermal technique that ablates tumor cells through the use of short, high-frequency bipolar electric field pulses. Previous work in our lab has demonstrated that H-FIRE can successfully ablate the primary tumor, and shift the tumor microenvironment from an immunosuppressive phenotype to an antitumor phenotype, by increasing inflammatory cell death signaling and the production of local inflammatory cytokines.

The research proposed here will further expand on the mechanisms of immune system activation seen after treatment of breast cancer with H-FIRE. Key goals of this research are to 1) characterize the release of tumor-associated antigens and damage-associated molecular patterns following H-FIRE treatment, 2) assess immune cell recruitment and activity in the tumor microenvironment post-H-FIRE treatment, and 3) determine the ability of H-FIRE treatment to induce a systemic immune reaction and the efficacy of this reaction in eliminating metastatic lesions. This research will be conducted utilizing the well-established 4T1 orthotopic murine breast cancer model, as well as patient derived tumor models and in vitro culture systems. An additional intriguing avenue of investigation would be to evaluate the in vivo therapeutic efficacy of H-FIRE in combination with other immunotherapies, like checkpoint blockade or adoptive cell transfer.

P20
Decoding Chronic Neuroinflammatory and Behavioral Impacts of Traumatic Brain Injury: Exploring Regional Neuroimmune Dynamics and Bone Marrow Contributions

Pinar S. and Theus MH.

[1]*Department of Biomedical Sciences & Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061, USA*

Traumatic brain injury (TBI) often leads to chronic cognitive, motor, and neuroinflammatory deficits, yet the long-term mechanisms driving these impairments remain insufficiently understood and require extensive investigation. This study examines the distinct cognitive, motor, and neuroinflammatory outcomes associated with moderate diffuse and focal TBIs over a chronic period of 90 days post-injury. Using a combination of behavioral assaysóincluding T-maze (spatial memory), rotarod (motor function), open field (anxiety), novel object recognition (declarative memory), and splash tests (depression)óthe research aims to quantify functional deficits and recovery trajectories in male and female mice. To uncover the regional neuroimmune responses driving these outcomes, brain cryosections were analyzed through immunohistopathology using markers of astrogliosis and microgliosis. Advanced imaging techniques (Imaris) are used to assess glial morphology and neuroimmune cell activation. To evaluate whether TBI induces chronic alterations in the bone marrow (BM)-derived circulatory system that could directly impact behavioral outcomes, we generated adult chimeric mice by transplanting BM from sham and TBI mice at 90 days post-injury (dpi). Behavioral assessments were conducted longitudinally, extending up to 8 months post-adoptive transfer, to investigate the potential long-term influence of BM-derived factors on recovery and behavior. By correlating behavioral deficits with regional neuroinflammatory changes, this study seeks to elucidate the underlying mechanisms of chronic TBI pathophysiology.

P21

Bubble therapy: Histotripsy for pancreatic tumor ablation modulates the tumor microenvironment and enhances systemic anti-tumor immunity in an in-vivo murine model

Tamalika Paul[1], Victor Lopez[5], Cora Youngs[6], Carley Elliott[2], K.M. Imran[2], Benjamin Tintera[3], Tyler Moore[6], Eli Vlaisavljevich[4,5], Irving Coy Allen[1,2,3,5,6]

[1]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, USA.
[2]Graduate Program in Translational Biology, Medicine and Health, Virginia Tech, Roanoke, VA, USA
[3]Virginia Tech Carilion School of Medicine, Roanoke, VA, USA
[4]Department of Biomedical Engineering and Mechanics, Virginia Tech, Blacksburg, VA, USA
[5]Institute for Critical and Applied Science Center for Engineered Health, Virginia Tech, Blacksburg, VA, USA
[6]College of Science, Virginia Tech, Blacksburg, VA, USA

Pancreatic cancer is one of the leading causes of cancer-related deaths, highlighting the urgent need for innovative treatment strategies. Histotripsy is a non-invasive, non-ionizing, non-thermal, image-guided focused ultrasound ablation treatment method that uses high-pressure pulses to create acoustic cavitation, a ìbubble cloud,î at the target. The rapid expansion and collapse of this bubble cloud ablates the tumor into an acellular homogenate. The objective of the study is to evaluate the hypothesis that histotripsy ablation of pancreatic tumors reduces tumor burden, activates systemic immune responses, and induces an abscopal-like effect. Using a contralateral in-vivo study in C57BL/6J mice, we employed a customized 1 MHz, 8-element histotripsy transducer for partial tumor ablation (50ñ60%) to stimulate immune responses and transform the ìcoldî pancreatic tumor microenvironment to ìhotî tumor microenvironment. Treatment parameters included 250 Hz pulse repetition frequency, and 250 pulses per focal point. Mice were analyzed at five timepoints (2 hours,1 day,7 days,14 days,21 days) for ablation zones, immune cell dynamics, and systemic transcriptomic/proteomic changes. The results show that Histotripsy significantly reduced tumor volumes in both treated and untreated (contralateral) tumors compared to controls. Tumor diameters in the control groups were approximately 70 mm, while the treated groups had diameters around 20 mm. Histological examination confirmed accurate ablation zones, and flow cytometry revealed notable changes in immune cell populations across treated, untreated, and control groups. The results denote significant alterations in the population of cytotoxic T cells between the treated and control groups. Transcriptomic analyses identified differentially expressed genes and pathways associated with systemic anti-tumor immune responses, while mass spectrometry highlighted antigens presented by antigen-presenting cells. Histotripsy induces localized tumor ablation and systemic immune activation, effectively modulating the pancreatic tumor microenvironment. The resulting immune modulation highlights histotripsyís potential as a therapeutic modality, particularly in combination with immunotherapies, to enhance treatment efficacy and achieve durable anti-tumor responses in pancreatic cancer.

P22
Exploring the Impact of High- and Low-Dose Community Resilience Model (CRM) Training at Virginia Tech

Samar Elsaadawy[1], Emma Hare[1], Erica Coate[2]

[1]Virginia Tech Graduate Student | MPH
[2]CRM Coordinator ñ CLAHS Embedded Counselor

Introduction:
In the 2020ñ2021 school year, over 60% of college students reported at least one mental health issue, according to the Healthy Minds Study, which surveyed 373 campuses. This underscores the need for interventions like the Community Resilience Model (CRM), which builds individual and collective resilience. Few studies have explored the impacts of high- and low-dose training sessions. This study examines how CRM exposure affects resilience at Virginia Tech.

Methodology:
Low-dose training consisted of a 45-minute to 3-hour session for individuals from various departments. High-dose training involved a workshop with weekly brief reinforcement of concepts for departmental groups.
Data were collected through open-ended survey questions of both low-dose trainees (n = 837) and high-dose trainees (n = 392). Low-dose trainees were asked about their biggest takeaway from the training, while high-dose trainees were asked about the benefits of using CRM skills and the 'Zones' language (emotional states) taught over multiple sessions.
The responses were analyzed using qualitative inductive coding. Themes were then developed by grouping broader concepts or relationships between codes.

Results:
Analysis of low-dose traineesí responses identified four key themes: Awareness, Understanding, Emotional Regulation, and Techniques & Skills. Trainees learned to notice how their bodies react. They used zone language to understand the connection between their mind and body. They also got better at managing their emotions by using techniques like deep breathing, grounding, and paying attention to their feelings.
Analysis of high-dose traineesí responses revealed three key themes: Communication & Social Interaction, Emotional & Personal Development, and Practical Application. Trainees highlighted improvements in emotional expression within the group, stronger relationships, enhanced self-awareness, and the skills they gained that made them better prepared for various situations.

Conclusion:
The low-dose data suggest that trainees developed better self-regulation, which can increase personal resilience. The high-dose data indicate improved communication, group cohesion, and the use of a shared language. These results highlight the value of both CRM training sessions, with high-dose training offering additional social benefits.

P23
The role of substance abuse in the lives of incarcerated women in a rural Virginia jail

Emma Hare and Sophie Wenzel

Introduction: Existing research suggests large numbers of actively and formerly incarcerated individuals struggle with substance use disorders (SUDs), with one study finding that 49.4% of incarcerated women have a history of substance abuse. This, compared to 14.3% of women in the United States experiencing substance use disorder, suggests a need for intervention. SUDs in incarcerated populations lead to increased mortality risks post-release, recidivism, in-prison violence and victimization.

Methods: To investigate this, fifteen women at the New River Valley Regional Jail were interviewed and asked to speak about their families and loved ones, experiences with domestic violence, experiences with substance abuse, and resources needed. Interview transcripts were recorded, cleaned and coded by three separate researchers and a thematic analysis was conducted.

Results: The thematic analysis uncovered nine primary themes: childhood exposures to substance abuse, environmental pressures, negative influential relationships, positive influential relationships, patterns of substance abuse, impacts of substance abuse, impacts of substance abuse on children, hope and resiliency, and interactions with broader systems.

Conclusion: These findings suggest a lack of accessible substance abuse treatment and mental health support. Various circumstances and exposures drastically impacted the lives of many of these women from an early age and contributed to patterns of recidivism and substance abuse. Improved and accessible mental health and substance abuse treatment options are necessary within carceral settings and post-release to reduce likelihood of relapse and recidivism. Increased community education and substance abuse prevention programs in rural areas will reduce exposures to substance abuse at an early age to stop the generational substance abuse cycle.

P24
Development of Yeast-Engineered Nanobody for Norovirus and Rotavirus Immunoprevention, Therapy, and Early Detection

Sai Navya Vadlamudi[1], Ernawati Giri-Rachman[1,2], Lijuan Yuan[1]

[1]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, USA
[2]School of Life Science and Technology, Institut Teknologi Bandung, Indonesia

Human Norovirus (HuNoV) and Rotavirus (HRV) are leading causes of viral gastroenteritis worldwide, contributing to substantial morbidity and mortality, particularly among young children and the elderly. Norovirus alone is responsible for approximately 685 million infections annually, with an estimated 136,000 to 278,000 associated deaths. Currently, no FDA-approved vaccines exist for Norovirus, although multiple candidates are under development. While vaccines for Rotavirus are available, the disease remains prevalent in low- and middle-income countries, where it accounts for approximately 29% of all diarrhea-related deaths, leading to an estimated 453,000 fatalities per year. The current treatment options are limited to rehydration therapy and supportive care, highlighting the urgent need for novel therapeutic and preventive strategies to combat HuNoV and HRV induced gastroenteritis.

In this study, we utilize yeast-engineered nanobodies targeting HuNoV and HRV. Nanobodies, derived from the variable heavy domain (VHH) of camelid single-domain antibodies, represent a promising class of virus-binding ligands. These VHH nanobodies exhibit distinct advantages, including small size, high solubility, and remarkable stability under diverse environmental conditions. Their simple structure facilitates efficient expression in microbial systems such as bacteria and yeast. Yeast species like Saccharomyces cerevisiae var. boulardii, serve as probiotics and can be genetically modified to display nanobodies fused to cell surface-associated proteins, enabling cost-effective and scalable production.

We engineered S. boulardii to display nanobodies targeting HuNoV and HRV, using AGA1P and AGA2P as surface anchoring proteins. Plasmids (pCEV-G4-Km) carrying the nanobody sequences for NbM5 (anti-HuNoV), Nb2kD1 (anti-HRV), and a combination of both were assembled using Gibson assembly and verified by gel electrophoresis and Sanger sequencing. These plasmids were then successfully transformed into S. boulardii. The functionality of the displayed nanobodies is currently being evaluated through ELISA-based viral binding assays.

This platform may contribute to the development of complementary strategies for the prevention, treatment, and diagnosis of HuNoV and HRV infections, and could be adapted for targeting other foodborne pathogens in the future.

P25
Establishing a Murine Model of Rift Valley Fever Virus (RVFV) Encephalitis Using the MP12 Strain in a BSL-2 Setting

Abraham Adeyemo[1], Caitlin Woodson[1,2], Maryna Akhrymuk[1,2], Kaylee Petraccione[1,2], Todd Bell[3], Kylene Kehn-Hall[1,2]

[1]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA
[2]Center for Emerging, Zoonotic, and Arthropod-Borne Pathogens (CeZAP), Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA
[3]Department of Veterinary Pathology, Iowa State University, Ames, IA 50011, USA

Rift Valley Fever Virus (RVFV) has caused multiple outbreaks in humans across Egypt, Saudi Arabia, South Africa, Uganda and Kenya, with most infections being asymptomatic. However, a subset (8ñ10%) of symptomatic cases progress to severe disease, including ocular complications, hemorrhagic fever, and meningoencephalitis, leading to visual impairment, vision loss, lasting neurological deficits, and sometimes death. Despite its clinical significance, the sporadic nature of outbreaks and limited clinical data hinder a comprehensive understanding of its neuropathogenesis, necessitating a well-characterized animal model. This study aims to establish a BSL-2-compatible mouse model of RVFV encephalitis using the MP12 strain to facilitate mechanistic studies and provide a platform for testing potential antiviral interventions. BALB/c mice were infected intranasally with the MP12 strain of RVFV and monitored for clinical signs, weight loss, and disease progression for up to 28 days post-infection (pi). A subset of mice was sacrificed at days 4 and 8 pi for tissue collection, and viral titers were measured in serum, brain, and liver. Brain and liver samples were analyzed for cytokine expression and histopathological changes. Symptoms appeared on day 7 pi, peaking at days 8ñ10 pi, with infected mice exhibiting significant weight loss (~20%) before being euthanized. Infectious virus was detected in the brain at day 8 pi, coinciding with peak clinical symptoms but was absent in serum and liver. Gene expression assays carried out on brain tissue revealed increased expression of MMP9 (days 9ñ10 pi), IL-1? (days 8ñ10 pi), IFN-? (days 8ñ10 pi), and CXCL10 (days 8ñ10 pi), indicating a robust neuroinflammatory response, while no significant cytokine changes were detected in the liver. Histopathological analysis showed lesions in the olfactory bulb, cerebral cortex, and hippocampus, with peak pathology observed at days 8ñ10 pi. To further characterize this model, a follow-up study is planned, involving intranasal infection of mice with the MP12 strain, monitoring clinical symptoms, weight loss, and collecting serum, liver, and brain samples at days 2, 4, 6, 8, 9, and 10 pi for viral titration and cytokine expression analysis. This study will further refine our understanding of RVFV-induced encephalitis and enhance the utility of this model for future research and therapeutic development.

ABSTRACTS

P26
First successful engraftment of human pancreatic cancer cells in immunocompromised porcine model to test tumor ablation by High-frequency irreversible electroporation (H-FIRE).

Manali Powar[1,2], Tamalika Paul[2], Edward Jacobs[3], Levente Gal[4], Maxx Steimann[4], Mackenzie Woolls[1,2], Carley Elliott[1,2], Poole Cassandra[2], Madeline Mott[1], Katie Orr[4], Christopher Byron[5], Sherrie Clark- Deener[5], R.V. Davalos[3], Sheryl Coutermarsh-Ott[2], Kiho Lee[6], Irving Coy Allen[2]

[1]Graduate Program in Translational Biology, Medicine, and Health, Virginia Tech, Roanoke, VA
[2]Department of Biomedical Sciences and Pathobiology, VA-MD College of Veterinary Medicine, Blacksburg, VA, USA
[3]Coulter School of Biomedical Engineering, Georgia Tech and Emory Medical School, Atlanta, Georgia, USA
[4]College of Science, Virginia Tech, Blacksburg, VA, USA
[5]Department of Large Animal Clinical Sciences, Virginia?Maryland College of Veterinary Medicine, Blacksburg, VA, USA
[6]Division of Animal Science, College of Agriculture Food and Natural Resources, University of Missouri, Columbia, MO, USA

Background: Late diagnosis, critical location of the tumor, limited eligibility of candidates for resection and metastasis make pancreatic tumors very challenging to treat. High frequency Irreversible electroporation(H-FIRE) is a non-thermal ablation technique used to destroy cancer cells by applying short, high-voltage electric pulses creating permanent nanopores in the cell membranes, leading to cell death. These permanent pores then allow the influx and outflux of ions through the cells, which causes instability in homeostasis and, eventually cell death. As most studies are done in rodents, well-established large animal models for assessing the safety and efficacy of H-FIRE in pancreatic tumors are lacking.

Objective: This study aimed to establish the feasibility of successfully ablating pancreatic tumors by H-FIRE using an immunocompromised porcine model.

Methods: This was done by generating Panc1 human pancreatic cancer cell line tumors in the pancreas of the immunocompromised pigs. The orthotopic porcine model was established using RAG2/IL2RG double-knockout immunocompromised pigs. Panc1 cells were injected orthotopically into the pancreas of the pigs. The tumors were treated by the 2 needle probe Nanoknife. The histopathology assessment were done by Hematoxylin and eosin stain.

Results: Three weeks after the injections, the invasive treatment showed the successful engraftment and growth of pancreatic tumors in the pancreas. The study showed feasible access of the probes to the tumor, delivering 2000V/cm, 2us positive - 5us delay - 2us negative pulse, 90 bursts accross 2 axis with minimal local muscle contraction being observed. The histopathological images show immediate and delayed response and clear boundaries of ablation zone by the treatment while preserving the surrounding tissue.

Conclusion: Our preliminary study could demonstrate, for the first time, a robust model of human pancreatic cancer for H-FIRE in a large animal model. Future work will explore the potential of these immunocompromised porcine models for developing advanced therapeutic strategies to enhance tumor ablation by H-FIRE.

P27

Interpersonal violence as a precursor to incarceration in a rural Virginia jail

Mikhala Stafford and Sophie Wenzel

[1]*Center for Public Health Practice and Research*

Introduction: Research suggests that currently and formerly incarcerated women experience alarmingly high rates of domestic violence (DV), sexual violence (SV), and intimate partner violence (IPV). Compared to 27% of all women reporting some form of violence by a partner in their lifetime, up to 98% of incarcerated women report these experiences of violence. Additionally, incarcerated women report significantly more extensive histories of violence in childhood than never-incarcerated women. This disparity of DV, SV, and IPV in incarcerated women highlights the need for further research into their unique experiences and needs.

Methods: To further examine the histories of violence in the lives of incarcerated women, fifteen women at the New River Valley Regional Jail were interviewed and asked to describe their experiences with violence and substance use, as well as their relationships with loved ones and needs for resources. Interviews were recorded, transcribed using Transcribe Me software, and coded using ATLAS.TI. Each transcript was coded twice and reviewed by a tertiary coder, and a reflexive thematic analysis was conducted using Braun & Clarkeís method.

Results: Eight themes related to violence emerged from the thematic analysis: childhood exposures to violence, influential relationships, acute impacts of violence, cumulative impacts of violence, impacts of violence on children, dynamics with abusive partners, survival/resiliency, and systemic failures. Several of the women reported multiple instances of violence throughout their lives, and many of them reported violence experienced across multiple generations in their families.

Conclusion: These findings highlight the profound impacts of violence throughout the life course for these women, as well as a lack of adequate support before, during, and after incarceration. A deeper understanding of the complex pathways between violence and incarceration is critical to improving outcomes for incarcerated women. Immediate support services during and after incarceration, including mental health services, safe housing, and reliable transportation, are vital components of a trauma-informed approach to care for incarcerated women. In addition, primary prevention of violence and early intervention are necessary for mitigating the impacts of violence over the course of their lives.

P29
Cryptococcus neoformans: Do They Use Bias DNA Segregation to Select for Advantageous Mutations

Kimberly Martinez, Rachel Berg-Murante, and Kirsten Nielsen

[1]ID IGEP
[2]BMVS

Cryptococcus neoformans is a pathogenic yeast that is found throughout the environment and primarily impacts immunosuppressed individuals, in particular patients suffering from advanced HIV. C. neoformans infections are initially established in the lungs. In immunocompromised individuals, this initial infection in the lungs can disseminate through the blood stream and ultimately cross the blood brain barrier infecting the brain. Cryptococcal meningitis accounts for 10%-15% of AIDS-associated deaths worldwide.
C. neoformans virulence is associated with its ability to form titan cells. Titan cells are large polypoid cryptococcal cells that are formed when an unbudded G2 arrest occurs during the cell cycle, usually caused by stress. During this unbudded G2 arrest, the normally haploid cryptococcal cell retains the duplicated copy of its DNA instead of transferring the DNA copy to a daughter cell. Additional rounds of DNA synthesis within this unbudded G2 arrest produces the large polyploid titan cells. Titan cells can re-enter the cell cycle and produce typical-sized haploid daughter cells. Titan cells are unable to exit the lungs due to their increased size, however the small daughter cells can disseminate throughout the body. Previous studies showed the daughter cells exhibit new traits, leading to the hypothesis that the daughter cells are important to generate the disseminated infection.
One of the theories of stem cell self-renewal is the immortal DNA strand hypothesis, where asymmetrical chromatid segregation in the stem cells leads to daughter cells with exclusively newly synthesized DNA that may contain mutations and progenitor cells with exclusively old DNA. Due to the titan cells being able to return to a typical cell cycle and produce haploid daughter cells, we hypothesized that C. neoformans have biased DNA segregation, similar to stem cells, and that this biased DNA segregation leads to advantageous traits within the haploid daughter cells. To test our biased DNA segregation hypothesis, we incorporated a thymidine analog into the newly synthesized progenitor cell DNA and detected it using fluorescent antibody flow cytometry. Our preliminary data suggests that the typical cell cycle has unbiased DNA segregation but under stress a portion of the cells exhibit biased DNA segregation. Future studies will analyze DNA segregation in titan cells and test whether biased DNA segregation results in advantageous mutations in the haploid daughter cells.

P30
Plasma ADAMTS13 activity in dogs with severe thrombocytopenia and presumed immune thrombocytopenia

Sierrah D. Travis[1], DVM; Ashley R. Wilkinson[1], DVM, MS, DACVIM, Audrey Keebaugh[1], DVM, MS, DACVIM; Stefanie DeMonaco[1], DVM, MS, DACVIM; Timothy Bolton[2], DVM, DACVIM

[1]Virginia Maryland College of Veterinary Medicine, Veterinary Teaching Hospital, Blacksburg, VA 24060
[2]Purdue University College of Veterinary Medicine, Purdue University Veterinary Hospital, West Lafayette, IN 47907

Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by platelet destruction and impaired megakaryocyte and platelet production. Bleeding tendencies are unpredictable and do not correlate to platelet counts. There is a possibility of misclassification of a subset of dogs with ITP. In human medicine, ITP can be difficult to distinguish from immune-mediated thrombocytopenia thrombotic purpura (iTTP). The condition is due to reduced ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 motif, 13) activity. ADAMTS13 is a von Willebrand factor cleaving enzyme, which prevents the accumulation of ultra-large vWF multimers, which are highly thrombogenic. The treatment for ITP and iTTP differ and proper diagnosis is important.

The primary aim of this prospective study is to determine if a subset of dogs with presumptive primary ITP (pITP) have reduced ADAMTS13 activity. It will further determine if ADAMTS13 activity differs between dogs with presumptive pITP and healthy dogs. ADAMTS13 activity will be evaluated for correlation with clinical bleeding scores. We hypothesize that a subset of dogs with presumed pITP have reduced ADAMTS13 activity compared to healthy dogs and bleeding assessment scores (DOGiBAT) negatively correlate with ADAMTS13 activity.

Fifteen dogs met the inclusion criteria and were greater than or equal to one year of age with a platelet count of less than 20,000 platelets/uL. Diagnostic evaluation categorized the study dogs as pITP or ITP with concurrent disease. All dogs received a DOGiBAT score. The DiapharmaÆ RUO TechnozymeÆ ADAMTS13 Activity ELISA kit assessed plasma ADAMTS13 activity. Wilcoxon rank sum test was used to determine if ADAMTS13 was significantly different in the study dogs compared to healthy dogs. Spearmanís rank correlation rho test was used to determine if ADAMTS13 activity correlates with bleeding scores. The study dogs were compared to 40 healthy dogs enrolled in a separate study recruited from a hospital population.

Preliminary results showed that dogs with presumed ITP have reduced ADAMTS13 activity compared to healthy dogs (P = 0.039). Individually, plasma ADAMTS13 activity in dogs with pITP or ITP with concurrent disease is not different from healthy dogs (P = 0.428, P = 0.095, respectively). ADAMTS13 activity does not correlate with DOGiBAT scores (P = 0.069).

ADAMTS13 deficiency may be a mechanism for thrombocytopenia in some dogs with severe thrombocytopenia that have pre

P31
The Cell Cycle-Regulated Cytoplasmic Kinase CRCK1 Modulates Host Cell Invasion in Toxoplasma gondii

Dima Hajj Ali[1], Ramu Anandkrishnan[1,2], Rajshekhar Y. Gaji[1]

[1]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Blacksburg, Virginia 24061, USA
[2]Department of Biomedical Sciences, Edward Via College of Osteopathic Medicine (VCOM), Blacksburg, Virginia 24060, USA

Toxoplasma gondii is an obligate intracellular parasite which relies on a complex network of protein kinases to regulate essential processes including invasion, replication, and egress. However, the function of many kinases remains poorly understood. In this study, we characterized the role of CRCK1 in T. gondii biology and pathogenesis. Using endogenous tagging and immunofluorescence assays, we demonstrated that CRCK1 exhibited a cell cycle-dependent expression pattern and localized to the cytoplasm during specific cell cycle stages. Conditional depletion of CRCK1 using an auxin-inducible degron system impaired parasite growth, primarily due to defects in host cell invasion. This invasion defect correlated with delayed processing of the microneme protein M2AP, a key factor required for host cell attachment. Transcriptomic analysis of CRCK1-deficient parasites identified 93 differentially expressed genes, many of which are involved in motility, invasion, and intracellular signaling. Surprisingly, despite its clear importance in vitro, depletion of CRCK1 did not attenuate the parasiteís virulence in the T. gondii infection mouse model, suggesting the possibility of the presence of compensatory mechanisms that sustain parasite survival in vivo. Altogether, these findings identify CRCK1 as an important regulator of host cell invasion and underscore the complexity of kinase-mediated signaling in T. gondii. Further studies into its downstream targets may provide valuable insights into parasite pathogenesis and identify new avenues for therapeutic intervention.

P32
Veterinary Growth Promoter as a Dual-Action Inhibitor of Clostridioides difficile Virulence and Recurrence: A New Therapeutic Strategy

A. Abouelkhair[1], N. Abutaleb[1], and M. Seleem[1]

[1]Department of Biomedical Sciences and Pathobiology, Virginia Polytechnic Institute and State University

Objective

Clostridioides difficile is the leading cause of severe antibiotic-associated diarrhea in hospitalized patients, classified as an urgent public health threat causing approximately 12,800 deaths annually and imposing substantial healthcare costs. This led the United States Centers for Disease Control and Prevention (CDC) to announce C. difficile as the "most urgent public health threat". Current treatments suffer from high failure and recurrence rates, highlighting the urgent need for new, effective therapies. Using both laboratory and animal models, this study sought to assess the anti-C. difficile and anti-virulence activity of carbadox (CRX), a previously discovered veterinary growth promoter from screening of microbial metabolites library against C. difficile to create an alternative approach to traditional antibiotics.

Methods

In this study, we were able to identify carbadox from a collection of 527 metabolites that exhibited a powerful anti-C. difficile. Afterward, we assessed CRX's ability to inhibit C. difficile growth using minimum inhibitory concentration (MIC) testing across clinical isolates and time-kill assays to evaluate bactericidal effects and post-antibiotic action. Toxin production was analyzed at subinhibitory CRX concentrations, compared to vancomycin. Mechanistic studies included DNA synthesis inhibition (macromolecular synthesis assay) and measurement of oxidative stress via reactive oxygen species (ROS) production, with N-acetyl-l-cysteine rescue experiments to confirm oxidative damage. In vivo, efficacy was evaluated using a mouse model of C. difficile infection (CDI).

Results

CRX inhibited 50% of C. difficile clinical isolates with a MIC?? of 1 ?g/mL and rapidly killed bacteria within 4 hours, with a post-antibiotic effect lasting up to 14 hours. At subinhibitory doses, CRX reduced toxin production more effectively than vancomycin. Mechanistic studies showed that CRX blocked DNA synthesis and induced oxidative DNA damage, reversible with antioxidant supplementation. In vivo, CRX protected 100% of mice from lethal CDI and prevented infection recurrence, outperforming the control drug, vancomycin.

Conclusions

These results, collectively, demonstrate that CRX has potent antibacterial and anti-virulence activity against C. difficile in vitro and in vivo, including prevention of recurrent infection. These findings position CRX as a promising lead molecule for C. difficile infection (CDI), a critical gap in current therapeutics

P33
Targeting Vancomycin-Resistant Enterococci (VRE) with De Novo Designed Antimicrobial Peptides

Somaia Abdelmegeed[1,2], Mohamed Mohamed[1,2], Ahmed Abouelkhair[1,2], Abdullah Abdelsattar[1,2], Nader Abutaleb[1,2] and Mohamed Seleem[1,2]

[1]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA
[2]Center for One Health Research, Virginia Tech, Blacksburg, Virginia

Vancomycin-resistant Enterococci (VRE) present a significant challenge in clinical settings due to their resistance to conventional antibiotics. This study explores the antimicrobial efficacy of newly designed derivatives of a short ?-helical peptide, RR. Among the variants, RR4 and its D-enantiomer, D-RR4, exhibited over a 32-fold enhancement in antimicrobial activity against multidrug-resistant VRE strains. Notably, D-RR4 demonstrated superior biofilm disruption compared to traditional antibiotics. Mechanistic investigations revealed that these peptides depolarize and permeabilize bacterial membranes, leading to the leakage of intracellular contents. Additionally, the peptides displayed rapid bactericidal action. Similar to linezolid, D-RR4 demonstrated 100% survival of infected mice in a VRE peritonitis model. Interestingly, D-RR4 caused significant reduction of bacteria inside kidney and spleen compared to both untreated and linezolid. These results highlight D-RR4 as a promising therapeutic option in combating VRE infections.

P34
The novel transcription factor TgAP2X-7 is essential for the growth of Toxoplasma gondii

Mandadi P.[1], Anandakrishnan R.[1,2], Gaji R.Y.[1]

[1]Department of Biomedical Sciences and Pathobiology, Blacksburg, Virginia Tech, VA
[2]Department of Biomedical Sciences, VCOM, Blacksburg, Virginia Tech, VA

Toxoplasma gondii is an obligate intracellular parasite with the cat as its definitive host. It infects most warm-blooded animals, including humans, causing abortions in pregnant women and birth defects such as hydrocephalus. The disease has acute and chronic forms. In the acute (virulent) form, the parasite causes tissue destruction, while in the chronic form, it evades the immune system by forming cysts, which persist for life, primarily in the brain and muscles. No drugs eliminate these cysts, which can revert to the virulent stage. Understanding molecular mechanisms regulating this stage conversion is crucial for developing better therapeutics.

Transcription factors, including the AP2 familyófirst discovered in plants and protozoaóplay key roles in T. gondii stage transitions. My research focuses on TgAP2X-7, a hypothesized interactor of TgTKL1, a protein studied in our lab. To investigate TgAP2X-7, we tagged it with an HA epitope and performed immunofluorescence assays (IFA), revealing it as a nuclear protein with peak expression in G1, disappearing in S/early M, and reappearing in late M phase. Given its essential role in parasite survival, we used the auxin-inducible degron (AID) system to conditionally degrade TgAP2X-7 upon auxin treatment. Immunoblots on extracellular parasites demonstrated degradation within 2 hours.

Plaque assays showed a complete loss of plaque formation upon TgAP2X-7 depletion. Lytic cycle assays revealed that while TgAP2X-7 had no role in egress, its depletion led to slower replication and a 65% reduction in parasite invasion efficiency. Although plaque formation ceased entirely, no single step of the lytic cycle was completely blocked. IFAs using various markers confirmed normal parasite growth in untreated wells, whereas auxin-treated parasites became non-viable and misshapen by day four.

RNA-seq analysis revealed 377 downregulated and 183 upregulated genes upon TgAP2X-7 depletion, mainly affecting invasion, metabolism, signaling, and protein trafficking. CUT&TAG assays identified an 8-bp octamer motif (C/TGCATGCA/G) in both up- and downregulated genes, suggesting TgAP2X-7 functions as an activator or repressor depending on its co-factors.

Our future experiments will focus on understanding the role of each AP2 domain of the TgAP2X-7 protein in its overall function, identifying the co-factors or interactors of TgAP2X-7, and investigating its contribution to virulence and cyst formation, both in vitro and in vivo.

P35
Assessing Avian Influenza Spillover Risks: A Methodological Approach to Linking Migratory Bird Flyways and Human Mobility

Kirsten Masters, Nick Ruktanonchai†

[1]*Virginia Tech Population Health Sciences*

Avian influenza is an endemic virus in poultry, with multiple migratory bird species as natural hosts. The current strain (H5N1 clade 2.3.4.4b) circulating in North America has affected over 111 million poultry since 2022. Since March 2024, confirmed detections in dairy cattle have led to over 900 herds being infected. Such a rapidly spreading virus raises concerns about how this virus could spill over into the human population. Though the public health risk for humans with this current strain of avian influenza is low, it is crucial to develop a methodology that allows us to model migratory bird flyways and human mobility together to discern spillover risks. This developed methodology can be used to model disease propagation through the human population, should the virus become readily susceptible to humans.

This project is dedicated to developing a methodology that allows the overlay of migratory bird and human mobility networks with poultry and cattle farms as the bridge between the two networks. This developed methodology can be challenged with seasonality, various migratory bird networks, and other potential risk factors to discern the spillover risk into the human population. Our findings will help determine the counties where spillover is most likely to occur and subsequently affect large communities.

P36
Determining the adaptation of recent Haitian strains of Mayaro virus to urban vector transmission

Cereghino C.[1,2], Weger-Lucarelli J.[1,2]

[1]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA
[2]Center for Emerging, Zoonotic and Arthropod-borne Pathogens, Virginia Tech, Blacksburg, VA

Mayaro virus (MAYV) is an emerging alphavirus responsible for a febrile illness characterized by arthralgia and fatigue that can last years after the acute phase of infection. MAYV is endemic in South America where it is transmitted by jungle-dwelling vectors, but it has been detected in urban Aedes aegypti populations and has caused small outbreaks in urban areas. Recent introductions of MAYV to Haiti caused outbreaks in schoolchildren. The genotypes responsible were new strains of genotype D, found throughout South America, and genotype L, largely restricted to Brazil. Haiti is experiencing rapid deforestation and lacks the ecological conditions that would favor populations of jungle-dwelling vectors. Given this, we sought to determine whether MAYV is becoming more adapted to transmission by urban mosquito vectors by comparing transmission of the Haitian strains to a historical strain isolated from Trinidad and Tobago. Using reverse genetics, we constructed infectious cDNA clones of genotype D and L strains of MAYV isolated in Haiti and generated virus stocks from these clones. We will expose Aedes aegypti and Anopheles albimanus, two urban vectors of MAYV found in both Haiti and the U.S., to the two genotypes of MAYV. Transmission rates, saliva titers, oral infectious doses, and length of transmission windows will be measured to determine whether the Haitian strains have an advantage in transmission compared to the historical strain. Future experiments will examine MAYV transmission in additional mosquito species found in Haiti and determine the viral genetic drivers of transmission.

P37

Advancing One Health forecasting: extreme weather and pathogen exposures from concentrated animal feeding operations

Mahabub Chowdhury[1], Mark E. Borsuk[2], Celine S. Robinson[2], Christopher Krapu[2], Ryan S.D. Calder[1,2]

[1]Dept. of Population Health Sciences, Virginia Tech, Blacksburg, VA
[2]Dept. of Civil and Environmental Engineering, Duke University, Durham, NC

Modern agriculture depends on concentrated animal feeding operations (CAFOs), but these sites present many One Health risks and challenges. For example, heavy rainfall or other extreme weather events can mobilize pathogens (e.g., via sewage lagoon overflow), increasing risks of disease to downstream communities. Such events may be increasing with frequency under climate change. Exposures are hard to model and forecast because of sparse observations of extreme events and complex interactions between management decisions (e.g., frequency of lagoon drainage), natural processes governing fate and transport (e.g., precipitation intensity and duration, landscape features), and social processes governing ultimate exposure (e.g., evacuation during a storm). We develop a model for Duplin County, NC, home to one of the densest concentrations of CAFOs in the United States. We characterize how human pathogen exposures may be affected by changing hydrometeorological conditions and alternative management regimes.

We use the Soil and Water Assessment Tool (SWAT) and the Hydrologic Engineering Center's River Analysis System (HEC-RAS). Streamflow and flooding results for a variety of climate and management scenarios are overlaid onto a land use map to understand implications for human exposure risks. The model developed illustrates the complexity of modeling One Health risks and provides a starting point for screening priorities for intervention and surveillance.

P38
Efficacy of a Live Attenuated HSV-1 Vaccine Candidate for Protection against HSV-2

Matthew D. Irwin[1], Jillian Green[1], Jonathan Ellis-Smith[1], Edward Gershburg[2], Andrea Bertke[1,3]

[1]BMVS
[2]PHS
[3]CeZAP

Herpes simplex viruses (HSV) establish lifelong latent infection from which the virus can periodically reactivate to cause recurrent painful skin lesions, as well as more serious neurological disorders and disseminated disease in newborns. HSV-2 is the primary cause of genital herpes. HSV-1, typically associated with oral lesions, can also cause genital infection but has a much lower risk of recurrences. No licensed vaccines exist for HSV-1 or HSV-2, for either prophylactic or therapeutic use, and treatment relies on antiviral medications that are only partially effective. We report the results of a preclinical efficacy study of a live attenuated vaccine candidate derived from a recombinant HSV-1 virus. Guinea pigs were intradermally administered two doses of RVx1001, in two different formulations (n=11 each), 3 weeks apart. Monitoring of clinical signs, weight and body temperature indicated no safety concerns in response to either formulation of RVx1001. Following challenge with HSV-2 (MS), evaluation of clinical manifestations for 14 days post inoculation (dpi) demonstrated the efficacy of both formulations of RVx1001 against acute disease. Recurrent genital lesions and viral load in vaginal swabs were quantified beginning 15 dpi to assess protection against symptomatic recurrences and asymptomatic viral shedding; both parameters were reduced in vaccinated animals. Neutralization assays with sera collected pre and post vaccination, as well as post challenge with HSV-2, showed high anti-HSV titers in vaccinated animals. Finally, viral load was quantified in lumbosacral dorsal root ganglia to assess the vaccineís impact on the ability of HSV-2 to establish latent infection. The differences in efficacies between two formulations are discussed. The development of a safe and effective vaccine that provides protection against HSV genital disease would have broad public health impacts, with the potential to alleviate painful and stigmatizing symptoms in up to 50 million people in the US and a half billion worldwide.

P39
Nitroxoline: An Old Drug with New Antifungal Potential Against Filamentous Fungi

Khan A.[1,2], Seleem M.[1,2]

[1]*Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA*
[2]*Center for One Health Research, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA*

Invasive fungal infections caused by Aspergillus and Mucor species pose significant challenges due to high mortality rates among immunocompromised patients. Current treatment options, such as azoles, are increasingly limited by rising resistance rates, while polyenes like amphotericin B are associated with significant toxicities. Therefore, identifying drugs that can act as potent antifungals both alone and in combination with standard therapies is critical to improving patient outcomes. Nitroxoline, a broad-spectrum antibiotic used for over 50 years to treat urinary tract infections, was identified through screening the Antimicrobial Library as a promising antifungal candidate. In combination with voriconazole, nitroxoline demonstrated synergistic activity against Aspergillus species, significantly enhancing antifungal efficacy. Time-kill assays revealed that these combinations markedly inhibited A. fumigatus growth over 48 hours. Additionally, nitroxoline exhibited potent standalone activity against Mucor species, highlighting its potential as an effective treatment for mucormycosisóa particularly lethal infection. These findings suggest that repurposing nitroxoline could offer a novel approach to managing invasive fungal infections, warranting further mechanistic and in vivo investigations.

P40
Evaluation of Natral Product ?-mangostin as a Potent and Rapidly Killing Anti-C. difficile Compound

Brice J. Stolz, Ahmed A. Abouelkhair, Nader S. Abutaleb, Mohamed N. Seleem

[1]*Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, United States of America*
[2]*Center for One Health Research; Virginia Polytechnic Institute and State University, Blacksburg, Virginia, United States of America*

Clostridioides difficile (C. difficile) is the leading hospital-associated diarrhea and has remained a consistent threat for older patients and those with comorbidities or vulnerabilities. C. difficile infections (CDI) have high rates of treatment failure and recurrence leading to escalating treatment costs and decreased rates of survival. It is imperative to discover an antibiotic that is both efficient in clearing C. difficile, and cost. ?-mangostin is a natural compound isolated from edible mangosteen fruit pericarp that has previously known antimicrobial activity but is often overlooked due to its poor pharmacokinetics. For CDI, an infection which takes place primarily in the colon, this is ideal as poor absorption and metabolization is necessary for a drug to reach the site of infection in high concentrations. To assess its efficacy against C. difficile, we found that it possesses rapid bactericidal activity completely clearing C. difficile in vitro within 2 hours at a concentration similar to drug choice for CDI vancomycin. This is most likely due to its activity against the bacterial membrane of C. difficile which we confirmed through assays assessing the leakage of DNA and ATP post-exposure to the compound. ?-mangostin also possesses other beneficial characteristics for CDI treatment such as little to no cytotoxicity and stability in high inoculum, pH, and intestinal fluids. These findings demonstrate a potent potential drug scaffold or standalone antibiotic with pharmacokinetic characteristics that are rarely ideally found for CDI.

P42
Bacterial Growth and Infiltration of Polyacrylamide Gels and Viscosupplementation Products Used in Equine Medicine

Jessica Gilbertie, Lauren Trager Burns, Sophie Bogers, Christopher Byron, Claire Read, Lauren Schnabel

[1]*Virginia-Maryland College of Veterinary Medicine*

Osteoarthritis and degenerative joint disease are common disorders in horses, especially sport horses, and can lead to significant pain and decreased performance. Treatment of osteoarthritis in equine medicine has progressed over the years, and the aim to identify and utilize disease modifying agents versus traditional symptom modifying agents, such as steroids, has been a driving force in altering equine practice. Equine clinicians are now utilizing orthobiologics, viscosupplementation, and polyacrylamide gels that have the potential to enhance joint health and slow or alter progression of disease. Although understanding of the pathophysiology of osteoarthritis has evolved, and aids in guiding targeted therapy, the use of novel biomedical devices such as polyacrylamide gels has outpaced scientific study.

Increased use of biomedical devices has been partially guided by observation of positive outcomes on a clinician-to-clinician basis; however, actual data collection has been scant. Due to the paucity of solid scientific evidence, many clinicians shy away from the use of polyacrylamide gels and other viscosupplementation products. One main concern is that based on demonstrated properties of polyacrylamide material following intra-articular injection; i.e. that Noltrex 4% Polyacrylamide gel mainly coats the articular surface and Arthramid 2.5% Polyacrylamide integrates into the synovium and joint capsule, there is potential for persistent joint infection and septic arthritis following intra-articular injection.

The general safety and function of polyacrylamide gel products have been studied, mainly in vitro, or in healthy joints, although there is some published data on use in diseased joints. Overall little adverse reaction has been reported following injection. However, there has been very little data published in regard to the potential for joint infection and biofilm formation associated with the devices. Although joint infection is rare following joint injections in general (if proper sterile technique is used), the potential of an implant associated infection with a device that is integrated into the joint and cannot be removed is of great concern. The aim of this study is to fill a major gap in knowledge by studying the ability of bacteria to form biofilms and even potentially penetrate and grow within polyacrylamide gels and other

P43
Cooperation between mucoid and nonmucoid Pseudomonas aeruginosa during biofilm formation in the cystic fibrosis lung

Chad Artman[1], Preston J. Hill[2], Stacie E. Deaver[1], Erin S. Gloag[1]

[1]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA
[2]Department of Microbial Infection and Immunity, The Ohio State University, Columbus OH, USA

Background: Cystic fibrosis (CF) is a genetic disease which results in chronic pulmonary infections due to abnormal mucus biology, and subsequent decreased mucosal expectoration. Chronic Pseudomonas aeruginosa infections are associated with the emergence of evolved variants that coexist with the parental strain. Isolation of P. aeruginosa mucoid variants from CF clinical samples is associated with increased treatment complications and worsened lung function. We hypothesize that cooperative mechanisms between nonmucoid and mucoid subpopulations that alter key physical biofilm properties contribute to persistent CF infections and pulmonary exacerbation.

Methods: To assess cooperation between mucoid and nonmucoid P. aeruginosa during biofilm formation, mixed biofilms were grown with a starting inoculum of 1, 10, 50 and 90% mucoid cells, and compared to single biofilms. Biophysical cooperation of biofilms was determined by rheological testing. Creep-recovery, and dynamic oscillatory analyses were performed to measure biofilm viscoelastic behavior. To determine spatial interactions, biofilms were grown in flow cells and imaged by confocal laser scanning microscopy (CLSM). Images were analyzed by COMSTAT and BiofilmQ to quantify changes in biofilm biomass, and subpopulation localization. Carbazole assay was used to determine alginate concentrations of lawn grown biofilms. Agar hydrogel mimics were used to determine penetration of fluorescently tagged LL-37 by CLSM.

Results: Material strength significantly increased for mixed biofilms with 50% mucoid cells, compared to single biofilm controls. Creep-recovery revealed that mixed biofilms had significantly increased viscoelasticity and oscillatory frequency sweep analysis showed that 50% mucoid cells are more resistant to cough clearance than single mucoid biofilms. Carbazole assay determined that alginate is significantly reduced when strains are grown together. CLSM of mixed biofilms grown in flow cells revealed that mucoid cells localized specifically to the substratum of the biofilm, with the nonmucoid cells forming microcolonies on top. Nonmucoid and mixed population hydrogel mimics showed reduced antimicrobial peptide penetration.

Conclusion: Results indicate that mucoid and nonmucoid P. aeruginosa display unique joint biofilm development, forming biofilms that have altered biomass, architecture, and mechanical properties, which may lead to a more adapted, recalcitrant infection in the CF lung.

P44

Evaluation of the effectiveness of the antimycobacterial compound SQ109 against the human pathogen Cryptococcus neoformans.

Nour M. Alkashef [1,2], Ehab A. Salama [1,2], Ramu Anandakrishnan [1,3], Tony R Hazbun [4,5], Christopher B. Lawrence[1,2], Mohamed N. Seleem[1,2]

[1]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 24061, USA
[2]Center for One Health Research, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, 24061, USA
[3]Department of Biomedical Sciences, Edward Via College of Osteopathic Medicine (VCOM), Blacksburg, Virginia, USA
[4]Purdue Institute for Cancer Research, Purdue University, West Lafayette, Indiana, USA
[5]Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN, USA

Cryptococcosis is a global health threat that primarily affects individuals with a weakened immune system. This opportunistic infection is predominantly caused by inhaling fungal cells from Cryptococcus neoformans and to a lesser extent, from C. gattii. The rising use of immunosuppressive agents has raised concerns about the risk of this infection, even among immunocompetent individuals. Additionally, the limited accessibility to effective treatments, emerging resistance, and treatment-related toxicities underline the pressing need for more effective therapeutic options. In this study, we conducted a whole-cell screening of ~ 3700 clinical molecules against the C. neoformans H99 strain. The anti-mycobacterial agent, SQ109 was identified among the most potent hits, with a broad antifungal activity, being more potent against Cryptococcus with MIC90 of 4 ?g/mL. In the time-kill assay, SQ109 exhibited fungicidal activity on the proliferated cryptococcal cells in a concentration-dependent manner. In contrast to fluconazole (FLC) and flucytosine (5-FC), Cryptococcus showed a negligible tendency to overwhelm the lethality of SQ109 during frequent passaging. Delving into its mechanism of action, the transcriptomic analysis highlighted the impact of SQ109 on the ergosterol biosynthesis pathway. Furthermore, SQ109 exhibited substantial in vivo efficacy in the murine model of a cryptococcal infection. Treating with a dose of 25 mg/kg for 10 consecutive days resulted in a 50% survival rate among the infected mice. Our findings highlight the therapeutic potential of SQ109 in combating cryptococcal infections as a standalone treatment.

P45
Brilacidin: A Novel Peptide Mimetic For Combating Multidrug- Resistant Neisseria gonorrhoeae

Abdallah S. Abdelsattar[1,2], Nader S. Abutaleb[1,2], and Mohamed N. Seleem[1,2]

[1]*Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA*
[2]*Center for One Health Research, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA*

The Centers for Disease Control and Prevention (CDC) has classified Neisseria gonorrhoeae as an urgent public health threat due to the increasing incidence of infections and the rapid development of resistance. N. gonorrhoeae has progressively developed resistance against all FDA-approved therapeutics. Ceftriaxone is currently the only recommended treatment for gonococcal infections. Yet, isolates that exhibit high-level resistance to ceftriaxone have been reported worldwide. Without new treatments, untreatable gonorrhea could soon become a reality. The present study utilized a drug repurposing approach to identify the peptide mimetic, brilacidin, as an effective anti-gonococcal agent. Brilacidin is currently in Phase 2 clinical trials for the treatment of skin infections and COVID-19. Brilacidin displayed a potent anti-N. gonorrhoeae activity inhibiting a panel of multidrug-resistant clinical isolates at concentrations ranging from 1 to 4 ?g/mL. The peptide exhibited a fast bactericidal activity against N. gonorrhoeae, completely eradicating the high bacterial burden within two hours. Moreover, brilacidin was superior to the drug of choice, ceftriaxone, in completely eliminating the intracellular N. gonorrhoeae harbored inside endocervical cells. Additionally, brilacidin showed high tolerability to mammalian cells and did not show hemolytic activity to human red blood cells up to 128 ?g/mL. Altogether, the results clearly demonstrate that brilacidin is a highly promising anti-gonococcal agent that warrants further in-depth investigation.

P46
Determining the Importance of Casein Kinase 1 Activity in Rift Valley Fever Virus Infection

Flor R.[1,2], Akhrymuk I.[1,2], Akhrymuk M.[1,2], Stocker T.[1,2], OíMaille P.[3], Omichinski J.[4], Silberfarb A.[5], Kehn-Hall K.[1,2]

[1]Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA
[2]Center for Emerging, Zoonotic and Arthropod-borne Pathogens, Virginia Tech, Blacksburg, VA
[3]Biosciences Division, SRI International, Menlo Park, CA, USA
[4]Department of Biochemistry and Molecular Medicine, UniversitÈ de MontrÈal, MontrÈal, QC, Canada
[5]Artificial Intelligence Center, SRI International, Menlo Park, CA, USA

Rift Valley Fever Virus (RVFV) is an arthropod-borne RNA virus from the genus Phlebovirus, order Bunyavirales. It causes mild to moderate febrile illness in humans, but can also progress to hemorrhagic liver necrosis and encephalitis. RVFV represents an important pathogen due to a lack of preventative measures and treatments, combined with its rising threat to public health and agriculture, and its ability to cause a phenomenon described as abortion storms in ruminants. The RVFV RNA-dependent RNA polymerase (L protein) is responsible for viral transcription and replication. Previous mass spectrometry (MS) analysis identified 5 phosphorylated amino acids within the L protein, 4 serine (S) and 1 threonine residues. Further MS data focused on L protein-protein interactions and showed that casein kinase 1-alpha (CK1?) is a potential host enzyme involved in the regulation of L protein phosphorylation. We hypothesize that RVFV makes use of this host enzyme in order to regulate its own replication and transcription cycles through the modification of the L proteinís phosphorylation states. To test this hypothesis, we utilized two experimental drugs, D4476, a pan CK1 inhibitor, and BTX-A51, a CK1? specific inhibitor. Treatment was performed in Vero cells and human small airway epithelial cells (HSAECs) which were infected with RVFV at a multiplicity of infection (MOI) of 0.1 or 1. Supernatants were collected at 24 hours post infection and infectious titers measured via plaque assay. Treatment with BTX-A51 in HSAEC cells resulted in one-log reduction of infectious virus titers at both MOIs, while the same treatment in Veros resulted in over a log and a half reduction, also at both MOIs, when compared to DMSO control samples. D4476 treatment resulted in a two-log reduction in HSAEC cells infected at MOI 0.1. In addition, treatment with D4476 inhibited L protein phosphorylation. These results demonstrate the importance of CK1 for L protein phosphorylation and RVFV replication, highlighting CK1? inhibition as a potential antiviral treatment. Future studies will determine mechanistically how CK1? is utilized to facilitate RVFV infection, in addition to its involvement in L protein phosphorylation. We will test the potential of BTX-A51 as an antiviral treatment in a BALB/c mouse model. Overall, this information can be used to better understand key aspects of the viral cycle and for the rationale design of host directed therapeutics for the treatment of RVFV infection.

P47
Directed evolution of Semliki Forest virus to improve oncolytic efficacy in glioma cells

Ian Taylor, James Weger-Lucarelli

[1]*Virginia-Maryland College of Veterinary Medicine, Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg VA*
[2]*Center for Emerging, Zoonotic and Arthropod-borne Pathogens, Virginia Tech, Blacksburg VA*

Glioblastoma (GBM) is the most common form of highly aggressive brain cancer, and, while other forms of cancer have seen significant increases in patient life expectancy in recent decades, GBM continues to be a terminal diagnosis. Oncolytic viral therapy, the use of viruses to kill cancer cells, shows promise as a cancer treatment and may finally improve GBM treatment outcomes. Semliki Forest virus (SFV) A774 is avirulent, but is able to cross the blood brain barrier in mice and has shown promise at treating GBM in preclinical models. However, while the virus readily kills GBM cells in vitro, it has failed to show such promise in immunocompetent in vivo models. To improve SFVís ability to target GBM cells, we sought to develop a strain of SFV that is more cytotoxic to GBM cells and less cytotoxic in healthy brain cells. To that end, we serially passaged SFV A774 in GL-261 murine glioma cells for 10 passages, tested them for cytotoxicity against the parent strain of SFV, and sequenced the most cytotoxic virus populations, with the goal of identifying mutations that increase the oncolytic efficacy of SFV. We found a promising mutation in the E1 fusion protein, D327G. This mutation shows significant increases in GL-261 cytotoxicity, with no greater degree of cytotoxicity in a non-cancer mouse microglia cell line. We plan to further explore this mutation with fusion assays and test the safety of this mutant in normal human astrocytes. Further, we will infect immunocompetent mice with the mutant to determine its safety in vivo. Finally, we will test this virus in GBM mouse models to determine its oncolytic activity.

P48
Histotripsy as a Dual-Function Therapy: Optimizing Tumor Ablation and Immune Activation in Breast Cancer

Carley Elliott[1,3], Cassandra Poole[2,3], Tamalika Paul[2,3], Irving Coy Allen[1,2,3,4,5]

[1]Graduate Program in Translational Biology, Medicine and Health, Virginia Tech, Roanoke, Virginia, USA
[2]Graduate Program in Biomedical and Veterinary Sciences
[3]Department of Biomedical Sciences and Pathobiology, Virginia-MD College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia, USA
[4]Institute for Critical and Applied Science Center for Engineered Health, Virginia Tech, Blacksburg, Virginia, USA
[5]Virginia Tech Carilion School of Medicine, Roanoke, Virginia, USA

Breast cancer is among the most commonly diagnosed cancers in women worldwide, with over 2.3 million new cases annually. Despite therapeutic advancements, current treatments are limited by incomplete tumor control, adverse effects, and variable immune responses, particularly in aggressive subtypes. Conventional tumor ablation techniques, like radiofrequency and microwave ablation, rely on thermal energy, which can damage surrounding tissue and restrict immune activation. Histotripsy, a novel, non-thermal ultrasound-based ablation method, mechanically disrupts tumors via cavitation, preserving critical structures while enabling precise ablation. Real-time image guidance enhances treatment precision, ensuring effective tumor destruction while minimizing unintended tissue damage and procedural risks.

Beyond mechanical precision, histotripsy has the potential to enhance systemic anti-tumor immunity by promoting antigen release and immune cell recruitment. Tumor disruption through cavitation induces the release of damage-associated molecular patterns (DAMPs) and other anti-tumor mediators, stimulating antigen-presenting cells and triggering downstream immune activation. Early studies suggest histotripsy reshapes the tumor microenvironment, shifting it from an immunosuppressive (ìcoldî) state to an immunologically active (ìhotî) one. These localized immune effects may also drive a systemic immune response, leading to an abscopal effect in which distant metastatic sites regress alongside the primary tumor. However, the extent and durability of these immune responses remain unclear.

This research aims to optimize histotripsy parameters for tumor ablation and assess its immunomodulatory effects in murine 4T1 breast cancer models. By characterizing immune cell infiltration, DAMP and anti-tumor mediator release, and systemic immune activation, this work will further elucidate histotripsyís role in reshaping the tumor microenvironment and promoting long-term anti-tumor immunity. Findings will provide critical mechanistic insights into histotripsyís potential as a dual-function therapy. By integrating non-invasive tumor ablation with immune activation, histotripsy could revolutionize breast cancer treatment, particularly for patients with advanced disease, limited surgical options, or high recurrence risk. Understanding its immunological impact will inform clinical translation and combination therapies, positioning histotripsy as a transformative approach in oncology.

P49
Inhibition of Stress Hormone Receptors Reduces Clinical Recurrences of Herpes Simplex Virus 1 In Guinea Pigs

Green, J. C.[1,4], Moore, G. A.[1,4], Irwin, M.[1,4], Joyce, J. D.[2,4], Bertke, A. S.[3,4]

[1]*Biomedical and Veterinary Sciences, Virginia Tech, Blacksburg, VA*
[2]*Translational Biology, Medicine, and Health, Virginia Tech, Blacksburg, VA*
[3]*Department of Population Health Sciences, Virginia Tech, Blacksburg, VA*
[4]*Center for Emerging, Zoonotic and Arthropod-borne Pathogens, Virginia Tech, Blacksburg, VA*

Herpes simplex virus 1 (HSV1) establishes lifelong latency in sympathetic and sensory neurons and can cause painful oral and genital lesions upon reactivation. The World Health Organization estimates approximately 67% of individuals globally have HSV1 and many unknowingly transmit the virus due to asymptomatic shedding. Stress is one of the commonly known stimuli that can induce HSV1 reactivation from latency. The stress hormone epinephrine mediates the ìfight or flightî response by binding to adrenergic receptors (AR) found on numerous cell types throughout the body, including sympathetic and sensory neurons. We previously showed in vitro that epinephrine causes reactivation of HSV1 in sympathetic neurons, but not sensory neurons, and reactivation requires the activation of multiple adrenergic receptors, specifically ?2-, ?1-, and ?2-ARs. We hypothesized that blocking these ARs pharmaceutically would decrease the frequency of clinical recurrences of HSV1 in vivo. Following intravaginal infection, Hartley guinea pigs were either untreated or treated with atipamezole (?2-AR antagonist), propranolol (non-selective ?-AR antagonist), or a combination of atipamezole/propranolol. Treatment with atipamezole alone reduced recurrences by 29.26% (p=0.148), but recurrences were further and significantly reduced by treatment with propranolol alone (43.55% decrease, p=0.035) or both atipamezole and propranolol (39.12% decrease, p=0.047). Blocking adrenergic receptors, particularly beta blockers, may be useful for reducing the frequency of HSV1 recurrences.

P51
Determining the Role of Viral Structural Genes that Drive ZIKV Shedding in Semen

Nolen, T.[1], Marano, J.[1], Weger-Lucarelli, J.[1], & Duggal, N. [1]

[1]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA

Zika virus (ZIKV; Flaviviridae, Flavivirus) is a mosquito-transmitted virus that normally causes flu-like symptoms but can cause microcephaly in newborns exposed in utero. In the recent 2015 pandemic in the Americas, sexual transmission of ZIKV was reported and was estimated to cause 3-23% of ZIKV cases. Active replication of ZIKV occurs in the epididymis which contributes to viral shedding in semen. The most closely related virus to ZIKV, Spondweni virus (SPOV), displays poor shedding in semen; however, it is still able to disseminate to the epididymis. Currently, it is unknown what viral genes of ZIKV contribute to sexual transmission of the virus and which epididymal cell types contribute to viral shedding during acute infection. Here, we present a tool to investigate the mechanism of ZIKV shedding in semen. First, we generated a SPOV infectious clone. Then, using bacteria-free cloning techniques, we generated chimeric viruses in which prM/E structural genes were swapped between ZIKV and SPOV. Future studies with these chimeras will determine whether these genes drive viral shedding in semen and identify epididymal cell types that help facilitate acute viral shedding using an in vivo mouse model. These studies will be crucial in identifying factors that dictate viral shedding in semen and will increase our understanding of how viruses are sexually transmitted.

ABSTRACTS

P52
Alphavirus infection causes chronic neurobehavioral outcomes, cellular remodeling, and hippocampal single-cell transcriptomic changes similar to neurodegenerative diseases

Morgen VanderGiessen[1,2], Elizabeth Harris[1], Liduo Yin[1], Brittany Heath[1,2], Caitlin M. Woodson[1], Xiaowei Wu[3], Erik Johnson[4], Hehuang Xie[1], Michelle Theus[1], Kylene Kehn-Hall[1,2]

[1]*Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA*
[2]*Center for Emerging, Zoonotic, and Arthropod-borne Pathogens, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA*
[3]*Department of Statistics, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA*
[4]*United States Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland, USA*

Viral infections, including those caused by neuroinvasive pathogens such as Venezuelan equine encephalitis virus (VEEV), may lead to persistent neurological symptoms that can mimic or potentially trigger the onset of neurodegenerative disorders. Despite its significant public health implications, including its potential as a biological weapon, VEEV remains understudied. To this end, we characterized the pathology, behavior, and transcriptomic changes caused by VEEV infection in C57BL/6 mice. Mice were intranasally infected with a sublethal dose of VEEV TC-83 (~80% survival), weighed, and monitored for clinical scores for 106 days post infection. By Day 9, 65% of mice displayed signs of neurological illness including circling, head tilt, head pressing, and altered gait or imbalance. At 30, 60, and 90 DPI mice appear to have hyperexcitability, anxiety-like behavior, and memory changes at 60 and 90 DPI with some signs of neurological disease emerging at 30 DPI. At both acute (Day 7), and chronic (Day 106) days post-infection (DPI), mice had reduced hilar interneurons (NeuN), reduced inhibitory (Reln) neurons, and prolonged activation of microglia (Iba1) and astrocytes (GFAP) in the hippocampus. Transcriptomic analysis highlighted persistent alteration in signaling pathways including immune response, synaptogenesis, and glutaminergic signaling, which appear to be correlated with persisting neural activation. Finally, we utilize a combination of transcriptomic, proteomic, and drug discovery resources to identify potential neuroprotective drug targets. Pifithrin-? (PFT-?), an inhibitor autophagy and apoptosis which targets heat shock protein 70 (HSP70) interactions with many proteins critical for viral pathogenesis, including p53 and Bcl-2, was selected because of neuroprotective activity in other models of neurological injury. Treatment with PFT-? resulted in a significant reduction in viral replication, viral capsid protein expression, and autophagy markers such as LC3 and p62. These results emphasize the therapeutic potential of targeting autophagy pathways, particularly in primary cell culture and in vivo. By bridging the fields of cell biology, virology, and neuropathology, this research offers innovative strategies to understand and combat alphavirus-induced neuronal damage and valuable framework for developing neuroprotective interventions, with broader implications for understanding viral impacts on cellular function and neurodegeneration.

P53
Modulation of cellular transcriptomics by Venezuelan equine encephalitis virus capsid protein

Abdullahi Jamiu[1,2], Ivan Akhrymuk[1], Kenneth Foreman[3], Dmitri Klimov[4], Mikell Paige[3], Kylene Kehn-Hall[1,2]

[1]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA
[2]Center for Emerging, Zoonotic, and Arthropod-Borne Pathogens (CeZAP), Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA
[3]Department of Chemistry and Biochemistry, George Mason University, Manassas, VA 20110, USA
[4]School of Systems Biology, George Mason University, Manassas, VA 20110, USA

Venezuelan equine encephalitis virus (VEEV), a mosquito-borne, single-stranded RNA virus infects both equines and humans, with neurological complications occurring in ~14% of human cases. VEEV is also classified as a select agent by both the CDC and USDA. However, there are currently no FDA-approved therapeutics or licensed vaccines for VEEV infection. The VEEV capsid protein is a key virulence factor, binding simultaneously to the host nuclear import receptors (importin ?/?1) and the nuclear export receptor (CRM1) to form a tetrameric complex. This complex accumulates at the nuclear pore, disrupting nucleocytoplasmic trafficking, suppressing host transcription and antiviral responses, and ultimately inducing cell death. Notably, the VEEV Cm mutant, which lacks a functional nuclear localization sequence (NLS) in the capsid and is unable to block nucleocytoplasmic trafficking, fails to suppress cellular transcription and antiviral responses, marked by strong Type I interferon response in infected cells. However, the global gene expression and pathway changes induced by VEEV Cm remain uncharacterized. To address this, bulk RNA sequencing was performed to analyze transcriptomic changes in human microglial HMC3 cells infected with VEEV or VEEV Cm at 3, 9, and 18-hours post-infection, capturing early, mid, and late transcriptional responses. RNA sequencing was conducted using the NovaSeq platform, followed by Ingenuity Pathway Analysis. Comparative analyses between the VEEV group relative to the VEEV Cm group revealed time-dependent transcriptional alterations. At 3 hpi, pathways related to cellular development, differentiation, and immune response were amongst the top canonical pathways. By 9 hpi, pathways associated with cell cycle regulation, immune response, apoptosis, oxidative stress, and protein translation were significantly altered. At 18 hpi, the top pathways were linked to antiviral responses and cell death. Among key upstream regulators, NUPR1, a stress-response transcription factor involved in ER stress, oxidative stress, apoptosis, autophagy, and cell cycle, was predicted to be significantly inhibited in the TC83 group relative to Cm (z-score: -8.848). Future studies will focus on elucidating the role of NUPR1 and its downstream targets in VEEV pathogenesis. Taken together, this project will elucidate the mechanisms by which VEEV capsid suppresses host transcription, providing critical insights that will aid in vaccine and therapeutic development.

P54
Depletion of the gut microbiota reduces inflammatory cytokines in murine lupus mice

Rana Estaleen[1], David Oakland[2], Hilary Montano[1], Ran Lu[1], Arpita Das[3,4], Pavly Amin[5], Christopher M. Reilly [1,6], Xin M. Luo[1]**

[1]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech
[2]Translational Biology, Medicine and Health Graduate Program, Virginia Tech
[3]School of Neuroscience, College of Science
[4]Department of Chemical Engineering, College of Engineering
[5]Department of Biological Sciences, College of Science, Virginia Tech
[6]Department of Biomedical Sciences, Edward Via College of Osteopathic Medicine
Corresponding authors: xinluo@vt.edu; chreilly@vt.vcom.edu

The gut microbiota contributes to the health and disease of host and an imbalance of the gut microbiota is linked to several diseases, including systemic lupus erythematosus (SLE). The gut microbiome is composed of bacterial cells residing in the human gastrointestinal tract. CX3CR1 is expressed on various types of cells including lymphocytes, monocytes, granulocytes, and leukocytes. Cx3cr1+/+ MRL/lpr (WT) and Cx3cr1-/- MRL/lpr (KO) female mice were treated with antibiotics for 12 weeks and the mice were euthanized at 15 weeks of age. Antibiotics treatment reduced kidney CD4 T cells and double negative (DN) T cells expressing RORgt, proinflammatory cells. Antibiotics treatment also depleted CD4 T cells and DN T cells producing IL-17A, interestingly, IL-17F production was not affected by the antibiotics treatment. To examine bacterial translocation from the gut to peripheral tissues, kidney single cell suspension was plated on blood agar plates for 72-hours in 37oC. We observed no significant differences between WT and KO mice treated with antibiotics; however, we speculate that the identity of bacteria will differ in the antibiotics mice compared to the control mice. This data suggests that antibiotics treatment may be beneficial for patients with SLE.

P55

Role of gut microbiota in Cx3cr1 deficiency-mediated exacerbation of murine lupus

Rana Estaleen[1]Ü, Aida Shakeri[2]Ü†, David Oakland[3], Razan Alajoleen[1], Ran Lu[1], Hilary Montano[1], Pavly Amin[2], Christopher M. Reilly [1,4], Xin M. Luo[1]**

[1]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech
[2]Department of Biological Sciences, College of Science, Virginia Tec
[3]Translational Biology, Medicine and Health Graduate Program, Virginia Tech
[4]Department of Biomedical Sciences, Edward Via College of Osteopathic Medicine
*Corresponding authors: xinluo@vt.edu; chreilly@vt.vcom.edu
Ü Co-First Authors
†Presenting author

The health of a host is dependent on a balanced gut microbiota. When there is an imbalance, this can be linked to systemic lupus erythematosus (SLE). A chemokine receptor, CX3CR1, initiates intracellular signaling cascades. These cascades contain three main roles: survival, proliferation, and regulation of cellular activity. CX3CR1 is expressed on various types of immune cells. In a previous study, we have shown that Cx3cr1 deficiency intensifies lupus-like disease in the MRL/lpr mouse model, hence in the case of its absence, it changes the gut microbiota. The focus of this study was based on the hypothesis that CX3CR1 regulates lupus nephritis through gut microbiota-mediated renal infiltration of T cells which produce IL-17. The effects of the microbiota on glomerulonephritis were analyzed in wild-type (WT) and Cx3cr1-knockout (KO) MRL/lpr mice over a twelve-week co-housing experiment. Co-housing of WT and KO mice together allows mice to share the gut microbiota through coprophagy. Proteinuria levels in KO MRL/lpr cohoused with WT mice were significantly reduced. This reduction in proteinuria suggests that the WT microbiota attenuates proteinuria in KO mice. In the renal lymphocytes, there was also a significant reduction of T helper cells producing IL-17A in KO MRL/lpr mice co-housed with WT mice. There was no significant effect on IL-17F production in these mice. Additionally, KO MRL/lpr mice cohoused with WT mice moderately decreased the levels of naÔve CD4+ T cells. However, the introduction of KO microbiota in WT mice induced the production of cytotoxic T cells in WT MRL/lpr mice. The experimental results indicate that the gut microbiota may impact different T cell subsets in the kidney and intensify lupus nephritis in Cx3cr1-deficient MRL/lpr mice.

P56
Paraspeckle Protein NONO Regulates Active Chromatin by Allosterically Stimulating NSD1

Chen-I Hsu, Shenglin Mei, Justin Demmerle, Sarah Ruttenberg, Mahnue Sahn, and Jia-Ray Yu

[1]*Graduate Program in Biomedical and Veterinary Sciences (BMVS), Virginia Tech*
[2]*Virginia Tech Fralin Biomedical Research Institute Cancer Research Center DC, Childrenís National Research & Innovation Campus*
[3]*Department of Biomedical Sciences and Pathobiology (DBSP), Virginia-Maryland College of Veterinary Medicine, Virginia Tech*
[4]*Center for Cancer and Immunology Research, Childrenís National Hospital*

NSD1 is a key lysine methyltransferase for di-methylation of lysine 36 of histone H3 (H3K36me2) essential for the establishment of active chromatin domains. While the loss of NSD1 catalytic activity halts embryonic development and a gain of that drives oncogenesis in leukemia and glioma, the regulatory mechanisms that control NSD1 activity in these processes remain poorly understood. Here, we uncover that NSD1 requires allosteric activation through the aromatic pocket of its PWWP2 domain. Surprisingly, we identify that NSD1-PWWP2 binds to a noncanonical target, nuclear paraspeckle protein NONO, and this protein-protein interaction allosterically stimulates the catalytic activity of NSD1. Mouse embryonic stem cells (mESC) engineered with mutations in the aromatic pocket of NSD1-PWWP2 cannot differentiate into neural progenitor cells (NPC), and genetic depletion of NONO partially phenocopied this defect at cellular and transcriptional levels, potentially explaining the neurodevelopmental disorder phenotypes in NSD1- or NONO-deficient patients. Our work revealed a novel mechanism driving active chromatin domain formation and has critical implications in the interplay between nuclear paraspeckles and active chromatin, and a vulnerability of NSD1 for therapeutic interventions.

P57
A Potential Therapeutic Role of Adenosine Receptor Modulation on Systemic Lupus Erythematosus Disease Progression

Hilary Montano, Emma Poehler, Tyler McCormick, Shawn Dziepak, Rana Estaleen, David N. Oakland, Amrie Grammer, Peter Lipsky, and Christopher M. Reilly

[1]*Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech*
[2]*Department of Biomedical Sciences, Edward Via College of Osteopathic Medicine*
[3]*Translational Biology, Medicine and Health Graduate Program, Virginia Tech*
[4]*AMPEL BioSolutions LLC, Charlottesville, VA*

Adenosine receptor dysregulation has been linked with lupus disease exacerbation as it plays a crucial role in immune modulation. We hypothesized that either activation of A2AR or inhibition of the A2BR would decrease lupus disease severity. Lupus prone NZB/W female mice were intraperitoneally injected daily for 12 weeks with ADO-5024 (A2BR antagonist) or ADO-6001 (A2AR agonist) beginning at 20 weeks-of-age. Mice received dexamethasone as a standard control and PBS as the negative control. As they aged, urine was assessed for proteinuria and inflammatory cytokines were measured in the sera. At 32 weeks-of-age, the mice were euthanized, and spleen and kidney tissue were assessed. Our findings showed that mice treated with the ADO-5024 had overall attenuated lupus disease not statistically different from dexamethasone treatment, while those treated with the ADO-6001 and PBS were statistically different from the former across all parameters including proteinuria, renal pathology, and inflammatory cytokine expression. Mice treated with ADO-5024 showed decreased inflammatory profiles as observed by multiplex assay measuring cytokines like BAFF, TNF-alpha, and more. The ADO-5024 and dexamethasone treated mice showed reduced IgG and C3 deposition and decreasing trends of glomerular and interstitial inflammation score compared to the PBS treated or ADO-6001 treated animals. Overall, our data suggests that inhibition of the A2BR may have therapeutic potential in treating lupus disease and more specifically lupus nephritis.

P58
Spatial transcriptomic analysis of low-grade intestinal T cell lymphoma and lymphoplasmacytic inflammatory bowel disease in cats

Laura V. Quishpe Contreras[1], Santiago Diab[1], Priscila Serpa[1,2]

[1]Virginia-Maryland College of Veterinary Medicine
[2]Cornell University College of Veterinary Medicine

Background: Feline idiopathic inflammatory bowel disease (IBD) and low-grade intestinal T cell lymphoma (LGITL) are two common diseases that affect the gastrointestinal tract of cats with symptoms that are often nonspecific and overlap. Routine tests and even biopsies and histopathology will often lead to an inconclusive diagnosis.

Objective: To transcriptionally profile IBD and LGITL using spatial sequencing in FFPE tissues of feline jejunum and ileum.

Hypothesis: This study hypothesizes that IBD will have altered expression of genes associated with inflammation, while LGITL will present alterations in genes involved with cell survival, proliferation, and repair.

Methods: Fifteen formalin-fixed and paraffin-embedded (FFPE) samples were collected, 6 for cats with LGITL, 6 for cats with IBD (3 ileum, 3 jejunum per group) and 3 control samples (ileum). Controls are full-thickness samples from necropsy cats without gastrointestinal or neoplastic diseases. The LGITL and IBD cases are from the Virginia Tech Animal Laboratory Service archives (2017-2024). Quality of the samples was assessed by the student and three board-certified pathologists. Selected samples were sent for PARR, and those with confirmatory results (monoclonal for LGITL and polyclonal for IBD) were used for transcriptomic analysis.
The spatial RNA transcriptomic analysis was performed using the platform GeoMx (Nanostring). To identify the cells of interest in a structural context, GeoMx uses fluorescent antibody detection as morphology marker. Validation of primary and secondary antibodies was conducted in cat spleen and ileum FFPE samples.
This study is also a pilot for the application of the platform in cat tissues. A commercially available canine oncopanel (GeoMxÆ canine cancer atlas) composed of probes for 1962 genes was used. For adequate performance, homology over 85% is required, and 1574 genes from the panel attained the minimum homology. Antibodies for T and B cells were used as morphology markers to locate the regions of interest, and a library is created for sequencing using Illumina.

Statistical analysis: Data generated from GeoMx is currently being analyzed using R Studio, involving normalization, dimensionality reduction, and clustering, followed by downstream bioinformatics pipelines to explore key aspects of cell-signaling and cell micro-environment aanalysis.

Current state of the project and next steps: The samples were sequenced and are currently being analyzed.

P59
Characterization of acute neuropathogenesis in a sublethal-sequelae mouse model of EEEV infection

Carney S. K.[1,2], Woodson C.[1,2], VanderGiessen M.[1,2], de Jager C.[1], Harris E.[1], LeRoith Tanya[1,2], Bishop B.[3], Theus M.[1], Kehn-Hall K.[1,2]

[1]Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA, USA
[2]Center for Emerging, Zoonotic and Arthropod-borne Pathogens, Virginia Tech, Blacksburg, VA, USA
[3]Department of Chemistry, George Mason University, Manassas, VA, USA

Eastern equine encephalitis virus (EEEV) is a mosquito-borne encephalitic alphavirus that causes disease in both equines and humans, infection with which results in death in 50-75% of patients, and of the survivors, 50-90% experience debilitating neurological sequelae. These include seizures, paralysis, intellectual disability, and permanent mood and behavioral changes. Currently, there are no therapeutic options available. While EEEV is known to cause disease and death in mice, few studies have performed a detailed analysis of neuropathology and there is no neurological sequelae mouse model currently available. We aim to address this deficiency by developing and characterizing a novel neurological sequelae model of EEEV infection that will be used to characterize innate immune and neuroinflammatory gene expression within the brain. We hypothesize that animals, which appear to have recovered from EEEV infection, still suffer substantial neuropathology resulting from neurodegeneration associated with viral infection. In order to afford animal survival, EEEV FL939-39 was mutated in three regions important for virulence and nano luciferase was inserted to measure viral replication via bioluminescence (EEEV TM-nLuc). Mice were intranasally infected with 10^3, 10^4, or 10^5 plaque forming units of virus, IVIS imaging performed on days 3, 5, and 7 post-infection (pi), and their brains collected at day 14 pi to assess neuropathology. Viral replication was observed in 5/5 mice in the 10^4 and 10^5 PFU dose groups by day 7 pi and 100% of mice survived infection. Significant neuropathology was observed at day 14 pi, including neuronal necrosis, gliosis, meningitis, and perivascular cuffing. With this triple mutant, we achieved neurovirulence with correlated neuropathology and survival in mice, which is favorable to study neurological sequelae. Ongoing studies aim to recapitulate these results and compare neuropathology and gene expression in mice during acute infection with parental EEEV nLuc and attenuated EEEV TM-nLuc in order to assess EEEV TM-nLuc as an appropriate sublethal model of EEEV infection. With these results we will be able to characterize the host response to EEEV infection and understand the associated neuropathology and potential therapeutic targets to prevent neurological damage and disease.

P60
Identification of recurrent mutations in canine mast cell tumors using PCR amplification on cytologic specimens

Marlie Nightengale, Natalia Strandberg, Kevin Lahmers, Kurt Zimmerman

[1]*Virginia-Maryland College of Veterinary Medicine*

Cutaneous mast cell tumors (MCTs) are the third most prevalent type of tumor in dogs, manifesting either as isolated or as multiple, distinct, or progressing lesions. These tumors frequently metastasize to local lymph nodes, the liver, spleen, and other organs, particularly in cases of poorly differentiated tumors. In contrast, well-differentiated tumors can often be effectively treated with surgical excision. Given the significant variability in the biological behavior of MCTs, accurately predicting this behavior is crucial for determining appropriate treatment strategies and setting realistic expectations for pet owners.

Traditionally, histopathology has been the standard for grading these tumors, but comparable grading on cytologic samples is not commonly practiced despite the frequent use of cytology for quick and definitive diagnosis. Moreover, prognostic panels on formalin-fixed tissues that detect mutations indicative of aggressive behavior are available, yet the emergence of new chemotherapy protocols that diminish the role of surgical intervention necessitates non-invasive predictive methods.

This study aims to detect specific recurrent mutations in MCTs using DNA extracted from cytologic specimens. Our primary objective is to employ PCR amplification on archived cytologic samples from mast cell tumors to identify mutations linked to specific biological behaviors. Selected PCR products will undergo nanopore sequencing to verify the presence of these mutations. We hypothesize that these genetic markers will correlate with the histologic grades of the tumors, providing valuable prognostic information for managing clinical cases. Our secondary objective is to explore the relationship between the identified mutations and the histologic grade of the tumors, with the goal of developing a prognostic panel applicable to cytologic samples, thus potentially obviating the need for surgical biopsy.

P61
Comparison of cardiac output measurements utilizing two-dimensional (2D) and three-dimensional (3D) transesophageal echocardiography with pulmonary artery thermodilution

Annie Showers, BS, DVM; Giulio Menciotti, DVM, MS, PhD, DACVIM (Cardiology); Vaidehi Paranjape, BVSc, MVSc, MS, DACVAA

[1]*Department of Small Animal Clinical Sciences*
[2]*Virginia Maryland College of Veterinary Medicine*
[3]*Virginia Tech*

Cardiac output (CO) can be estimated utilizing transesophageal echocardiography (TEE). This technique is minimally invasive and negates the need for right heart catheterization, which is required for obtaining gold standard CO measurements. However, echocardiographic aortic area measurements have been identified as one of the main sources of inaccuracy of echocardiographic CO measurements. The objective of this study was to compare CO estimates calculated with real-time 3D TEE (3DTEECO) and 2D TEE (2DTEECO) with CO measurements obtained via pulmonary artery thermodilution (PATDCO). This was a prospective diagnostic comparison study in which six healthy male beagles underwent two different experiments. Dogs were randomly administered four different vasoactive drugs in the first experiment. For the second experiment, their blood volume was manipulated using phlebotomy, autotransfusion, and colloid bolus. In addition, the dogs underwent a modified passive leg raise maneuver during each blood volume state to assess fluid responsiveness. Hemodynamic data for †PATDCO, 2DTEECO and 3DTEECO was recorded after each intervention and their respective return to baseline hemodynamic state. A total of 120 CO measurements were therefore obtained by each method. Bland-Altman analysis was performed for the general dataset to assess variability between the different diagnostic approaches for CO measurement or estimation. Additionally, the data was stratified by the various vasoactive drugs and blood volume states to assess diagnostic variability in different hemodynamic scenarios. A greater measurement bias was appreciated between PATDCO and 3DTEECO (-1.35, [-1.57 to -1.13], $p<0.0001$) vs PATDCO and 2DTEECO (0.25, [0.13-0.38], $p<0.0001$), though both biases were significant. Analysis of Bland-Altman Plots revealed a trend of 3DTEECO overestimating CO at lower values and underestimating CO at higher values compared to PATDCO. The correlations between 2DTEECO and PATDCO, and PATDCO and 3DTEECO were significant but not strong (0.64, $P<0.0001$; 0.73, $p<0.0001$). Only 3DTEECO showed significant bias from PATDCO measurements when stratifying the data by vasoactive drug conditions. In conclusion, while both 2DTEECO and 3DTEECO show moderate-strong correlation with PATDCO, both modalities are prone to bias.

P62
Classification of Canine Primary Sensory Neurons in Dogs

Leanne Jankelunas DVM, MSc[1]; Shamsuddin A. Bhuiyan, PhD[3]; William Renthal, MD, PhD[4]; Rell Parker DVM, PhD, DACVIMñNeurology[2]

[1]Department of Biomedical and Veterinary Sciences, VA-MD College of Veterinary Medicine
[2]Department of Small Animal Clinical Sciences, VA-MD College of Veterinary Medicine
[3]Renthal Lab, Neurology, Brigham and Womenís Hospital
[4]John R. Graham Headache Center, Department of Neurology, Brigham and Womenís Hospital

Like people, dogs experience acute and chronic pain; however, there are still opportunities to better understand pain pathways in these species. To study the sensory systems of the dog, we have decided to focus on the cells that are the primary sensory neurons, to determining the cell types present. This can be done by measuring RNA expression in individual neurons and comparing them to previously established cell types; thus, developing a cell atlas. Once cell types are identified, this research can then be expanded to evaluate alterations in gene expression correlated with various types of acute and chronic pain. At this time, studies developing cell atlases and evaluating gene expression within the primary sensory neurons of the dorsal root ganglia have been conducted in a variety of species, including rodents and humans, but not in dogs. Given this, the primary goal of this study was to perform single nuclei RNA sequencing of lumbosacral dorsal root ganglia and develop a cell atlas for this region in the dog. The secondary goal was to compare this atlas with known human and rodent models to determine if the dog is a good translational model for pain studies in humans and to understand fundamental species differences from rodents as well. The L5, L7, and S1 DRG from four canines that were euthanized for non-orthopedic and non-neurologic conditions had single nuclei RNA sequencing performed using Genomics 10X Single Cell sequencing reagents and a cell atlas was generated from these samples which was then compared to available rodent and human models.

P63
Characterizing the Glioblastoma "Ablatosome" Treated with High-Frequency Electroporation

James May, Jonathan Hinckley, Dr. John Rossmeisl Jr. DVM, MS, DAVCIM

[1]*Veterinary and Comparative Neuro-oncology Laboratory, Virginia-Maryland College of Veterinary Medicine, Virginia Tech*

Glioblastoma (GBM) is a highly invasive and treatment refractory brain tumor in humans and dogs that is associated with short post-treatment survivals. Successful treatment of GBM will require eradication of diffusely invasive tumor cells without damaging the infiltrated brain; modulating the highly immunosuppressive tumor microenvironment (TME); overcoming the blood brain barrier (BBB) that inhibits delivery of therapeutics to the tumor; and minimizing adverse effects associated with current standard-of-care treatments. To address these limitations, our laboratory developed pulsed electrical field therapies (PEF) for cancer, including irreversible electroporation (IRE) and high-frequency irreversible electroporation (H-FIRE). The treatment configurations can be manipulated as needed to a range of downstream biological effects. Using canine spontaneous and rodent orthotopic models of GBM, our current objective is to characterize the molecular landscape of the TME (the ablatosome) to elucidate mechanisms by which H-FIRE causes tumor cell death as well as contributes to tumor control through the initiation of host anti-tumor immune responses. Our primary hypothesis is that H-FIRE treatment, due to its nonthermal and lipid membrane modifying mechanisms of action, induces cell death via pyroptosis and ferroptosis, and initiates adaptive and innate immune signaling pathways. Treatment naÔve and acute post-H-FIRE treatment tumor biopsies from canine and rat GBM models will be utilized for a cross-species analyses. Select transcriptomic and proteomic markers of pyroptosis, ferroptosis, and ICD will be further validated using immunohistochemical and gene expression analyses on tumors from both species. Through in vitro characterization of dose-dependent effects of H-FIRE on DAMP expression and immune cell recruitment in GBM, we hypothesize that 1) HFIRE treatment of GBM will result in expression of ICD-associated DAMPs that are positively correlated to the strength of the applied electrical field; and 2) H-FIRE associated DAMP expression will induce immune cell recruitment in a dose-dependent fashion. The immediate impact of this proposal is to generate data needed to define and refine mechanisms of PEF therapies in animal models of GBM prior to human clinical trials. The potential long-term impact is the development of innovative combinatorial ablative, BBB permeating, and immunotherapeutic approaches that fill a current and critical need for GBM patients.

P64
Novel Cell Segmentation Method for Spatial Transcriptomics Data with Subcellular Resolution

Amanda Moore[1], Yu Lin[1,2], Xiguang Xu[1,2], Min Liu[1,2], Timothy Jarome[3], Hehuang Xie[1,2,4,5]

[1]*Department of Biomedical & Veterinary Sciences, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061, USA*
[2]*Epigenomics and Computational Biology Lab, Fralin Life Sciences Institute, Virginia Tech, Blacksburg, VA 24061, USA*
[3]*Department of Animal and Poultry Sciences, College of Agriculture and Life Sciences, Virginia Tech, Blacksburg, VA, 24061, USA*
[4]*Graduate Program in Translational Biology, Medicine, and Health, Virginia Tech, Blacksburg, VA, 24061, USA*
[5]*School of Neuroscience, Virginia Tech, Blacksburg, VA, 24061, USA*

New technologies allow us to study RNA activity within cells (and within the context of the cellís surrounding tissue). New technologies call for new methods to facilitate analysis. A novel method for defining the cell boundaries in such data improves the ability of researchers to study these large-scale datasets.

P65
Monocyte-specific loss of ephrin receptor A4 alters chronic pathological changes in neuronal architecture in the dentate gyrus associated with cognitive decline following TBI

Elizabeth A. Harris[1], Sabrina Budianto[3], Michelle H. Theus[1,2]

[1]Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA 24061, USA
[2]Center for Engineered Health, VT, Blacksburg, Virginia, 24061, USA
[3]College of Science, Virginia Tech, Blacksburg, VA 24061, USA

Traumatic brain injury (TBI) is a major cause of morbidity and mortality in the US, often leading to serious chronic conditions including post-traumatic epilepsy, cognitive impairment, and mood disorders. We recently revealed significant derangements in the inhibitory interneuron population and excitatory granule cells in the dentate gyrus (DG) following TBI, which have been implicated in driving post-traumatic sequalae. These findings correlated with the influx of blood-borne monocytes expressing the ephrin receptor A4 (EphA4). The aim of this study is to characterize the role of monocyte-specific EphA4 in mediating chronic alterations in interneuron subtypes, aberrant neuroblast migration, and cognitive impairment in a model of controlled cortical impact (CCI) injury using conditional Epha4f/f/Ccr2-CreERT2 (monocyte-specific KO) and Ccr2-CreERT2 (WT) mice. Histopathology was performed at 7, 30 and 120 days post-CCI injury (dpi) or sham craniectomy and behavioral assessments were performed at 30, 60, 90dpi. Non-biased stereological quantification revealed a significant loss in DG hilar interneurons after CCI injury, coinciding with chronic spatial memory decline and cortical tissue damage in WT mice. These outcomes were attenuated in monocyte-specific EphA4 KO mice, including an observed decrease in the incidence of chronic glial activation and acute levels of phospho-NF-kB in the DG. These findings are the first to demonstrate that EphA4-expressing blood-derived monocytes drive acute and chronic pathological changes in the neuronal architecture in the DG and suggest their pro-inflammatory properties contribute to cognitive decline following TBI. These findings highlight monocytes as potential therapeutic targets for chronic neuroinflammation and cognitive decline.

P66
Comparative Study of Heel Movement and Foot Biomechanics in Aluminum Nail-On and Indirect Glue-On Fabric Cuff Shoes in Horses

Fabian J Roa, Amy Santonastaso, Travis Burns, Christopher Byron, Rebecca Funk, Lauren Trager, Raffaela De Vita

[1]*Virginia-Maryland College of Veterinary Medicine*

The equine hoof is a dynamic structure that undergoes deformation during locomotion, contributing to shock absorption and vascular perfusion. Traditional shoeing methods have been implicated in restricting natural hoof movement, potentially altering biomechanics and increasing the risk of hoof pathologies. This study aimed to compare the effects of two aluminum shoeing techniques nail-on shoes and indirect glue-on fabric cuff shoes on heel movement in horses of various breeds and disciplines.

Fifteen healthy horses were evaluated under three conditions: barefoot, aluminum nail-on shoes, and indirect glue-on fabric cuff shoes. A displacement sensor was affixed to the heels of one forelimb to measure total heel displacement over 20 strides at both the walk and trot. Testing was conducted on both hard (asphalt) and soft (arena footing) surfaces. Mixed-model ANOVA was used to assess differences between shoeing conditions.

Results demonstrated significantly greater heel expansion in barefoot horses compared to both shoeing conditions ($P < 0.0001$). While indirect glue-on shoes allowed for more heel movement than nail-on shoes at the trot ($P = 0.0005$), no significant difference was observed between the two at the walk ($P = 0.1742$). These findings confirm that both shoe types restrict heel expansion, though to differing degrees.

Limitations of this study include the absence of an absolute zero baseline measurement, preventing differentiation between heel expansion and contraction, as well as data loss for some horses on soft footing due to low heel conformation.

This study contributes to the understanding of equine hoof biomechanics and the impact of shoeing techniques on heel movement. The findings support the need for further investigation into alternative materials and methods that optimize hoof function. Additionally, they have implications for therapeutic farriery, particularly in cases requiring hoof stabilization, such as coffin bone fractures.

P67

Optimization techniques for Deep Branch Lateral Plantar Neurectomy and Plantar Fasciotomy in the Equine

Nadine Altmann DVM; Jessica Gilbertie MS, DVM, PhD; Elaine Claffey DVM, DACVS-LA; Teresa Southard DVM, PhD, DACVP; Sophie Bogers BVSc, MVSc, PhD, DACVS-LA

Proximal suspensory ligament (PSL) desmitis affects athletic horses. Neurectomy of the deep branch of the lateral plantar nerve (DBLPN) and plantar fasciotomy (PF) doubles return to work rate vs no surgery. However, complex anatomy and poor visualization results in difficult identification of the DBLPN increasing risk of tissue trauma, infection and hemorrhage. Moreover, Metzenbaum scissors traditionally used to cut the plantar fascia result in PSL damage in 90% of cases. Our objectives are to optimize identification of the DBLPN using ultrasound (US) guided perineural Methylene Blue (MB) injection and compare damage to the PSL between 3 fasciotomy techniques. We hypothesize that US guided perineural MB stain will reduce time to DBLPN identification for inexperienced surgeons, and fasciotomy using a MPR double-shielded carpal tunnel knife (CTK) or Evans CTK and guide will result in a lower frequency and degree of PSL damage compared to Metzenbaum scissors. Sixteen paired (32 total) cadaveric equine hindlimbs free of orthopedic disease will be positioned to mimic a live surgery. Each limb will be randomly assigned to one of four conditions (8 per group): 1) MB stain + inexperienced surgeon, 2) MB stain + experienced surgeon, 3) no stain + inexperienced surgeon, 4) no stain + experienced surgeon. A 2nd year ACVS resident will act as an inexperienced surgeon and an ACVS diplomate with 10 years experience will act as the experienced surgeon. For MB groups, a US guided injection of 0.1 mL MB is placed in the location of the DBLPN. Surgical isolation of the DBLPN will be performed as described for both stained and unstained groups. Timed identification of the DBLPN will be recorded from incision start until isolation. One of three randomly assigned PF techniques will be performed by the experienced surgeon (10 per group): 1) Metzenbaum scissor, 2) MPR CTK, 3) Evans CTK. Post procedure, the plantar metatarsus will be dissected and the PSL inspected under dissection microscope. PSL damage will be graded by gross measurement of length and depth. PSL sections will undergo histological assessment. We have performed MB stain guided (n = 3) and MPR CTK (n = 2) or Evans CTK (n = 1) in 3 pilot limbs. MB stain increased subjective ease of DBLPN identification by the inexperienced surgeon, requiring less soft tissue dissection. PF was successful with the CTKs with no gross evidence of PSL damage. The study is ongoing.

P68
Intrapancreatic Gas in a Dog: A Case Report and Literature Review of Etiologies

Andrianna Krippaehne, DVM, Michael Edwards, DVM, MS, Ph.D., DAVCR, Michael Ciepluch, DVM, MS, DACVR

[1]*Virginia-Maryland College of Veterinary Medicine, Department of Small Animal Clinical Sciences*

This case report describes an unusual presentation of gas identified via ultrasound within the pancreas of a dog, who was also diagnosed with pancreatitis.

Pancreatitis has a variable clinical presentation in the dog, depending on severity and acuity. No clinical sign or combination of signs has been identified as pathognomonic for acute pancreatitis in dogs, but common signs include anorexia, vomiting, evidence of nausea, weakness, abdominal pain, diarrhea and possibly signs of dehydration and shock. Ultrasound findings in acute pancreatitis commonly include an enlarged, or mass-like pancreas with hypoechoic or heterogeneous parenchyma, adjacent hyperechoic mesentery, and peritoneal effusion. However, a lack of changes on sonographic exam of the pancreas does not rule out pancreatitis.

A 13 year old castrated male Dachshund presented for suspected pancreatitis and hemorrhagic gastroenteritis. On ultrasound, the pancreas was heterogeneous, lobular, and surrounded by hyperechoic mesentery and a small volume of peritoneal effusion, consistent with pancreatitis. There was also linear, branching, gas within the pancreas and the accessory pancreatic duct. The patient responded well to medical management and was discharged 5 days later.

Intrapancreatic gas is not described in the veterinary literature, however, it is documented in human literature. In humans, gas may be found within and around the pancreas secondary to emphysematous pancreatitis, as a complication of necrotizing pancreatitis, or pancreatic abscessation, often associated with high mortality rates. It can also be associated with a pancreatic fistula, occurring secondary to ulceration, pseudocyst rupture, pancreatitis, surgery, or trauma. Gas is rarely found within the ductal system of the pancreas, associated with biliary-pancreatic or duodenal-pancreatic reflux due to dysfunction of the papillae sphincters, which may occur secondary to regional surgery, papillitis, often as a complication of biliary, duodenal, or pancreatic disease, including pancreatitis, or spontaneously.

In this patient, the gas is suspected to be within the pancreatic ductal system. Human literature suggests this gas may arise from biliary-pancreatic or duodenal-pancreatic reflux, potentially associated with the known pancreatitis, however, this is not documented in dogs. Additional study is necessary to identify the potential etiologies and clinical significance of pancreatic gas in veterinary patients.

P69

Simulation of pooling optimization methods for flexible application responsive to scarcities, surges, and lapses in testing capacity in surveillance testing

Catharine Burgess[1,2,4], Alan Curran, Duan Loy[3], Dustin Loy[3], Alessanro Ceci[4], Carla Finkielstein[4,5,6], Kevin Lahmers[1,7]

[1]Department of Biomedical Sciences and Pathobiology, Virginia Maryland College of Veterinary Medicine, Virginia Tech
[2]Graduate Program in Translational Biology, Medicine and Health
[3]Nebraska Veterinary Diagnostic Center, School of Veterinary Medicine and Biomedical Sciences, University of Nebraska Lincoln, Lincoln, NE 68583, USA
[4]Molecular Diagnostics Laboratory, Fralin Biomedical Research Institute at VTC, Virginia Tech, Roanoke, VA, USA
[5]Fralin Biomedical Research Institute at VTC, Virginia Tech, Roanoke, VA, USA
[6]Center for Zoonotic and Arthropod-borne Pathogens, Virginia Tech, Blacksburg, VA, USA
[7]Virginia Tech Animal Laboratory Services (ViTALS), Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, USA

Sample pooling is a critical strategy to meet increased testing demand and conserve resources in surveillance testing. Much of its effectiveness depends on how well optimized the pool size is to the prevalence of infection in the sampled population, which can be difficult to anticipate in many circumstances, including the infection dynamics and sampling practices for a given target pathogen. Multiple methods exist to better optimize pooling, with unique trade-offs. Understanding the limitations and advantages of different optimization methods to apply each method appropriately under different testing circumstances is not well understood, and suboptimal pooling can add time to sample processing with less benefit to testing economy or can create additional tests and cost if prevalence is misestimated to a high degree. Using Monte Carlo and Discrete Event simulations, this study aimed to develop parameters for applying different optimization methods in a flexible way to make laboratories more responsive to resource scarcities, testing surges, and lapses in laboratory capacity, as well as determining their effect on processing time during surges and outbreaks, and costs to clients when frequent re-sampling is critical for disease management. Different infection dynamics were represented through surveillance data of Theileria orientalis Ikeda in the Eastern United States, Bovine Viral Diarrhea Virus (BVDV) in Nebraska, and COVID-19 in Southwest Virginia. Simulation results showed contrasting performance of historical optimization between T. orientalis with cumulative herd prevalence and BVDV with fluctuating herd prevalence, wherein severe misestimation caused additional testing and cost in T. orientalis, but was highly accurate in BVDV, not statistically different from true optimal pooling ($p > 0.05$). COVID-19 simulations showed all pooling optimization methods reduced processing time during large scale surveillance events, but not during outbreak periods, except for when testing capacity was lowered. Prevalence estimation optimization was similar to true optimal pooling ($p > 0.05$) in resource conservation for all datasets, but added the most processing time, requiring changing optimization approaches when the dominant priority shifts between resource conservation and processing time. These results show a greater degree of adaptability when laboratories can flexibly apply multiple pooling optimization methods.

P70
Phenotypic Characterization Of Exhausted Monocytes In Lupus-Prone Mice

Armstrong C[1,2], Caldwell B[2], Lu R[1,2], Alajoleen R[1], Estaleen R[1], Oakland D[1,3], Montano H[1], Luo X[1], Li L[2]

[1]Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA
[2]Department of Biological Sciences, Virginia Tech, Blacksburg, VA
[3]Virginia Tech Carilion School of Medicine, Virginia Tech, Roanoke, VA

Autoimmune diseases are estimated to affect 10% of the population. Systemic lupus erythematosus (SLE), a common autoimmune disease, can affect multiple organs including the skin, kidneys, and heart. Despite progress in the development of targeted biologics, successful management of SLE typically still requires the use of immunosuppressive medications. Development of novel drugs with strong safety profiles requires an improved understanding of the underlying disease pathogenesis. Previous studies have highlighted the key role that immune exhaustion (IE) plays in the etiology of different diseases (e.g. chronic viral illnesses, cancer). The role of IE in autoimmune diseases (including SLE) is a developing area of research. Historically, IE was defined primarily in T cells, a key cell type of the adaptive immune system. Recent work has demonstrated that IE can also occur in the innate arm of the immune system, including in monocytes/macrophages. Notably, while IE is known to occur in T cells in autoimmune conditions (e.g. SLE), the role of monocyte exhaustion (ME) in this context is still poorly understood. Here, we used both conventional and spectral flow cytometry to characterize the ME phenotype in a in a murine model of SLE (MRL/lpr). This phenotype was characterized both in vivo and following administration of an ex vivo inflammatory challenge. Not only does ME track with disease severity in vivo, but it also precedes the onset of SLE symptoms, suggesting a potential role for ME in disease initiation. In addition, bone marrow-derived macrophages (BMDMs) taken from MRL/lpr mice exhibited heightened sensitivity to an inflammatory stimulus (lipopolysaccharide, LPS) administered ex vivo compared to controls, further suggesting an alteration in their functional state. Collectively, these findings define the distinct features of ME in a well-established murine model of SLE. The underlying mechanisms driving this phenotype warrant additional investigation, as they may serve as potential targets for novel SLE therapies.

AWARDS

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