33rd ANNUAL RESEARCH SYMPOSIUM

VIRGINIA-MARYLAND COLLEGE OF VETERINARY MEDICINE

March 18, 2024 • The Inn at Virginia Tech • 7:30 AM - 7:30 PM

Welcome to the 33rd Annual Research Symposium

Each year, the Virginia-Maryland College of Veterinary Medicine hosts this event to support the College's mission of providing education to a diverse population of professional and post-graduate students in preparation for careers in the broad areas of veterinary medicine, biomedical sciences, and public health. The event serves to showcase the research of our graduate and training programs. The MS and Ph.D. degree curriculum operates as a single multi-disciplinary Biomedical and Veterinary Sciences (BMVS) graduate program.

For more information about our program, please visit our program website:
bmvs.vetmed.vt.edu

BIOMEDICAL AND VETERINARY SCIENCES
VIRGINIA TECH™

PROGRAM

Forging ahead: Pathways toward novel clinical approaches

7:30 am	**Registration and Continental Breakfast**
8:20 am	**Welcome - Dr. Coy Allen** *Chair of VMCVM Research Committee*
8:30 am	**Public Health Block** *Faculty Speaker: Dr. Julia Gohlke* *Faculty Speaker: Dr. Ryan Calder* *Full Talk: Greyson Moore* *Flash Talks: Rylee Matheson, Mols Kwitny, Kirsten Replogle*
9:45 am	**Break/Networking**
10:00 am	**Biomedical Sciences and Pathobiology Block** *Faculty Speaker: Dr. X.J. Meng* *Faculty Speaker: Dr. Kevin Lahmers* *Full Talk: Yehia Elgammal* *Flash Talks: Sai Navi Vadlamudi, Rachel Persinger, Hilary Montano*
11:15 am	**Break/Networking**
11:30 am	**Keynote Speaker Dr. John Rossmeisl** **"The role of comparative medicine in the evolution of clinical practice"**
12:15 pm	**Lunch**
1:00 pm	**Poster Session** *1:00PM to 1:45PM Even numbered posters* *1:45PM to 2:30PM Odd numbered posters*

PROGRAM

Forging ahead: Pathways toward novel clinical approaches

2:30 pm **Large Animal Block**
Faculty Speaker: Dr. Dan Givens
Faculty Speaker: Dr. Jess Gilbertie
Full Talk: Dylan Easley
Flash Talks: Catherine Jula, Emilee Lacey, Fabian Jimenez Roa

3:45 pm ***Keynote Speaker Dr. Yanjin Zhang***
"Zika Virus Perturbs Importin for Efficient Replication."

4:30 pm **Break/Networking**

4:45 pm **Small Animal Block**
Faculty Speaker: Dr. Joanne Tuohy
Faculty Speaker: Dr. Rell Parker
Full Talk: Alessandra Franchini
Flash Talks: Camille Brassard, Christina Vezza, Andy Hsueh

6:00 pm **Roundtable and Closing Remarks**

6:30 pm **Dinner and Awards**

KEYNOTE SPEAKER

Dr. John Rossmeisl

“The role of comparative medicine in the evolution of clinical practice”

Associate Department Head
Department of Small Animal Clinical Science
Dr. and Mrs. Dorsey Taylor Mahin Professor of Neurology and Neurosurgery

John H. Rossmeisl is the Dr. and Mrs. Dorsey Taylor Mahin Endowed Professor of Neurology and Neurosurgery at Virginia Tech. He received a DVM degree from Auburn University, a MS from Virginia Tech, and is board certified by the American College of Veterinary Internal Medicine (ACVIM) in the specialties of small animal internal medicine and neurology. Dr. Rossmeisl has worked for 20 years on elucidating the mechanisms of brain tumor formation, developing novel systems for the delivery of drugs to the brain, and conducting clinical trials in dogs with brain cancers. His research has been funded by the NIH, Focused Ultrasound Foundation, American Brain Tumor Association, American Kennel Club, and private foundations. He serves on several national research advisory boards, including the National Cancer Institute’s Comparative Brain Tumor Consortium Steering Committee, and is a past-president of the ACVIM specialty of neurology.

KEYNOTE SPEAKER

Dr. Yanjin Zhang

"Zika Virus Perturbs Importin for Efficient Replication."

Associate Professor
University of Maryland
Department of Veterinary Medicine

Yanjin Zhang is an associate professor in the Department of Veterinary Medicine (Maryland Campus of Virginia-Maryland College of Veterinary Medicine), University of Maryland, College Park. His research interests are on molecular virology, virus-cell interactions, viral pathogenesis, antiviral drug and vaccine development. His current projects are on hepatitis E virus (HEV), porcine reproductive and respiratory syndrome virus (PRRSV), Zika virus (ZIKV), and the coronavirus SARS-CoV-2. His research focuses on elucidating mechanisms of viral replication in the host cells, viral interference with innate immunity, especially interferon production and interferon-activated JAK-STAT signaling, and other aspects of virus-cell interactions. He is also a faculty member of the Molecular and Cell Biology Program (MOCB). He serves as an Associate Editor for Virology Journal and an Academic Editor for BioMed Research International. He has over 70 peer-reviewed publications, two book chapters, and several patents or invention disclosures.

Before he joined the University of Maryland, he was an assistant professor at the Center for Pediatric Research, Eastern Virginia Medical School. He graduated from Iowa State University with a Ph.D. degree in immunobiology and received postdoctoral training at the University of Texas Health Science Center in San Antonio. He got his DVM training at Shandong Agricultural University, China.

ORAL PRESENTATIONS

Public Health Block

8:30am - 9:45am

MODERATED BY:

Chad Artman

Laura Contreras

FACULTY SPEAKERS

DR. JULIA GOHLKE, Associate Professor Environmental Health

-

"LEVERAGING COMMUNITY-ENGAGED AND SECONDARY DATA ANALYSIS APPROACHES TO ELUCIDATE HEALTH IMPACTS ASSOCIATED WITH CLIMATE CHANGE"

DR. RYAN CALDER, Assistant Professor Environmental Health and Policy

-

"ADVANCING THE ENERGY TRANSITION THROUGH COMMUNITY VALUATION OF PUBLIC HEALTH OUTCOMES"

ORAL PRESENTATIONS

Public Health Block

8:30am - 9:45am

MODERATED BY:

Chad Artman

Laura Contreras

STUDENT SPEAKER

Greyson Moore

Mentor - Dr. Andrea Bertke

FLASH TALKS

Rylee Matheson

Mentor - Dr. Audrey Ruple

Kirsten Replogle

Mentor - Dr. Nick Ruktanonchai

Mols Kwitny

Mentor - Dr. Sophie Wenzel

ORAL PRESENTATIONS

Biomedical Sciences and Pathobiology Block

10:00am - 11:15am

MODERATED BY:

Brice Stolz

Amanda Moore

FACULTY SPEAKERS

DR. X.J MENG, University Distinguished Professor of Molecular Virology, VMCVM

-

“NOVEL VACCINE CANDIDATES AGAINST PORCINE EPIDEMIC DIARRHEA VIRUS”

DR. KEVIN LAHMERS, Clinical Professor Anatomic Pathology

-

“A LESS DIRECT PATH TO APPLIED PRODUCTIVITY”

ORAL PRESENTATIONS

Biomedical Sciences and Pathobiology Block

10:00am - 11:15am

MODERATED BY:

Brice Stolz

Amanda Moore

STUDENT SPEAKER

Yehia Elgammal

Mentor - Dr. Mohamed Seleem

FLASH TALKS

Sai Navi Vadlamudi

Mentor - Dr. Priscilla Serpa

Rachel Persinger

Mentor - Dr. Nisha Duggal

Hilary Montano

Mentors - Drs. Xin Luo and Christopher Reilly

ORAL PRESENTATIONS

Large Animal Clinical Science Block

2:30pm - 3:34pm

MODERATED BY:

Ny Luong

Claire Read

FACULTY SPEAKERS

DR. DAN GIVENS, Dean & Professor

-

"CAREER-IMPACTING INTERACTIONS IN LARGE ANIMAL RESEARCH"

DR. JESS GILBERTIE, Assistant Professor for Microbiology and Biomedical Sciences, VCOM

-

"NOVEL APPROACHES TO ORTHOPEDIC INFECTION AND INJURY"

ORAL PRESENTATIONS

Large Animal Clinical Science Block
2:30pm - 3:34pm

MODERATED BY:

Ny Luong

Claire Read

STUDENT SPEAKER

Dylan Easley

Mentor - Dr. Vincent Wang

FLASH TALKS

Catherine Jula

Mentor - Dr. Harold McKenzie

Emilee Lacey

Mentor - Dr. Katherine Wilson

Fabian Jimenez Roa

Mentor - Dr. Amy Santonastaso

ORAL PRESENTATIONS

Small Animal Clinical Science Block

4:45pm - 6:00pm

MODERATED BY:

Mahfuzul Islam

FACULTY SPEAKERS

DR. JOANNE TUOHY, Assistant Professor
Surgical Oncology
-
"NOVEL STRATEGIES TO IMPROVE OSTEOSARCOMA THERAPY"

DR. RELL PARKER, Assistant Professor
Neurology and Neurosurgery
-
“COMPARATIVE AND TRANSLATIONAL RESEARCH OF NOCICEPTION AND PAIN”

ORAL PRESENTATIONS

Small Animal Clinical Science Block

4:45pm - 6:00pm

MODERATED BY:

Mahfuzul Islam

STUDENT SPEAKER

Alessandra Franchini

Mentor - Dr. Michelle Borgarelli

FLASH TALKS

Camille Brassard

Mentor - Dr. Ashely Wilkenson

Christina Vezza

Mentor - Dr. John Rossmeisl

Andy Hsueh

Mentor - Dr. Nick Dervisis

POSTER PRESENTERS

Session 1

1:00 - 1:45pm

Even numbered posters

2. Jatia Mills
4. Camille Brassard
6. Christina Vezza
8. Xiaoran Wei
10. Janice O'Brien
12. Yehia Elgammal
14. Emilee Lacey
16. Sai Navya Vadlamudi
18. Alejandra Tellez Silva
20. Rachel Persinger
22. Rana Estaleen
24. Ahmed Abouelkhair
26. Elizabeth Harris
28. Dylan Easley
30. Rafaela Flor
32. Christina Pacholec
34. Abdellah Abdelsattar
36. Dao Xu
38. Daniel Rothschild
40. Mitch Caudill
42. James May
44. Dima Hajj Ali
46. Alessandra Franchini
48. Shannon Carney
50. Amir Mortazavigazar
52. Hilary Montano
54. Abdullahi Jamiu
56. Brie Trusiano
58. Michael Brooks
60. Bhargava Teja Sallapalli

POSTER PRESENTERS

Session 2

1:45pm - 2:30pm

Odd numbered posters

1. Jing Ju
3. Brittany Heath
5. Kirsten Replogle
7. Pallavi Rai
9. Tian Xu
11. Rylee Matheson
13. Chelsea Cereghino
15. Mols Kwitny
17. Josefa Garcia-Mora
19. Fabian Jimenez Roa
21. Tamalika Paul
23. Andy Hsueh
25. Charlotte Nyblade
27. Pavly Amin
29. Mia Grzywinski
31. Md Shakhawat Hossain
33. Samantha McCarter
35. Brice Stolz
37. Sierrah Travis
39. Jillian Green
41. Padmaja Mandadi
43. Babatomiwa Kikiowo
45. Morgen VanderGiessen
47. Katherine Gottleib
49. Greyson Moore
51. Catherine Jula
53. Nour Alkashef
55. Lauren Helber
57. Caitlin Armstrong
59. Madusudan Timilsina

ACKNOWLEDGMENTS

We are delighted to host our 33rd Annual Graduate Research Symposium event themed "Forging ahead: Pathways towards novel clinical approaches." This event is a collaboration of the Office of Research and Graduate Studies and the VMCVM Research Committee, chaired by Dr. Coy Allen.

On behalf of the College of Veterinary Medicine, we want to thank our invited speakers, Dr. John Rossmeisl and Dr. Yanjin Zhang, for agreeing to deliver the keynote talks. We would also like to thank the following VMCVM faculty for presenting talks during our departmental blocks: Drs. Gohlke, Calder, Meng, Lahmers, Givens, Gilbertie, Tuohy, and Parker. Our Research Symposium is designed to celebrate and showcase VMCVM faculty research programs primarily through the graduate students' research project presentations. This year we have 60 poster presentations, 4 traditional research talks, and 12 research flash talks being presented by our graduate students. Many thanks and congratulations to all of our students for attending and participating in the Symposium.

We sincerely thank personnel from the Office of Research and Graduate Studies, Dr. Jessica Crawford, Andrea Green, and Monica Taylor, our Graduate Teaching Assistant, Brittany Heath, as well as the entire Research Committee for their efforts in planning the Symposium. We also take this opportunity to thank the graduate students and postdoctoral researchers who reviewed student abstracts and our many dedicated faculty members who helped to provide feedback for graduate student presentations. We would also like to acknowledge and express our appreciation to the entire College for your support of this symposium.

Dr. Margie D. Lee, Associate Dean of Research and Graduate Studies

Dr. Audrey Ruple, BMVS Program Director

SESSION SCHEDULE

POSTER SESSION I
1:00 PM - 1:45 PM

P2 Jatia Mills, Eman Soliman, Jing Ju, and Michelle H. Theus
Characterization of Temporospatial Changes in Resident Microglia Following Traumatic Brain Injury

P4 Camille Brassard, DVM, IPSAV, Ashley Wilkinson, DVM, MS, DACVIM, Stefani DeMonaco, DVM, MS, DACVIM
Evaluation of a feline optimized TSH assay in cats with hyperthyroidism and with non-thyroidal illness

P6 Christina Vezza, Lauren Ruger, Maya Langman, Francesco Prada, Eli Vlaisavljevich, Rell L. Parker, John H. Rossmeisl
First-In-Dog Histotripsy for Intracranial Tumors Safety Trial: The FIDOHIST Study

P8 Xiaoran Wei, Jiangtao Li, Zuolin Cheng, Songtao Wei, Guoqiang Yu, Michelle L. Olsen
Decoding the Epigenetic Landscape: Insights into 5mC and 5hmC patterns and impacts on gene regulation in mouse cortical cell-type

P10 Janice O'Brien, DVM; Alex Varela, DVM; Ingrid Luo; Maryanne Murphy DVM, PhD, DACVIM-Nutrition; Angela Rollins DVM, PhD, DACVIM-Nutrition; Matt Kaeberlein, Dog Aging Project Consortium; Audrey Ruple, DVM, MS, PhD, DACVPM, MRCVS; Katie Kerr, PhD; M. Katherine Tolbert, DVM, PhD, DACVIM-SAIM
Diet Types, demographics, and health outcomes in pet dogs in the United States

P12 Yehia Elgammal, Ehab A. Salama, Mohamed N. Seleem
Evaluation of the combinational therapy of atazanavir and saquinavir with azole antifungals against Candida auris

P14 Emilee Lacey, Katherine Wilson, Jennifer Davis
Pharmacokinetics of oral mirtazapine in healthy adult alpacas

P16 Sai Navya Vadlamudi, Andrea P. Santos, Santiago Diab, Priscila B. S. Serpa
Artificial Intelligence-Driven Macrophage Identification in Canine Diffuse Large B Cell Lymphoma: A Pilot Study to Explore Prognostic Significance

P18 Alejandra Tellez, Ester Yang, Marlie Nightingale, Nikolaos Dervisis, Shawna Klahn
Interim Analysis of a Novel Chemotherapy Protocol THOP (Temozolomide, Doxorubicin, Vincristine, Prednisone) for the Treatment of naïve B-cell Lymphoma in Dogs

P20 Rachel Persinger, Nisha Duggal
North American Culex mosquitoes are competent vectors for Usutu virus

P22 Rana A. Estaleen, David N. Oakland, Razan Alajoleen, Hilary Montano, Ran Lu, Pavly Amin, Aida Shakeri, Christopher M. Reilly, Xin M. Luo
Double The Trouble: CX3CR1 Modulates Double Negative T Cells and IL-17 Production in Systemic Lupus Erythematosus

P24 Ahmed A. Abouelkhair, Nader S. Abutaleb, Mohamed N. Seleem
Ionomycin demonstrates potent activity against Clostridioides difficile via calcium accumulation and disruption of membrane polarization

P26 Elizabeth A. Harris, Sabrina Budianto, Erwin Kristobal Gudenschwager Basso, Michelle H. Theus
Characterizing altered cellular architecture in the dentate gyrus and the chronic role of EphA4 on peripheral immune cell activity in the neurogenic niche following TBI

SESSION SCHEDULE

POSTER SESSION I
1:00 PM - 1:45 PM

P28 Dylan C. Easley, Megan Gulian, Kyle Cooper, Robert W. Grange, P. Gunnar Brolinson, Linda A. Dahlgren, Vincent M. Wang
Treatment Duration and Load Magnitude Differentially Affect Biomechanical Properties in a Preclinical Model of Achilles Tendinopathy

P30 Flor R., Ivan Akhrymuk, Paul O'Maille, James Omichinski, Andrew Silberfarb, Kylene Kehn-Hall
Characterization of Rift Valley fever virus L polymerase interaction with protein phosphatase-1 alpha

P32 Christina Pacholec, Kurt Zimmerman
Evaluation of Image Magnification on The Accuracy of Artificial Intelligence Models

P34 Abdallah S. Abdelsattar, Nader S. Abutaleb, Mohamed N. Seleem
Repurposing valnemulin for combating multidrug-resistant Neisseria gonorrhoeae.

P36 Dao Xu, Christopher M. Reilly
HDAC6 knockout alleviates pristane-induced lupus

P38 Daniel Rothschild, Renata Ramos, Ian Herring
Indocyanine green photodynamic therapy as a potential treatment for fungal keratitis: An in vitro investigation of antimycotic effect.

P40 Mitchell T. Caudill, Stephen T. Stoyanof, Clayton C. Caswell
Brucella abortus maintains a cryptic quormone response

P42 May, JL, Rossmeisl, JH
Characterizing the Glioblastoma "Ablatosome" Treated with High-Frequency Irreversible Electroporation

P44 Ali, D., and Gaji R.
Functional Characterization of a Novel Kinase TgTKL1 in Toxoplasma Pathogenesis

P46 Franchini, Menciotti, Hyeon, Lahmers, Borgarelli
Three-dimensional echocardiographic determinants of the age of onset of myxomatous mitral valve disease in Cavalier King Charles Spaniels dogs

P50 Amir Gazar, Ryan Calder, Richard B. Howarth, Chloe Jackson, Georgia Mavrommati
Canadian hydroelectricity imports to the U.S.; Modeling of hourly carbon emissions reduction in New England

P52 Hilary Montano, Chris Reilly
Dissecting the Role of NF-KB Inducing Kinase as a Possible Therapeutic Target for Systemic Lupus Erythematosus

P54 Abdullahi Jamiu, Ivan Akhrymuk, Kenneth Foreman, Dmitri Klimov, Mikell Paige, Kylene Kehn-Hall
Exploiting capsid-importin interactions to develop novel inhibitors against Venezuelan equine encephalitis virus

P56 Brie Trusiano, Rebekah Smith, Cassandra Poole, Maxx Steinmann,Dilruba Yeasmen, I.C. Allen
NIK influences the course of eosinophilopoiesis dependent on environmental factors

P58 Michael Brooks, Yuchin Albert Pan
Regulation of developmental cell death in hypothalamic corticotrophin-releasing hormone neurons through DSCAML1 and cortisol signaling

P60 Bhargava Teja Sallapalli, Peixi Chang, and Yanjin Zhang
Characterization of PRRSV nps5 that induces STAT3 degradation

SESSION SCHEDULE

POSTER SESSION 2
1:45 PM - 2:30 PM

P1 Jing J., Wang X., and Theus M.
The emerging role of peripheral-derived immune cells in the remodeling of pre-existing pial collateral vessels following ischemic stroke

P3 Brittany Heath, Caitlin Woodson, Tanya LeRoith, Jonathan Jacobs, Amy Reese, Kylene Kehn-Hall
Characterization of STING's role in Venezuelan equine encephalitis virus (VEEV) infection

P5 Kirsten Replogle, Nick Ruktanochai
Unraveling Avian Influenza Transmission Through Understanding Migratory Bird Dynamics

P7 Pallavi Rai, Jeffrey M Marano, Lin Kang, Sheryl Coutermarsh-Ott, Andrea R Daamen, Peter E Lipsky, James Weger-Lucarelli
Obesity fosters severe disease outcomes in a mouse model of coronavirus infection associated with transcriptomic abnormalities

P9 Tian Xu, Qin Xu, Ran Lu, David N. Oakland, Song Li, Liwu Li, Christopher M. Reilly, Xin M. Luo
Application of Deep Learning Models on Single-Cell RNA Sequencing Analysis Uncovers Novel Markers of Double Negative T Cells

P11 Matheson, R., Sexton, C.L., O'brien, J., Wise, C., Keyser, A., Kauffman, M., Dunbar, M., DAP consortium, Stapleton, H., Ruple, A.
Using a passive sampling technique to quantify environmental chemical exposures in dogs enrolled in the dog aging project

P13 Chelsea Cereghino, James Weger-Lucarelli
A risk assessment for Mayaro virus spread in the Caribbean and North America

P15 Kwitny, Mols; Richards, Quinn; Cann, Natalie; Lewis, Jasmine; Vaught, Kayla; Bejoy, Arushi; Gutierrez, Matos, Fernanda; DiGirolamo, Grace; Loving, Chloe; Neveldine, Tegan; Weekes, Sakina; Wenzel, Sophie
People of a Pandemic

P17 Josefa K. Garcia-Mora, Rell L. Parker, Kurt Zimmerman, Gregory Daniel, Jhon L. Robertson, Jhon H. Rossmeisl
Exploring radiomics on Magnetic resonance Imaging for the diagnosis of glioma in dogs.

P19 Amy Santonastaso, VMD, MS, DABVP (Equine Practice). Fabian Jimenez-Roa, DVM. Christopher Byron, DVM, MS, DACVS. Travis Burns, MSc, CJF, TE, EE, FWCF Raffaella De Vita, PhD. Lauren Trager-Burn, DVM, MS, DACVSMR-Equine. Rebecca Funk, DVM, MS, DACVIM.
Comparative study of the influence on heel movement and foot biomechanics between aluminum nail on shoes and commercial fabric-cuff indirect glue on shoes.

P21 Tamalika Paul, K.M. Imran, Jessica Ganon, Brie Trusiano, Kirsten Eden, Hannah Ivester, Madeline Mott, Manali Powar, Michael Edwards, Christopher Byron, Sherrie Clark-Deener, Kiho Lee, Tim Ziemlewicz, Eli Vlaisavljevich, Irving C. Allen
First successful engraftment of human liver cancer cell line in highly robust immunocompromised porcine model to test the tumor ablation efficacy by histotripsy

P23 Andy Hsueh, AeRyon Kim, Lauren Ruger, Marlie Nightengale, Nikolaos Dervisis, Eli Vlaisavljevich,
Natasha Sheybani, Shawna Klahn
HeEV: Histotripsy-enabled Extracellular Vesicle characterization in canine soft tissue sarcoma patients

P25 Charlotte Nyblade, Peng Zhou, Lauren Lavoie, Brian King, Zeinab Aboezz, Zhihui Yang, Chantal A. Agbemabiese, Samantha Q. Wales, Viviana Parreño, Judy Y. Qiu, Xiao-Li Pang, Lijuan Yuan
A GII.6 strain of human norovirus is infectious and pathogenic in gnotobiotic pigs

P27 Pavly Amin, Rana A Estaleen, David N Oakland, Razan Alajoleen, Hilary Montano, Ran Lu, Aida Shakeri, Christopher M. Reilly, Xin Luo
Investigating the Role of CX3CR1 and Gut Microbiota in Lupus-Like Disease Progression

SESSION SCHEDULE

POSTER SESSION 2
1:45 PM - 2:30 PM

P29 Grzywinski, M., O'Brien, J., and Ruple A.
Geographic trends in environmental exposures among Dog Aging Project participants

P31 Md Shakhawat Hossain, Megan B. Vogt, Seth A. Hawks, and Nisha K. Duggal
Cross Protection Against Usutu Virus And Saint Louis Encephalitis Virus In Mice Treated With Human West Nile Virus Convalescent Plasma

P33 Samantha McCarter, Orsolya Balogh
Comparing puppy growth of overweight and lean bitches

P35 Brice Stolz, Ahmed Abouelkhair, Mohamed Seleem
Discovery of Potent Anticlostridial Compounds from an Antiviral Library

P37 Sierrah D. Travis, DVM; Ashley R. Wilkinson, DVM, MS, DACVIM, Audrey Keebaugh, DVM, MS, DACVIM; Stefanie DeMonaco, DVM, MS, DACVIM; Timothy Bolton, DVM, DACVIM
ADAMTS13 activity in dogs with presumptive idiopathic immune thrombocytopenia

P39 Jillian C Green, Amber RN Abbott, Poorna Goswami, Andrea S Bertke
HSV2 latency is maintained through RET and NCAM in sensory neurons

P41 Mandadi P., and Gaji R.
Determining The Role of a Novel Plant-Like Transcription Factor Tgap2x-7 in Toxoplasma Biology and Pathogenesis.

P43 Babatomiwa Kikiowo, Nader S. Abutaleb, and Mohamed N. Seleem
Nitroxoline: A Promising Candidate for Treating Gonococcal Infections

P45 Morgen VanderGiessen, Caitlin Woodson, Elizabeth Harris, Caroline de Jager, Xiaowei Wu, Michelle Theus, Erik Johnson, Hehuang (David) Xie, Kylene Kehn-Hall
Comparative Analysis of Equine Encephalitis viruses (EEV), Traumatic Brain Injuries (TBI), and Organophosphorus Nerve Agents (OPNA) as a Path to Neuroprotective Therapeutics

P47 Gottleib K, Trager-Burns L, Santonastaso A, Bogers S, Burns T, Byron C
Comparison of gait characteristics for horses without shoes, with steel shoes, and with aluminum shoes

P49 G. A. Moore, A. M. Ives, A. S. Bertke
Female Sex Hormones Enhance HSV Replication and Disease Severity

P51 Catherine Jula, DVM, Jennifer Davis, DVM, MS, PhD, DACVIM, DACVCP, Harold McKenzie III, DVM, MS, MSc (VetEd), FHEA, DACVIM
Single Dose Pharmacokinetics of Pimobendan and O-Desmethyl-Pimobendan (ODMP) in Healthy Adult Horses

P53 Alkashef N., Seleem M.
Pimvanserine, A Promising Adjuvant to Fluconazole Monotherapy Against Cryptococcosis

P55 Lauren Helber, Amy Johnson, Alayna N. Hay, Bettina Wagner, Caroline M. Leeth, Tanya LeRoith, Thomas E. Cecere, Kevin K. Lahmers, Frank M. Andrews, Stephen R. Werre, Carol K. Clark, Nicola Pusterla, Stephen M. Reed, David S. Lindsay, Sandra D. Taylor, Krista E. Estell, Martin Furr, Robert J. Mackay, Fabio Del Piero, Mariabo Carossino, Daniela Luethy, Jennifer K. Morrow, Amy J. Graves, Sharon G. Witonsky
Potential for a New Supplemental Diagnostic Assay to Identify Horses with Equine Protozoal Myeloencephalitis (EPM)

P57 Caitlin Armstrong, Blake Caldwell, Ran Lu1, Razan Alajoleen, Rana Estaleen, Noah Oakland, Hilary Montano, Xin Luo, Liwu Li
Phenotypic Characterization of Exhausted Monocytes in Lupus-Prone Mice

P59 Madhusudan Timilsina, Dhiraj Chundru, and Mostafa Ghanem
Characterization of Upper Respiratory Tract Microbiome of Chickens following Avibacterium paragallinarum Infection

ABSTRACTS

P1
The emerging role of peripheral-derived immune cells in the remodeling of pre-existing pial collateral vessels following ischemic stroke

Jing J., Wang X., and Theus M.

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

Stroke remains a significant health concern in the United States, with ischemic strokes comprising most cases and often resulting from middle cerebral artery (MCA) occlusion. Pial collateral vessels, pre-existing vascular redundancies, play a crucial role in retrogradely re-supplying cerebral blood flow to the penumbra following MCA occlusion, thereby mitigating tissue damage. Arteriogenesis, the remodeling of these pial collateral vessels, is essential for promoting reperfusion and preventing ischemic injury. Our recent studies highlight the pivotal role of EphA4 receptor tyrosine kinase in suppressing arteriogenesis in ischemic stroke. However, the specific contribution of peripheral-derived immune (PDI) cells in this process remains poorly understood. Our current investigation aims to elucidate the role of EphA4 in regulating PDI cell-mediated pial collateral remodeling using a mouse model of permanent MCA occlusion (pMCAO). Through the utilization of EphA4 bone marrow chimeric mice and selective arteriole labeling technique (vessel painting), our findings demonstrate that PDI-specific EphA4 negatively influences arteriogenesis and modulates PDI cell recruitment and function post-pMCAO. These results provide insights into how PDI-specific EphA4 contributes to the neuroinflammatory milieu and identify potential EphA4 targets for enhancing collateral function and promoting functional recovery in ischemic stroke. Overall, our study contributes to a deeper understanding of the cellular mechanisms underlying pial collateral remodeling in ischemic stroke and holds promise for the development of targeted therapeutic strategies aimed at improving outcomes in stroke patients.

***Support:** NIH NINDS R01 NS112541*

P2
Characterization of Temporospatial Changes in Resident Microglia Following Traumatic Brain Injury

Jatia Mills, Eman Soliman, Jing Ju, and Michelle H. Theus

[1]Department of Biomedical Sciences and Pathobiology, Virginia Tech, Blacksburg, VA, United States, [2]School of Neuroscience, Virginia Tech, Blacksburg, VA, United States, [3]Center for Engineered Health, Virginia Tech, Blacksburg, VA, United States

Traumatic brain injury (TBI) results in long-term cognitive deficits and is a risk factor for neurodegenerative disease such as dementia. Neuroinflammation is a key factor elicited by resident microglia and peripheral-derived myeloid cells. The aim of this study is to identify the various temporospatial microglial changes that occur following controlled cortical impact (CCI) injury and how these changes may be influenced by recruitment of peripheral immune cells. GFP chimeric mice were generated in order to identify resident vs infiltrating myeloid cells and histological evaluation was performed at 4hrs, 1d, and 3 days post-injury (dpi). Our findings indicate that there is a progressive increase in the quantity of both microglial and peripheral-derived macrophages (PDMs) in the lesion and peri-lesion cortex. Using Iba1 immunostaining, we find that both GFP+/Iba1+ and GFP-/Iba1+ cell types show an increase in proliferation at 3dpi compared to 1dpi or 4hrs. The morphological changes of microglia also indicate a greater presence of cells that are amoeboid near the lesion core compared to the adjacent or contralateral cortex, which were ramified. Arg1+ staining also revealed that there is a low percentage of microglia expressing this anti-inflammatory protein at 3dpi indicating that most cells were pro-inflammatory in nature. In addition, novel object recognition has been conducted to correlate the effects of microglial activation on memory function up to two months post-injury. These studies add to our understanding of the temporospatial activation state of microglia following TBI.

Support: NIH NS121103

P3
Characterization of STING's role in Venezuelan equine encephalitis virus (VEEV) infection

Brittany Heath[1,2], Caitlin Woodson[1,2], Tanya LeRoith[1], Jonathan Jacobs[3], Amy Reese[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
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Venezuelan equine encephalitis virus (VEEV) is a mosquito-borne pathogen that can cause encephalitis in both humans and equine. This single-stranded, positive-sense RNA virus is listed as a Category B priority pathogen by the CDC due to ease of dissemination, high morbidity rates (90-100%), and low mortality rates (<1%). VEEV-induced neurological sequelae is observed in 4-14% of human cases, with symptoms ranging from confusion to convulsions and cognitive impairment. The innate immune pathway, cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING), has been shown to be active in response to single-stranded RNA viruses and has implications in neuroprotection as well as neurodegeneration. cGAS is a pattern recognition receptor which canonically recognizes dsDNA and DNA:RNA hybrids in the cytosol. cGAS produces a secondary messenger molecule that binds to and activates endoplasmic reticulum-bound protein STING. STING activation results in production of type-I interferons and leads to transcriptional regulation of interferon stimulates genes. Previous studies have shown cGAS-STING to be involved in limiting viral replication in the arthritogenic alphavirus Chikungunya virus, but little is known about encephalitic alphaviruses (i.e. VEEV). To address this gap, we aim to characterize STING's involvement in VEEV pathogenesis in vitro and in vivo. Here, we tested the direct infection of microglia by VEEV TC-83 and fold changes in gene expression were measured. Microglia exhibited transcriptional upregulation of cGAS at 16- and 24-hours post-infection (hpi). In vivo, C57Bl/6J wild-type mice and STING-deficient mice on a C57Bl/6 background were either uninfected or infected with VEEV TC-83 at 2x10^7 PFUs and serially sacrificed at days 2 and 7 post-infection (dpi). Brains were collected and halved into formalin or TRIzol. Histopathology scores, absolute quantification of viral RNA, and fold changes in gene expression were measured. STING-deficient mice had significant upregulation of interferon-stimulated genes, CXCL10 and IFIT2, at 7dpi compared to wild-type mice. Additionally, cGAS and STING are upregulated in wild-type mice at Days 2 and 7 post-infection. Our findings validate STING's activation during VEEV pathogenesis. Ongoing studies are evaluating the impact of STING on neuropathology due to chronic VEEV infection.

Support: FY24 229 Agricultural Research Project

P4
Evaluation of a feline optimized TSH assay in cats with hyperthyroidism and with non-thyroidal illness

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Background:
Since approximately 10% of hyperthyroid cats have a normal total T4 (TT4), thyroid stimulating hormone (TSH) may be needed to confirm the diagnosis. Until recently, only a canine TSH assay (cTSH) was available, which could not differentiate between subnormal and low-normal TSH concentrations in cats. A novel feline optimized TSH assay differentiates better between euthyroid and hyperthyroid cats compared to cTSH but the effect of non-thyroidal illness (NTI) on fTSH is unknown.

Hypothesis/Objectives: Comparison of fTSH and cTSH concentrations among hyperthyroid cats, NTI cats, and healthy cats. Evaluation of sensitivity and specificity of fTSH to diagnose hyperthyroidism.

Animals:
The study enrolled 102 client-owned cats, including 37 hyperthyroid, 33 healthy, and 32 NTI cats.

Methods:
Prospective cross-sectional study. TT4, cTSH (Immulite 2000) and fTSH (fTSH, Truforma by Zomedica) were measured in all cats. Hyperthyroidism was confirmed with thyroid scintigraphy. TT4 was repeated 3 months after enrollment if available in healthy and NTI cats to rule out subclinical hyperthyroidism. TSH was compared among groups using Kruskal Wallis followed by Wilcoxon pairwise method. Significance was set at $P < 0.05$.

Results:
The sensitivity and specificity of fTSH are 78% (62-90%) and 97% (84-100%), respectively. There is a significant difference between hyperthyroid cats and healthy and NTI cats with both assays ($P<0.01$), and the latter euthyroid groups are not different. Eight (21.6%) hyperthyroid cats have a normal fTSH but undetectable cTSH. Twelve (4 healthy, 8 NTI) euthyroid cats (18.5%) have an undetectable cTSH vs. only 2 (1 healthy, 1 NTI) (3%) have an undetectable fTSH.

Conclusions:
The fTSH is a useful tool to diagnose feline hyperthyroidism because it has a high specificity, identifies normal TSH in healthy cats more often, and appears to not be affected by NTI

Support: Virginia Tech Foundation Inc, Zomedica

P5
Unraveling Avian Influenza Transmission Through Understanding Migratory Bird Dynamics

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Avian influenza is an endemic virus in poultry with multiple migratory bird species as a natural host. The current strain (H5N1 clade 2.3.4.4b) circulating North America has affected over 90 million poultry since 2022. This research project is dedicated to understanding how migratory birds are linked to disease propagation and identifying high-risk regions for both poultry and human populations. Comparing connectivity can develop understanding for how migratory flyways correlate to outbreaks in poultry populations. This can further discern where spill-over cases into humans are of higher probability and should be closely monitored. The identification of high-risk regions can be utilized as starting points for vaccination to maximize efficiency of disease mitigation as well. Additionally, we aim to discern the differences in migratory patterns between birds known to carry avian influenza and non-carriers. To achieve this, we employ various network theory methodologies to analyze migratory bird data, enabling us to quantify the rationale behind heightened risk levels in specific counties across North America using bird banding data from USGS.

Our analysis has shown avian influenza carriers are more likely to migrate long distances, which may indicate higher amounts of contact and potential transmission with other birds. We also found that carriers exhibit less spatial clustering in their connectivity network, potentially leading to faster spread of avian influenza continent-wide.

Support: NSF

P6
First-In-Dog Histotripsy for Intracranial Tumors Safety Trial: The FIDOHIST Study

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Histotripsy is a non-invasive, image-guided technique that uses focused ultrasound waves to ablate tissues through acoustic cavitation. Histotripsy has been successfully used to treat a variety of cancers in humans and animal models, but has not been extensively evaluated in brain tumors, at least in part due to significant acoustic attenuation by the skull. The objectives of the prospective FIDOHIST clinical trial were to: 1) assess the safety and feasibility of histotripsy to ablate naturally occurring brain tumors in dogs, and 2) characterize local and systemic imaging, clinical, and immunologic responses to histotripsy treatment. The study design utilized a treat and resect paradigm, where tumors were approached using craniotomy, partially ablated with histotripsy delivered through the cranial defect, and then resected. Dogs were evaluated with clinical, brain magnetic resonance imaging (MRI), laboratory, immunopathologic, and genomic examinations before treatment, intraoperatively, and 1, 14, and 42 days post-treatment. Here we report the results of the first three dogs completing the trial, all of which had intracranial meningiomas. Histotripsy was successfully delivered to all dogs, resulting in histopathologic evidence of ablations that were sharply demarcated from untreated tumor, with measured treatment zones approximating planned volumes in 2/3 dogs. One dog experienced an adverse event consisting of transient neurological deterioration due to cerebral edema that was possibly attributable to histotripsy. Histotripsy ablations could be grossly visualized and identified on MRI, with features consistent with hemorrhagic necrosis. Proteomic and genomic analyses revealed significant expression or upregulation of HMGB1, calreticulin, and S100B damage associated molecular patterns and necroptosis pathways in histotripsy treated tumors. Precision ablation of canine meningiomas with histotripsy was feasible, clinically well tolerated, and associated with immunogenic tumor cell death responses.

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P7

Obesity fosters severe disease outcomes in a mouse model of coronavirus infection associated with transcriptomic abnormalities

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Obesity has been identified as an independent risk factor for severe outcomes in humans with coronavirus disease 2019 (COVID-19) and other infectious diseases. Here, we established a mouse model of COVID-19 using the murine betacoronavirus, mouse hepatitis virus 1 (MHV-1). C57BL/6 and C3H/HeJ mice exposed to MHV-1 developed mild and severe disease, respectively. Obese C57BL/6 mice developed clinical manifestations similar to those of lean controls. In contrast, all obese C3H/HeJ mice succumbed by 8 days post-infection, compared to a 50% mortality rate in lean controls. Notably, both lean and obese C3H/HeJ mice exposed to MHV-1 developed lung lesions consistent with severe human COVID-19, with marked evidence of diffuse alveolar damage (DAD). To identify early predictive biomarkers of worsened disease outcomes in obese C3H/HeJ mice, we sequenced RNA from whole blood 2 days post-infection and assessed changes in gene and pathway expression. Many pathways uniquely altered in obese C3H/HeJ mice post-infection aligned with those found in humans with severe COVID-19. Furthermore, we observed altered gene expression related to the unfolded protein response and lipid metabolism in infected obese mice compared to their lean counterparts, suggesting a role in the severity of disease outcomes. This study presents a novel model for studying COVID-19 and elucidating the mechanisms underlying severe disease outcomes in obese and other hosts.

***Support:** NSF*

P8
Decoding the Epigenetic Landscape: Insights into 5mC and 5hmC patterns and impacts on gene regulation in mouse cortical cell-types

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The DNA modifications, 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC), represent powerful epigenetic regulators of temporal and spatial gene expression across unique brain cell types in health and disease. Yet, how the cooperation of these genome-wide, epigenetic marks determine unique transcriptional signatures and alternative splicing across different brain cell populations is unknown. Here we applied Nanopore sequencing of native DNA to obtain a complete, genome-wide, single-base resolution atlas of 5mC and 5hmC modifications in neurons, astrocytes and microglia in the mouse cortex (over 40 million CpG sites quantified, 99% genome coverage). Our study revealed unique 5mC and 5hmC patterns across cell types, with microglia displaying the highest 5mC levels and astrocytes exhibiting the highest 5hmC levels. We also evaluate the role of both 5mC and 5hmC in the regulation of gene expression level and alternative splicing. Finally, we provide this quantitative, genome-wide, base resolution DNA methylation data as an interactive, online resource (NAM-Me, Neuronal, Astrocyte, Microglia Methylome) to serve as a benchmark dataset for those interested in the methylome landscape in pre-clinical murine models in health and disease.

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P9
Application of Deep Learning Models on Single-Cell RNA Sequencing Analysis Uncovers Novel Markers of Double Negative T Cells

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Double negative T (DNT) cells, a unique subset of CD3+TCRαβ+ T lymphocytes lacking CD4, CD8, or NK1.1 expression, constitute 3-5% of the total T cell population in C57BL/6 mice. Traditional machine learning models such as principal component analysis in single-cell RNA sequencing (scRNA-seq) analysis have been utilized to characterize this subset, but only deep learning models such as Single Cell Variational Inference (SCVI) can capture nonlinear gene expression in the sequencing data. In this study, using the deep learning approach, we have uncovered novel markers of splenic DNT cells in C57BL/6 mice. We classified DNT cells into two subgroups, naïve DNT (nDNT) cells and activated DNT (aDNT) cells, which could be differentiated by the expression of Ly6C and MHC-II, respectively. A previous study had predicted that CD137 encoded by Tnfrsf9 was highly expressed in aDNT cells, which was confirmed by our analysis. Innovatively, our data also identified CD153 encoded by Tnfsf8 as another unique marker for aDNT cells. Additionally, we classified two subgroups in nDNT cells and two subgroups in aDNT cells. SCVI analysis suggested, and flow cytometry analysis confirmed, that Ly49G2 encoded by Slamf7 was a marker for the nDNT0 group, whereas CCR2 encoded by Ccr2 was a marker for the nDNT1 subgroup. The same two surface proteins, Ly49G2 and CCR2, were determined as respective markers for the aDNT0 and aDNT1 subgroups after pre-gating on MHC-II+CD137+CD153+ aDNT cells. Furthermore, we validated MHC-II expression in DNT cells in human peripheral blood mononuclear cells. In addition to cell surface markers, we further discovered that nDNT cells expressed higher Rgs2 that is required for T cell activation and that aDNT cells expressed higher Rbpj involved in Notch signaling. Together, our comprehensive analysis has uncovered and validated novel markers for different subpopulations of DNT cells that can be used in the phenotypic and/or functional characterization of these relatively rare cells in health and disease.

P10
Diet Types, demographics, and health outcomes in pet dogs in the United States

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Introduction: Studying the nutrition of pet dogs in the United States presents many opportunities for veterinary practitioners to understand what foods owners are choosing, and how those choices impact pet health.

Methods: Over 40,00 initial surveys completed by US dog owners as part of the Dog Aging Project, from 2020 to 2022. Owners responded to many questions covering primary diet component, and the organic and grain-free status of that component, secondary diet component (if applicable), demographic information (both owner and dog demographics), and other comprehensive health questions. The primary and secondary diet component information was examined across demographic variables to determine which demographic factors appear to influence owner decisions regarding diet choice, and across health outcomes to find any potential correlations with primary diet type.

Results: Owner demographic variables had less influence on diet choice than dog demographic variables. The one owner demographic variable that meaningfully influenced diet choice was owner age. Owner income did not meaningfully influence diet choice in this population across all diet types. Smaller dogs tend to be fed diets other than extruded dry diet. Purebred and intact dogs were more likely to be consuming raw diets. Less active dogs tend to eat more canned and home cooked food. Active dogs tend to eat more extruded dry, commercial raw, and home raw diets. Rural dogs were more likely to be fed home raw diets, while urban dogs were more likely to eat commercial raw, canned, freeze-dried, and home cooked foods. A surprising percentage of therapy and service dogs were reported to be on raw diets, which is a concern as these dogs are more likely to interact with immunocompromised persons. Coastal states fed extruded dry diets at a lower rate compared to the central states. Home cooked diets statistically significantly correlated with GI, liver, and kidney disease diagnoses. Commercial raw diets statistically significantly correlated with respiratory diseases.

Conclusion: Dog and owner demographic variables impact the feeding choices that owners make for their pet dogs, which is an important consideration for veterinary nutrition research studying the impact of nutrition on diseases, and will be an important consideration for translational nutrition research. Raw and home prepared cooked diets require further investigation with respiratory, GI, liver, and kidney diseases.

Support: NIH T32 program, The Dog Aging Project is supported by U19 grant AG057377 from the National Institute on Aging, a part of the National Institutes of Health, and by additional grants and private donations

P11
Using a passive sampling technique to quantify environmental chemical exposures in dogs enrolled in the dog aging project

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OBJECTIVE: The Dog Aging Project (DAP) is a long-term, longitudinal study of over 45,000 dogs located throughout the United States. Dogs provide valuable insights as sentinels for human disease related to environmental exposures because dogs share human exposures and have shorter lifespans. The objective of this pilot study was to determine if passive samplers could be deployed to DAP participants to attach to their dog's collars to quantitatively assess chemical exposures within the shared home environment.

METHODS: Precleaned passive silicone monitoring devices were worn by DAP dog study participants (N=15) on their collar for 5 full days. Once removed, they were placed in a clean piece of aluminum foil and sealed in an airtight bag. All samplers and field blanks (N=3) were processed and analyzed for a suite of target compounds using gas chromatography–mass spectrometry. Field blanks were used to determine the method detection limits (MDLs) of each analyte and median values were calculated for all 120 specific compounds detected.

RESULTS: Analytes belonging to these chemical classes were detected using silicone dog tags placed on dog's collars: brominated flame retardants, organophosphates, PAHs, polychlorinated biphenyls, pesticides, phthalates, and personal care products. Differences in both the types and amounts of analytes detected varied significantly between participants.

CONCLUSIONS: The data in this study indicate that silicone dog tags are an effective means to measure chemical exposure in pet dogs. This supports the value of using silicone tags with dogs to investigate health impacts on humans from shared exposures.

Support: U19 Grant AG057377 from the National Institute on Aging

P12
Evaluation of the combinational therapy of atazanavir and saquinavir with azole antifungals against Candida auris

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Candida auris poses a pressing global public health threat that has been linked to numerous outbreaks worldwide and is associated with a significantly high mortality rate. The limited treatment options and the upsurge of drug resistance in C. auris, prompted us to evaluate a library of FDA-approved drugs for their ability to restore the anti-Candida activity of azole antifungal agents. We identified the HIV protease inhibitors atazanavir and saquinavir, as co-drugs that can overcome azole resistance in C. auris. Atazanavir and saquinavir displayed remarkable in vitro synergistic activity with itraconazole and posaconazole against all tested C. auris isolates. Moreover, both atazanavir and saquinavir restored the fungistatic activity of itraconazole and posaconazole against C. auris in an in vitro time-kill assay. Furthermore, in contrast to the individual drugs, the two combinations, atazanavir/posaconazole and saquinavir/posaconazole, demonstrated significant inhibition of C. auris biofilm formation, achieving reductions of 66.2% and 81.2%, respectively. The mechanistic investigation indicated that atazanavir and saquinavir significantly inhibited efflux pumps, glucose utilization, and ATP synthesis in Candida. When evaluated in a mouse model of disseminated candidiasis, the combinations of atazanavir/itraconazole, saquinavir/itraconazole, atazanavir/posaconazole and saquinavir/posaconazole, significantly reduced C. auris burden in murine kidneys. The reduction ranged from 93% to 99%, generating log10 colony forming unit (CFU) reductions of 1.15, 0.85, 2.04, and 1.44, respectively. Altogether, the data indicate that atazanavir and saquinavir are potent azole chemo-sensitizing agents that merit further investigation.

Support: National Institute of Health

P13
A risk assessment for Mayaro virus spread in the Caribbean and North America

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Mayaro virus (MAYV) is an emerging mosquito-borne pathogen responsible for a febrile illness characterized by arthralgia and fatigue that can last up to years after the acute phase of infection. MAYV is closely related to chikungunya virus (CHIKV), but the disease burden of CHIKV is significantly larger than MAYV which is in part explained by its transmission by the aggressively human-biting mosquitoes Aedes aegypti and Aedes albopictus. Recent outbreaks of MAYV in Haiti raise the question of whether the virus has adapted towards infection of these urban mosquito species since very few jungle-dwelling mosquitoes are found in Haiti that typically maintain the virus in a jungle transmission cycle in South America. Given the proximity of recent cases of Mayaro fever to the United States and the lack of approved therapeutics or vaccines available, we sought to characterize the adaptation of MAYV to Aedes aegypti and Aedes albopictus mosquitoes as a risk assessment for spread throughout the Caribbean and to the United States. Using three genetic sequences of MAYV strains from the Haiti outbreak in 2015, we constructed infectious cDNA clones of the virus and generated stocks of virus from these clones to use in our studies. We performed growth curves of these strains in Ae. aegypti and Ae. albopictus mosquito cell lines to determine how well the Haitian strains replicate compared to a more ancestral strain isolated in Trinidad in 1954. Future studies will involve infecting live mosquitoes to measure their transmission potential compared to the ancestral isolate. Given the ability of MAYV to infect a range of mammalian hosts and mosquito species found within the United States, the increasing geographic spread towards the United States, increasing case numbers, and the lack of therapeutics or vaccines available, MAYV poses a real threat that must be examined and mitigated.

Support: *NIH*

P14
Pharmacokinetics of oral mirtazapine in healthy adult alpacas

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Inappetence is a common clinical problem in hospitalized alpacas and is a risk factor for development of ketosis and hepatic lipidosis, thereby decreasing prognosis. Mirtazapine (MRZ) is a noradrenergic and specific serotonergic antidepressant, used in sick cats and dogs for appetite stimulation and weight gain. The pharmacokinetics of MRZ have been investigated in dogs, cats, and horses and show species-specific differences, but have not been investigated in alpacas. The aim of this study was to investigate the plasma concentrations and potential oral transmucosal (OTM) absorption of MRZ in healthy adult alpacas to determine the potential use of this drug in this species. 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 15, 30, 60, 90 minutes and 2, 4, 6, 8, 12, 24 hours after administration. Plasma MRZ concentrations were determined by ultra-pressure liquid chromatography with tandem mass spectrometry. Preliminary maximum plasma concentrations (Cmax) from the JV samples (148.88 ng/mL ± 90.26) are observed within 15 minutes and are substantially higher than the Cmax of the CV samples observed at 30 minutes (3.04 ng/mL ± 0.22), suggesting rapid OTM absorption of MRZ in alpacas. The JV concentrations in alpacas are similar to JV concentrations published in humans, cats, and dogs but CV concentrations failed to reach therapeutic concentrations reported in those species. Preliminary results of this study suggest that concentrations of MRZ known to be therapeutic in other species may be falsely elevated due to sample site used for collection. Additional information is needed before using oral MRZ in alpacas, including further studies of higher doses, altered formulations, or alternate routes of administration.

Support: American Board of Veterinary Practitioners Foundation's Research Grant

P15
People of a Pandemic

Kwitny, Mols; Richards, Quinn; Cann, Natalie; Lewis, Jasmine; Vaught, Kayla; Bejoy, Arushi; Gutierrez, Matos, Fernanda; DiGirolamo, Grace; Loving, Chloe; Neveldine, Tegan; Weekes, Sakina; Wenzel, Sophie

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People with marginalized identities were disproportionately affected by the COVID-19 pandemic. There was an increase in overdose-related mortality and a higher mortality rate in racial minorities. The pandemic showed an increase in isolation of older adults which has been linked to negative health outcomes. These issues were exacerbated in rural Appalachia.

In Spring 2021, we conducted 40 interviews with older adults (8), Latinx individuals (12), Black individuals (9), and people who use drugs (11), in Virginia's New River Valley, with the goal of understanding how the pandemic impacted these populations. A thematic analysis revealed major themes:isolation, technology and the internet, adherence to health policy, faith/religion, mental health, resilience, and access to resources.

These themes help better understand how marginalized populations experienced the pandemic. Telling their stories not only humanizes them but also gives insight into how resources in the community can be better leveraged to serve these populations, helping local community partners work to remove barriers to care.

Support: Town of Blacksburg

P16
Artificial Intelligence-Driven Macrophage Identification in Canine Diffuse Large B Cell Lymphoma: A Pilot Study to Explore Prognostic Significance

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Canine diffuse large B cell lymphoma (DLBCL) is the most common neoplasm in dogs. Within the tumor microenvironment, tumor-associated macrophages play a pivotal role and have been associated with worse prognosis in humans. These macrophages can engage in the phagocytosis and scavenging of tumor cells, resulting in the characteristic "starry-sky" pattern by the presence of tingible body macrophages (TBM). This distinctive pattern is a protective element by exerting control over tumor growth and a valuable indicator for prognostic investigations. This study aimed to apply artificial intelligence (AI) for histopathology image analysis and determine the degree of TBM infiltration into DLBCL for further correlation analysis of their impact on survival outcomes. The analysis included retrospective cases of canine DLBCL (present in lymph nodes or spleen), regardless of age, breed, or sex, submitted to the Virginia Tech Animal Laboratory Services (2013– 2023). From 924 cases, 43 were selected due to the availability of complete clinical history and histology slides. Pictures of ten fields within the neoplastic population were taken in a randomized manner at 20x magnification and used for annotation, training, and analysis using an AI based software QuPath. The software was trained using a selection of ten sample images, each thoughtfully annotated to highlight crucial features of TBM. The cases were further divided into two groups based on survival (<6 and ≥6 months). The AI successfully counted TBM in 34 cases (79%), while 9 cases were identified as outliers (exceptionally high or low counts). A comparative evaluation was then undertaken between the AI-generated results and the investigators counts. No significant correlation was observed between the numbers of TBM and survival outcomes (P=0.6402). The AI effectively identified TBM, although in a few instances excessive stroma and autolysis were confounding factors. Future studies will aim at AI refinement, confirmation of TBM with immunohistochemistry, and a larger data set to yield more precise results.

P17
Exploring radiomics on Magnetic resonance Imaging for the diagnosis of glioma in dogs.

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Background: On magnetic resonance imaging (MRI), qualitative characteristics lack biological specificity and show low sensitivities and specificities for the diagnosis of canine intracranial glioma (GM). As a result, prediction of GM type and grade and differentiation of GM from other intra-axial diseases that can mimic GM is difficult even for experienced radiologists. Recently, quantitative imaging analysis (qMRI) has been receiving more attention in veterinary medicine. The field is known as “radiomics” which combine diagnostic imaging techniques with artificial intelligence (AI)-based quantitative computational analysis to extract objective information from images associated with shape, texture, and intensity to find diagnostic/prognostic patterns that are not visible to the human eye.
Hypotheses/objectives: qMRI features will be more accurate than expert-defined qualitative imaging characteristics to classify the type and grade of canine GM and for the differentiation of GM from other diseases that can mimic glioma. Focal intra-axial diseases will each display a unique set of radiomic qMRI features that can be used to define these entities.
Animals and Methods: Three raters that were blinded and had distinct experience levels assessed a dataset of 186 brain MRI studies from dogs with focal intra-axial brain lesions, including 156 histologically confirmed intracranial GM (90 oligodendrogliomas, 47 astrocytomas, 9 undefined GM), 3 granulomas, 3 MUE and 24 CVA. Lesion features, signal characteristics and secondary effects were evaluated. Raters determined if the lesion was a tumor, if it was a tumor, which type and grade, and if not tumor, assigned an alternate diagnosis (abscess/granuloma, MUE, CVA, other). Results were compared to the histopathologic diagnosis, and inter-observer agreements were obtained. A low correlation was found for GM type (k=0.4) and low/moderate for GM grade (high: k=0.34 low: k=0.5). The same data set will be used to evaluate the performance of qMRI features using an open-source image analysis software (3Dslicer) starting with the pre-processing step (registration, field bias correction, denoising and interpolation and brain mask segmentation), following by intensity normalization using python and brain tumor segmentation in 3D slicer. qMRI features will be extracted on 3Dslicer using pyradiomics package. Machine learning classification models will be developed in Orange, an open-source data analysis and visualization software.

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P18
Interim Analysis of a Novel Chemotherapy Protocol THOP (Temozolomide, Doxorubicin, Vincristine, Prednisone) for the Treatment of naïve B-cell Lymphoma in Dogs

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Introduction: The gold standard for multicentric lymphoma in dogs is CHOP-based protocols. Current evidence suggests that disease control is most frequently lost following cyclophosphamide administration. The aim of this pilot prospective study is to determine the efficacy and toxicity profile of a novel, multi-agent protocol, THOP, as first-line treatment in dogs with intermediate-large B-cell lymphoma.
Materials and Methods: Dogs were enrolled if they were diagnosed with intermediate-large B-cell lymphoma and had not received >10 days of corticosteroids. THOP was administered on a three-week cycle, for five cycles.
Dogs were fully staged at screening, at week six and at the end of the protocol and monitored with monthly physical exams until cytologically confirmed relapse. Standard VCOG response and toxicity criteria were used. Response rate, time to best response, time to progression (TTP), and overall survival time (OS) were estimated using commercially available statistical software.
Results: Fourteen of twenty planned dogs have been enrolled. Three dogs classified as Stage III, five dogs stage IV, six dogs Stage V; eight were substage a and seven substage b. A total of 70 cycles of THOP have been administered. All dogs achieved complete remission (within a median of 26.5 days), with TTP of 269 days and OS of 433 days. There were five Grade III and four grade IV hematologic toxicities; Grade III and IV gastrointestinal toxicities were not observed.
Conclusions: THOP appears to be a well-tolerated and effective first-line protocol for the treatment of B- cell lymphoma in dogs.

Support: Veterinary Memorial Fund (VMF)

P19

Comparative study of the influence on heel movement and foot biomechanics between aluminum nail on shoes and commercial fabric-cuff indirect glue on shoes.

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The equine hoof is a flexible structure, and its deformation during locomotion is an important part of the hoof mechanism. The hoof mechanism is believed to play a vital role in absorption of ground reaction force and in vascular perfusion of the hoof. It has been speculated that shoeing may have a negative effect on the hoof mechanism by restricting natural movement of the hoof, but little research has been done to explore the effects of different types of horse shoes on the hoof mechanism.

This project's main goal was to measure the effects of two types of horse shoes on heel movement in the forelimb of the horse. We aimed to compare the effects on hoof heel movement of an indirect glue-on shoe with a fabric cuff, an open-heel aluminum nail-on shoe, and no shoe (barefoot).

We hypothesized that an open-heel aluminum nail-on shoe and an indirect glue-on shoe would restrict heel movement compared to no shoes (barefoot) conditions and that an indirect glue-on shoe would restrict heel movement to a greater degree than a nail-on shoe.

Nine horses of varying breeds and disciplines have been included, and the study was performed on asphalt (hard footing) and in a riding arena (soft footing). A displacement sensor was mounted directly on each horse's heels which measured expansion and contraction during each stride; data was collected and processed for a minimum of ten strides per each condition.

Data obtained from the different shoeing techniques (barefoot, aluminum nail-on shoe and the indirect glue-on shoe) will be compared using a mixed model ANOVA if the data is normally distributed or linear generalized estimating equations if data is skewed. Statistical significance will be set to $P<0.05$.

The data obtained for the first nine horses is currently being processed and organized for preliminary results. Six additional horses will be tested in the spring of 2024.

Support: ERC (Equine Research Competition)

P20
North American Culex mosquitoes are competent vectors for Usutu virus

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Usutu virus (USUV) is an emerging mosquito-borne virus belonging to the Flaviviridae family and is closely related to West Nile virus (WNV). It is maintained in an enzootic cycle primarily involving 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. To understand the emergence potential of USUV, we sought to investigate the vector competence of North American Culex tarsalis and Culex pipiens, both competent vectors for WNV. Cx. tarsalis and Cx. pipiens were exposed to artificial bloodmeals spiked with 7-8 log10 PFU/mL of USUV isolate Netherlands 2016 or Uganda 2012. Both species were able to transmit USUV, with 13% of Cx. pipiens and 4% of Cx. tarsalis having infectious virus in their saliva. We wanted to further assess vector competence of Cx. tarsalis and Cx. pipiens through a bird-to-mosquito enzootic transmission model. To do so, we first established a passerine bird model for USUV. We inoculated domestic canaries (Serinus canaria forma domestica), a species highly susceptible to WNV, with USUV isolate Netherlands 2016 or Uganda 2012. Canaries were susceptible to USUV, reaching viremias predicted to be transmissible to mosquitoes. Next, we plan to feed Cx. pipiens and Cx. tarsalis on USUV infected canaries and measure vector competence. These results indicate Cx. pipiens and Cx. tarsalis have the potential to be primary vectors of USUV in North America. Furthermore, we describe a passerine bird model that can be used to study USUV pathogenesis and enzootic transmission. Understanding species competent for USUV can better predict its emergence potential and maintenance.

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P21
First successful engraftment of human liver cancer cell line in highly robust immunocompromised porcine model to test the tumor ablation efficacy by histotripsy

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Liver tumor, commonly called as the Hepatocellular carcinoma (HCC) is the most common type of primary liver malignancy. It is the fourth leading cause of cancer-related deaths. Late diagnosis, location of the tumor, tumor burden, and metastases makes liver tumor very challenging to treat by liver transplantation, surgical procedures, and other ablation techniques like cryoablation and thermal ablation. Also, the absence of a preclinical animal model makes it challenging to develop and explore the feasibility of treatment modalities. 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 “bubble cloud,” at the target. The bubble cloud expands and collapses, which ablates the tissue into an acellular homogenate.

Pigs are ideal animal models as they closely resemble the human anatomy and are very significant in improving the translation to human patients. This study aimed to establish the feasibility of successfully ablating liver tumors by histotripsy using an immunocompromised porcine model to generate HepG2 human liver cancer cell line tumors in the liver of the immunocompromised pigs. The orthotopic porcine model was established using RAG2/IL2RG double knockout immunocompromised pigs. HepG2 cells were injected orthotopically into the liver of immunocompromised pigs. Three weeks after the injections, CT images and necropsy indicated successful engraftment and growth of liver tumors in the pigs. The ultrasound images and post-histotripsy treatment histology images showed confirmed ablation zones in the liver. Therefore, in conclusion, our preliminary results in the study could demonstrate, for the first time, a highly robust model of human liver cancer in a large animal model. Soon, we plan on conducting more such studies and exploring all the possible utilities of these immunocompromised porcine models to develop therapeutic strategies to increase the efficacy of liver tumor ablation by Histotripsy.

Support: *NIH*

P22
Double The Trouble: CX3CR1 Modulates Double Negative T Cells and IL-17 Production in Systemic Lupus Erythematosus

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The G-protein chemokine CX3CR1 receptor initiates intracellular signaling cascades responsible for modulating cell activity, proliferation, and survival. CX3CR1 loss-of-function leads to exacerbation of chronic kidney disease in patients. Renal disease is a severe complication that often occurs in patients with systemic lupus erythematosus (SLE). We have previously reported that Cx3cr1-deficiency exacerbated glomerulonephritis in lupus-prone MRL/lpr mice, when compared to their wildtype littermates. In our current studies, we sought to investigate the role of Cx3cr1-deficiency at different stages of SLE disease. We investigated glomerulonephritis development in MRL/lpr mice at 3, 7, 11, and 15 weeks (wks) of age. Cx3cr1-/- MRL/lpr mice had significantly higher proteinuria. Double negative (DN) T cells which are pathogenic in lupus were found to be significantly expanded in Cx3cr1-/- MRL/lpr mice at 3, 7, and 11 wks of age with alterations of production of the IL-17 isoforms. IL-17A and IL-17F are cytokines that play important roles in the immune system, particularly in the regulation of inflammation. Our data suggest that CX3CR1 plays a role in a balanced production of IL-17A and IL-17F by cytotoxic and DN T cells. Our studies suggest that Cx3cr1-deficiency alters disease pathogenesis through modulation of IL-17 isoforms.

Support: VCOM

P23
HeEV: Histotripsy-enabled Extracellular Vesicle characterization in canine soft tissue sarcoma patients

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Introduction
Histotripsy is a non-thermal technique that utilizes short high pressure pulses to generate targeted rapidly expanding and collapse bubbles causing mechanical disruption of the surrounding tumor tissue. Changes to the immune response has been demonstrated after treatment of research animal models and companion canines. Safety and feasibility has also been demonstrated in human clinical trials. The generation of an "ablatosome" of cellular and matrix components occurs after the mechanical destruction of targeted tissues.

Extracellular vesicles (EVs) serve as a form of intercellular communication, containing bioactive material including proteins, nucleic acids, lipids, and metabolites. The role of EVs in tumor metastasis, tumor-immune response, and targeted drug development is an area of active research.

Prior research has shown a shift in EV distribution and cargo profile following sublethal low-intensity focused ultrasound. However, to date, EVs have not been evaluated following histotripsy.

The purpose of our study is to characterize the vesicular ablatosome following histotripsy treatment of naturally-occurring canine soft tissue sarcomas (STSs). Additionally, serial sampling of plasma EVs in circulation will be cross-registered with ablatosome vesicles.

Methods
Canine patients diagnosed with STS were recruited. Computed tomography (CT) was performed pre- and 2 weeks post-treatment. Partial tumor ablation was performed with histotripsy, and the tumor ablatosome was collected immediately post-treatment. Plasma samples were obtained pre-treatment, and at 6 timepoints post-treatment. Control dog plasma EVs and EV mRNA from control dogs have been extracted with commercial kits. EVs were enumerated and characterized with ZetaView and ImageStream flow cytometry. EV mRNA will be evaluated with the NanoString Canine IO panel.

Results
All three planned dogs with STS have been recruited and treated. Post-treatment CT revealed ablated regions. Average plasma EV concentration from control dog patients with tumor was 5.16E+11 particles/mL and EVs were positive for CD9 and vimentin. EV mRNA average concentration was 6.6 ng/nL, average total amount was 178.9 ng with acceptable purity.

Conclusion
Histotripsy for spontaneous STS demonstrates ablative efficacy. Plasma EV characterization and EV mRNA extraction is feasible and yields sufficient and adequate material to perform the Canine IO panel.

Support: iTHRIVE Pilot Grant, Thrive Pet Healthcare

P24
Ionomycin demonstrates potent activity against Clostridioides difficile via calcium accumulation and disruption of membrane polarization

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Clostridioides difficile poses a severe global public health threat, marked by numerous infections and thousands of fatalities.. Current anti-C. difficile agents exhibit suboptimal outcomes, characterized by high treatment failure rates and recurrence. Hence, novel selective therapeutic agents are desperately needed to combat C. difficile. Herein, we report the discovery of ionomycin, a calcium ionophore metabolite produced by Streptomyces conglobatus as a potent anticlostridial agent. Ionomycin exhibited a potent activity against 30 C. difficile isolates, inhibiting the growth of 50% and 90% of the isolates tested at concentrations (MIC50 and MIC90) of 1 µg/mL and 2 µg/mL, respectively. In a time-kill assay, ionomycin outperformed the drugs of choice, vancomycin, and fidaxomicin, reducing the high bacterial inoculum by 3 log10 within 8 hours. At subinhibitory concentrations, ionomycin was superior to vancomycin and metronidazole in suppressing the toxins production and spores formation. Moreover, ionomycin exerted a sporicidal activity, clearing the C. difficile spore load within 4 days and significantly preventing the toxin production from the remaining germinating cells. Importantly, ionomycin demonstrated limited activity against representative members of the host's gut microbiota. Mechanistic investigations revealed that calcium supplementation enhanced the ionomycin's bactericidal activity, while the addition of calcium chelator reduced its activity. Ionomycin also altered C. difficile's membrane potential, an effect that was significantly enhanced by calcium supplementation. Taken as a whole, this improved ionomycin safeguarded 83% of mice from infection by C. difficile. Conversely, the drug of choice, vancomycin resulted in 33.3% survival of mice. These results, collectively, indicate that ionomycin represents a promising anticlostridial therapeutic that merits further investigation.

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P25
A GII.6 strain of human norovirus is infectious and pathogenic in gnotobiotic pigs

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GII.6 strains of human norovirus are among the top five most prevalent strains. Due to its clinical relevance, we aimed to evaluate if gnotobiotic (Gn) pigs are susceptible to GII.6 infection, as this model supports efficacy testing of candidate vaccines. The virus inoculum was prepared from an adult human stool sample, and the absence of other mammalian viruses was confirmed by DNA and RNA deep sequencing. Two neonatal Gn pigs were orally inoculated with 5.2x104 genome copies of the virus. From post-inoculation day (PID) 0-10, feces and rectal swabs were collected daily to monitor diarrhea and virus shedding (by RT-qPCR). At PID10, pigs were euthanized and serum samples were collected for evaluating GII.6 VLP-specific IgM antibody titers by ELISA. Both pigs shed high titers of the virus in feces with peak titers reaching 105 genome copies/g. Only one piglet developed diarrhea, which lasted for two days. Both pigs had GII.6-specific IgM titers (64) in serum. Next, we infected three 4-day-old and four 33-day-old pigs with 1x106 or 2x106 copies of the virus. Daily rectal swabs and feces were collected from PID0-10 to monitor diarrhea and virus shedding. In both groups, 100% of pigs developed diarrhea and shed virus in feces. Mean diarrhea onset day was 2 and 4.6 and the mean duration was 9 and 4.2 for 1x106 and 2x106 groups respectively. Virus shedding began on average at PID4 and 3.2 for the 1x106 and 2x106 groups respectively and lasted for 5 days on average for both groups. Mean peak titers reached 5.78x105 genome copies/g in neonatal pigs and 104 in older pigs. All pigs had IgM titers ranging from 16-256. Immunochemical staining of ileum revealed significant mononuclear cell infiltration. Passaging of the virus in pigs is being attempted. A Gn pig model of GII.6 infection and disease is established.

Support: NIH, ICTAS

P26
Characterizing altered cellular architecture in the dentate gyrus and the chronic role of EphA4 on peripheral immune cell activity in the neurogenic niche following TBI

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Traumatic brain injury (TBI) is a major cause of morbidity and mortality in the US, often leading to serious chronic sequalae including post-traumatic epilepsy, cognitive impairment, and mood disorders. The dentate gyrus, a specialized neurogenic region within the hippocampus, is particularly susceptible to injury. Using a moderate TBI model, our previous work has demonstrated a significant loss of inhibitory interneurons in the dentate gyrus after an injury and that this loss of inhibitory input is coupled with an increase in aberrant excitatory neuroblast migration. These changes to the cellular architecture were also associated with the development of a post-traumatic epilepsy phenotype. Neuroinflammation has been shown to drive secondary brain injury and subsequent neuropathology with influx of peripheral immune cells through the disrupted blood brain barrier perpetuating the inflammatory response in the injured region. The aim of this study is to characterize alterations in interneuron subtypes and neuroblast migration over time following a moderate TBI. Furthermore, we hypothesized that elimination of monocyte-specific EphA4 will ameliorate pathologic changes in the hippocampus as well as overall lesion volume. A controlled cortical impact (CCI) model was used to generate a moderate TBI in mice. Histopathology samples were collected at 7, 30 and 120 days post injury and behavioral assessments were performed at 30, 60, 90, and 120 days post injury. Immunohistochemistry and stereology were used to assess the cellular populations of the dentate hilus, revealing a significant reduction of reelin-expressing interneurons at 7 days post injury in CCI animals compared to shams, and this trend remained consistent until 120 days post injury. These findings correlate with spatial memory deficits, assessed via the object location task, at 90 and 120 days post injury. Epha4f/f/CCR2/CreERT2 (KO) mice were generated along with CCR2/CreERT2 (WT) controls to assess the role of monocyte-specific EphA4 in the development of chronic neuropathology. KO animals exhibited a smaller lesion volume at 7 days post injury compared to WT mice, establishing this model for further investigation of chronic neuroimmune interactions involving EphA4 in monocytes.

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P27
Investigating the Role of CX3CR1 and Gut Microbiota in Lupus-Like Disease Progression

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Systemic lupus erythematosus is an autoimmune disorder characterized by inflammation and tissue damage. The interaction between CX3CR1 and its ligand CX3CL1 is known to affect immune responses. Based on our previous observations, we hypothesized that Cx3cr1 deficiency would worsen lupus-like disease by modulating gut microbiota-mediated immune responses. We equalized the gut microbiota of Cx3cr1-wildtype (WT) MRL/lpr mice and Cx3cr1-deficient (KO) MRL/lpr mice by performing a co-housing experiment. Group 1 was WT, group 2 was WT co-housed with KO, group 3 was KO co-housed with WT, and group 4 was KO. KO MRL/lpr mice had significantly higher proteinuria than the other three groups. The gut microbiota of KO MRL/lpr mice promoted the expansion of cytotoxic T cells in WT MRL/lpr mice (group 2 compared to group 1). While the gut microbiota of WT MRL/lpr mice decreased IL-17A-producing CD4+ T cells in KO MRL/lpr mice (group 3 compared to group 4), it did not affect the production of IL-17F. Furthermore, the gut microbiota of WT MRL/lpr mice moderately decreased naïve CD4+ T cells in KO MRL/lpr mice (group 3 compared to group 4). These data suggest that the gut microbiota may influence different T cell subsets to exacerbate lupus in Cx3cr1-deficient MRL/lpr mice.

Support: VCOM

P28
Treatment Duration and Load Magnitude Differentially Affect Biomechanical Properties in a Preclinical Model of Achilles Tendinopathy

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Achilles tendinopathy (AT) is common and results in pain, reduced mobility, and decreased quality of life. Physical therapy (eccentric loading) of AT improves tendon biomechanical properties and clinical symptoms; however, the ideal "dose" of eccentric loading needed for optimal healing unknown. The objective of this study was to use an established pre-clinical mouse model to compare the effects of rehabilitative load magnitude and treatment duration on the biomechanical properties of injured tendon. We hypothesized that Increasing loading magnitude and treatment duration would improve mechanical properties of injured Achilles tendon. Under IACUC approval, Achilles tendinopathy was induced in 12-week-old, male mice using intratendinous TGFβ-1 injections. Mice were randomly assigned to 1 of 3 muscle loading groups (50%, 75% and 100% body weight (BW)), an age-matched injured/untreated (IU) group or a naïve (uninjured) group. Mice were anesthetized and eccentric loading simulated using an electrical stimulation model twice weekly for 1, 2, or 4 weeks. Following the final treatment, muscle strength was quantified, mice euthanized, and Achilles tendons harvested for uniaxial tensile testing for biomechanical properties. Outcomes were compared by 2-way ANOVA with significance set at $p<0.05$. Injured/untreated (IU) tendons had significantly increased mean cross-sectional area (CSA) compared to naïve and all loading groups at 2 and 4 weeks ($p<0.001$). Mean maximum stress of IU tendons was significantly lower compared to naïve and all loading groups after 4 weeks ($p=0.025$). Mean elastic modulus of the IU group was significantly decreased compared to naïve and 75% BW following 4 weeks of loading ($p<0.001$). Muscle strength was maintained over time as loading magnitude increased between the 3 muscle loading groups. Torque output was lower after 2 weeks at 100% BW loading compared to the naïve group, then recovered to naïve levels after 4 weeks. Cross-sectional area and biomechanical properties of injured tendons were influenced more by treatment duration than loading magnitude. Eccentric loading of injured Achilles tendons maintained normal CSA and resulted in improved biomechanical properties, regardless of loading magnitude after 2 and 4 weeks. As loading magnitude and treatment duration increased, muscle strength post-treatment improved. Ongoing histology and transcriptomics will enable more in-depth interpretation of these results as they apply to AT healing.

Support: VCOM/VMCVM Center for One Health Research, Virginia Tech Regenerative Medicine Interdisciplinary Graduate Education Program (RM IGEP)

P29
Geographic trends in environmental exposures among Dog Aging Project participants

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Several environmental exposures may vary for dogs across the United States. For example, dogs living in different regions may spend a different amount of time outdoors, may be exposed to different temperatures, and may be used to wetter or drier climates. The environment is known to play a role in the development of both acute and chronic diseases in humans and companion animals (Gaillard et al., 2022; Uddin et al., 2021; Wu et al., 2023).

Various differences in environmental exposures and resulting health outcomes have already been documented in dogs living in urban versus rural settings. For instance, rural dogs are likely to spend more time alongside livestock animals such as cattle compared to urban dogs, which can expose them to antibacterial resistant E. coli (Sealey et al., 2022). Season has also been seen to have an impact several health outcomes. In different seasons, drastic transitions in the environment can occur, including changes in temperature and rainfall. This can affect exposure to pathogens and other conditions that cause various adverse health outcomes (Uddin et al., 2021).

By better understanding the dogs' environments, we can be better prepared to predict and prevent poor health outcomes related to environmental exposures. The goal of this study is to characterize geographic differences in dogs' environments across the United States. This will lead to valuable insights related to how environmental exposures tend to vary geographically, and how these patterns could subsequently affect dog health across different regions.

P30
Characterization of Rift Valley fever virus L polymerase interaction with protein phosphatase-1 alpha

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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 in a smaller percentage of cases. RVFV represents an important pathogen of interest 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 work has shown that protein phosphatase 1 alpha (PP1α), a versatile serine/threonine host phosphatase, is important for RVFV replication at least partially through the enhancement of L protein-viral RNA interactions. We also found that PP1α regulates L protein phosphorylation, impacting viral replication. PP1α interacts with proteins via various binding motifs, most importantly the RVxF binding motif. Given this, we hypothesize that the RVFV L protein interacts with PP1α through an RVxF binding motif contributing to changes in L protein phosphorylation and alterations in viral replication. Bioinformatic analysis and AlphaFold structural modeling identified four putative RVxF binding motifs in the L protein, RVxF-1-4. Only two of these motifs, RVxF-1 and RVxF-4, were predicted to have favorable interactions with L protein. The key amino acid residues within these motifs were substitute for alanine and the impact on viral replication and protein-protein interactions determined. Mutations within the L protein RVxF-4 resulted in less efficient viral replication. However, this motif was not necessary for the interaction of PP1α with the L protein. Ongoing studies will determine the importance of RVxF-1 for interaction with PP1α and RVFV replication. The results of this study will provide mechanistic information regarding how PP1α interacts with RVFV L protein and modulates viral replication.

Support: Defense Threat Reduction Agency (DTRA)

P31
Cross Protection Against Usutu Virus And Saint Louis Encephalitis Virus In Mice Treated With Human West Nile Virus Convalescent Plasma

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Usutu virus (USUV), Saint Louis encephalitis virus (SLEV), and West Nile virus (WNV) are mosquito-borne flaviviruses within the Japanese encephalitis serocomplex. In humans, these viruses can cause fever, skin rash, and damage to the central nervous system, resulting in neuroinvasive diseases such as meningitis, encephalitis, and meningoencephalitis. These viruses share a similar transmission cycle among passerine birds and Culex spp. mosquitoes. Currently, USUV and WNV are co-circulating in many European countries, and SLEV and WNV are co-circulating in the United States. Thus, it is possible that an individual could get infected sequentially with these viruses. Previously we investigated if WNV vaccination protects against USUV infection in mice. We observed significant protection from USUV disease in WNV-vaccinated mice compared to mock-vaccinated mice. We also found that mouse serum from vaccinated mice cross-neutralized USUV in vitro. Therefore, we have been investigating whether human WNV convalescent plasma protects from USUV and SLEV disease. Five human WNV convalescent plasma samples cross-neutralized both USUV and SLEV in vitro. We have infected C57BL/6 mice with WNV and generated anti-WNV serum as a positive control. Next, we will conduct an in vivo study by treating wild-type mice (C57BL/6) with human WNV convalescent plasma, mouse anti-WNV serum, normal human plasma, or normal mouse serum followed by challenging them with WNV, USUV, and SLEV. Disease conditions will be observed by measuring their weights, viremia in blood, and observing histopathology, especially in the brain. This study will reveal if human anti-WNV antibodies protect against USUV and SLEV disease in mouse models. This study will help develop vaccines against WNV, SLEV, and USUV and predict disease consequences for individuals exposed to multiple flaviviruses.

Support: *NIH*

P32
Evaluation of Image Magnification on The Accuracy of Artificial Intelligence Models

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Diffuse large B cell lymphoma is a common and deadly neoplasm in canine patients. Therefore, monitoring its response to chemotherapy is an essential goal of care and critical to a patient's survivability. The current gold standard diagnostics for minimal residual disease (MRD) detection post-treatment includes PCR for Antigen Receptor Rearrangements (PARR) and flow cytometry (FC). However, associated costs for serial monthly monitoring with these diagnostics can be cost-prohibitive. The goals of this research are 1) to build an artificial intelligence model that can differentiate lymphoma from nonlymphoma using cytology lymph node aspirate images, 2) to determine which image magnification or magnifications leads to the best accuracy, 3) to demonstrate that we can determine the MRD status of dogs with lymphoma following treatment using artificial intelligence and digital cytology lymph node aspirate images, with the ground truth established by PARR and FC.

In the first part of this study, a CNN was built and trained using retrospectively collected cytology lymph node images to differentiate patients with lymphoma from patients without lymphoma. This study aims to determine which magnification, or set of magnifications, leads to the greatest accuracy of the CNN. The magnifications evaluated are 20x, 60x and 100x.

The second part of the study is ongoing. This part of the study will modify the artificial intelligence to detect MRD in dogs with lymphoma compared to PARR and FC.

Immediate impacts of this project will include earlier detection of disease remission, improved post-treatment monitoring, more affordable monitoring, and, ultimately, longer patient survivability. The longer-term impact of this work will lead to the development of online decision support tools using these methods for the initial cytologic detection and monitoring of lymphoma and other types of neoplasms.

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P33
Comparing puppy growth of overweight and lean bitches

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There are currently no studies comparing the impacts of a higher maternal body fat content on milk composition in dogs and its subsequent effect on the health of her puppies, despite a growing body of evidence for negative implications in other species. We hypothesized that maternal excess body fat conditioning will result in altered milk composition, such as higher fat content and increased gross energy, with no change to milk protein and sugar. Additionally, these changes in milk composition may increase puppy growth rate. Our objective was to analyze and compare milk composition between overweight/obese and lean bitches, and compare their puppies' growth rates during the first 3 weeks after birth. Two overweight (body condition score (BCS)=6/9) and 6 lean (BCS=4-5/9) otherwise healthy, 2-5 years old, client-owned bitches were included in our study so far. The average weight at enrollment was 26.3 ± 6.7kg. Breeds include Labrador Retriever (n=3), Weimaraner (n=2), Golden Retriever (n=1), Australian Shepherd (n=1), and English Shepherd (n=1). Milk samples were collected from all bitches on days 2-5, 7-10, 14, 21, and 28 after parturition, and frozen at -80°C. So far, milk was analyzed from 4 lean bitches and analyzed for crude protein, total fat, and sugar, with gross energy calculated. A physical exam, body weight, and BCS of the dams were performed at these intervals to confirm health. Body weights of all puppies were recorded daily for all dogs. Data was analyzed by a mixed model to compare weekly puppy growth rates between the overweight and lean groups. $P<0.05$ was set as significant. Milk analysis has only been completed on 4 lean dogs and thus could not be statistically analyzed for significance at this time. Our preliminary results for puppy growth show that there is no significant difference between lean and overweight dams on weekly puppy growth rate from birth until 3 weeks of age. While a conclusion cannot yet be drawn, preliminary data suggests that there may be no significant difference in the puppy growth rate between lean and obese canine mothers. This study is still ongoing.

Support: Society for Theriogenology

P34
Repurposing valnemulin for combating multidrug-resistant Neisseria gonorrhoeae.

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The Centers for Disease Control and Prevention (CDC) has categorized Neisseria gonorrhoeae as an urgent public health threat due to the increasing incidence of infections and the uprising bacterial resistance to antibiotics. With approximately 82.5 million annual infections, N. gonorrhoeae has developed resistance to all classes of antibiotics. Currently, ceftriaxone is the only recommended treatment, but the emergence of high-level resistance poses a serious challenge. Without developing new anti-gonorrheal treatments, the world faces the real possibility of an untreatable gonococcal infection. Drug repurposing represents an effective approach to drug discovery as it reduces the time, costs, and risks associated with traditional drug innovation. Adopting the drug repurposing approach, we identified the animal-approved drug valnemulin as a potent anti-gonococcal agent. Valnemulin is an FDA-approved pleuromutilin derivate used to treat swine dysentery, colitis and pneumonia. It displayed a potent activity inhibiting a panel of 24 multidrug-resistant clinical isolates of N. gonorrhoeae with concentrations ranging from 0.03 to 0.05 μg/mL. The drug exhibited a rapid bactericidal activity against N. gonorrhoeae, completely eradicating the high bacterial burden within 4 hours and demonstrated a prolonged post-antibiotic effect (> 6 hours). Importantly, no resistant mutants emerged even in the presence of a high bacterial inoculum. Valnemulin's advantages extend beyond its efficacy. In contrast to ceftriaxone, it demonstrated limited activity against the protective commensal vaginal microbiota, a crucial defense against gonococcal infection. Additionally, valnemulin was superior to ceftriaxone in killing the intracellular N. gonorrhoeae, completely clearing infected endocervical cells within 5 hours. Furthermore, valnemulin reduced the expression of IL-6, a pro-inflammatory cytokine contributing to the severity of gonococcal infection. Collectively, our findings indicate that valnemulin represents a promising anti-gonococcal agent that merits further investigation.

***Support:** NIH*

P35
Discovery of Potent Anticlostridial Compounds from an Antiviral Library

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Clostridioides difficile infections pose a significant burden on the health care system, contributing to nearly 29,000 deaths annually. C. difficile, an enteric pathogen that is responsible for colitis and potentially fatal diarrhea primarily in hospitalized patients. The bacterium takes advantage of microbial dysbiosis often caused by antibiotic use for another medical conditions, leading to high recurrence and treatment failure rates, thus limiting therapeutic options. The continued impact and severity of C. difficile indicates a need for novel molecules outside of standard antimicrobials and potentially new lead molecules which could serve as scaffolds for targeted C. difficile treatments. In this project, we report new molecules with potent anticlostridial activity from an antiviral library. A whole-cell-based screening of 618 compounds using a toxigenic strain of C. difficile, eight compounds with potent anticlostridial activity (MIC = 0.25-8μg/mL) were identified. Of these hits, Cenicriviroc, Rottlerin, and ITX5061 showed activity at concentrations below Vancomycin, a drug of choice for C. difficile infection. The minimum inhibitory concentrations (MICs) assays were further confirmed against various clinical isolates of C. difficile. Additionally, MIC assay against healthy microbiota, including Bacteroides sp., Bifidobacterium sp., and Lactobacillus sp. Were performed to determine the compounds effect on gut microflora. The observed anticlostridial activity of these compounds holds promise for addressing the pressing need for innovative strategies in the treatment and prevention of C. difficile.

Support: *NIH*

P36
HDAC6 knockout alleviates pristane-induced lupus

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by excessive inflammation and the production of pathogenic antibodies. Histone deacetylase 6 (HDAC6) is a class IIb histone deacetylase. It has been reported that selective HDAC6 inhibition decreases inflammation in lupus mice models. C57BL/6 mice develop SLE-like symptoms following pristane injection. In this study, sex and age-matched wild type and HDAC6-/- mice on the C57BL/6 background were injected with 0.5 ml pristane or PBS intraperitoneally (i.p.) at 8-12 weeks of age and were euthanized 10 days later. At sacrifice, body and spleen weights were measured, sera were collected, splenocytes and peritoneal cells were harvested for flow cytometry. We found pristane treatment increased the spleen weight with no difference between WT mice and HDAC6-/- mice. Flow cytometry results showed that there was no difference in T cell or B cell populations in the spleen. Pristane treatment promoted the population of CD11b+Ly6C++ inflammatory monocytes and CD11b+Ly6G+ neutrophils. Peritoneal recruitment of these inflammatory monocytes and neutrophils in HDAC6-/- mice was significantly decreased compared to the WT mice. Pristane administration also induced the interferon (IFN) signature genes, like Mx1, Oas1a, Irf7, Irf9, Cxcl10, and Isg15. qRT-PCR revealed that these IFN signature genes were decreased in HDAC6-/- mice compared to the WT mice. In vitro experiments showed HDAC6 inhibitor ACY-738 decreased NF-κB protein level in J774A macrophage cells after LPS/IFN-γ stimulation. In summary, our results show that HDAC6 knockout inhibits the recruitment of inflammatory monocytes and neutrophils in the peritoneum in early inflammation response to pristane. HDAC6 deletion also inhibited the expression of IFN signature genes with pristine stimulation. HDAC6 inhibitor suppressed the NF-κB signaling.

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P37
ADAMTS13 activity in dogs with presumptive idiopathic immune thrombocytopenia

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Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by both platelet destruction and impaired megakaryocyte and platelet production. Bleeding tendencies are quite variable, unpredictable, and do not correlate to platelet counts. The variation in clinical presentation may be due to misclassification of a subset of dogs with ITP. In human medicine, ITP can be difficult to distinguish from immune-mediated thrombocytopenia thrombotic purpura (ITTP), which is a life-threatening form of thrombotic microangiopathy that is characterized by hemolytic anemia, consumptive thrombocytopenia, hemorrhage, and diffuse microthrombi formation with subsequent organ damage. 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 is quite different with the former requiring immunosuppressive glucocorticoid therapy and the latter requiring therapeutic plasma exchange, anti-vWF therapy, and immunosuppressive therapy.

The primary aim of this prospective study is to determine whether a subset of dogs with presumptive primary ITP have reduced ADAMTS13 activity. The study will further determine if ADAMTS13 activity differs between dogs with presumptive primary ITP and healthy control dogs. ADAMTS13 activity will be evaluated for correlation with clinical bleeding scores. We hypothesize that a subset of dogs with presumed primary ITP has reduced ADAMTS13 activity and ADAMTS13 activity is overall reduced in dogs with presumed primary ITP compared to healthy control dogs.

Dogs with a platelet count of less than 20,000 with presumed ITP received a daily canine bleeding assessment tool score and plasma samples were obtained. ADAMTS13 activity will be measured using a chromogenic ELISA kit from Diapharma®.

The study is currently ongoing. At this time, seven dogs with presumed idiopathic ITP have been enrolled. Plasma ADAMTS13 activity has not yet been measured in the ITP dogs. Enrolled dogs have a median bleeding score 6 (range 3-8). Healthy control dogs (40) have a median plasma ADAMTS13 activity of 87.6% (range 55.5-103.1%).

The study is ongoing and further conclusions will be made once ADAMTS13 activity has been measured in all enrolled dogs.

Support: Veterinary Memorial Fund

P38
Indocyanine green photodynamic therapy as a potential treatment for fungal keratitis: An in vitro investigation of antimycotic effect.

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In both humans and veterinary patients, fungal keratitis is a serious ocular infection with potentially devastating consequences, including corneal destruction, globe rupture, vision loss and loss of the eye. Fungal keratitis is frequently challenging to resolve with conventional medical interventions and surgical options to preserve corneal integrity are both costly and pose significant attendant risks related to general anesthesia, which can lead to increased morbidity and mortality. Additionally, with ever-increasing antimicrobial resistance, alternative therapies are necessary to limit antimicrobial resistance and to treat resistant infections when they arise. Photodynamic therapy (PDT) is a minimally invasive technique that has been utilized for the treatment of a variety of conditions, including certain cancers and microbial infections in both human and veterinary patients. PDT has the potential to serve as a novel non-invasive therapeutic intervention for infectious fungal keratitis, although its role and efficacy as a potential treatment for this condition has yet to be fully elucidated. The objective of this study is to determine the ability of indocyanine green PDT to inhibit the in vitro growth of Aspergillus fumigatus, a common corneal fungal isolate in horses. The results of this study will inform future research that ultimately aims to translate the role of indocyanine green PDT as a potential novel therapeutic for equine fungal keratitis in vivo.

Support: VMF

P39
HSV2 latency is maintained through RET and NCAM in sensory neurons

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Herpes simplex viruses 1 and 2 (HSV1 and HSV2) preferentially establish latency in different types of neurons. Neurotrophic factors (NTFs), which support survival and homeostasis of neurons, contribute to the maintenance of HSV latent infections. Nerve growth factor (NGF) maintains HSV1 latency in sympathetic neurons, which are dependent on NGF. However, we previously showed that deprivation of neurturin (NTN) or glial-cell derived neurotrophic factor (GDNF) induces reactivation of latent HSV1 or HSV2, respectively, in primary adult sensory neurons. Upon binding to GDNF family receptors (GFRs), GDNF and NTN activate Ret, a receptor tyrosine kinase that regulates numerous intracellular signaling pathways involved in cell proliferation and differentiation. One of these signaling pathways, PI3K/Akt, has previously been implicated in maintaining HSV1 latency in embryonic sympathetic neurons and is regulated by Ret phosphorylation at Tyr1062. Our data shows that in uninfected sensory neurons RET is phosphorylated at Tyr981, activating the Src pathway, and Tyr1096, activating the Grb2 pathway, but not Tyr1062, when deprived of neurotropic factors for 15 minutes via western blot. This suggests that adult sensory neurons possess different intrinsic mechanisms regulating viral latency and neurotropic factor signaling when compared to embryonic sympathetic neurons. GDNF can also signal through neural cell adhesion molecule (NCAM). An NCAM-blocking antibody in the presence of GDNF caused HSV2 to reactivate, but not HSV1, as shown with qPCR. Our results suggest that HSV2 maintains latency in sensory neurons using alternative signaling pathways like Ret or NCAM compared to sympathetic neurons that maintain HSV1 latency via the PI3K/Akt pathway. Our next steps include testing neurotropic factor deprivation on latent HSV2 infected sensory neurons and examining phosphorylation sites on RET and key downstream signaling factors by western blot, supported by inhibition studies, to identify the specific pathways that regulate HSV2 latency and reactivation in sensory neurons.

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P40
Brucella abortus maintains a cryptic quormone response

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Brucellosis is a globally endemic, bacterial zoonotic disease with worldwide incidence approaching 2 million cases per year. Despite a century of effort, a safe and effective human vaccine remains unavailable and economic and administrative issues have forestalled disease eradication in many areas. A new generation of vaccine candidates do show promise and rely on genetic disruption of Brucella's quorum sensing system to generate live, attenuated strains. The Brucella bacteria utilize their quorum sensing to sense a self-generated quormone signal, C12-AHL. When in high cellular density or when entrapped in host cellular membranes, rising concentrations of C12-AHL are sensed by two proteins, BabR and VjbR, which then massively alter Brucella's gene transcription to allow it to survive in the new environment. We generated the first deletion of both quorum sensing proteins in the same bacterial strain and using RNA-seq analysis have found that Brucella still alters its transcription in response to C12-AHL. Furthermore, the double deletion strain is more highly attenuated than either BabR and VjbR, indicating a synergistic interaction in the role of the proteins. Studies are ongoing to determine the joint role of BabR and VjbR in responding to C12-AHL, as well as to determine potential mechanisms for how Brucella responds to C12-AHL in the face of BabR and VjbR deletion. The understanding generated by this work will allow for further refinement of quorum sensing based live attenuated vaccine strains.

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P41
Determining The Role of a Novel Plant-Like Transcription Factor Tgap2x-7 in Toxoplasma Biology and Pathogenesis.

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Toxoplasma gondii, a protozoan parasite, is recognized as a leading cause of adverse outcomes such as miscarriage in pregnant women, neonatal blindness, and fatal encephalitis in immunocompromised patients. In infected individuals, Toxoplasma replicates intracellularly through a process known as endodyogeny that involves generation of two daughter parasites within the confines of the mother parasite. After multiple divisions, the daughter parasites egress leading to destruction of the host-cell. This lytic mode of parasite propagation is the primary cause of pathology in toxoplasmosis. Hence, identifying distinct parasite factors critical during any stages of the lytic cycle including invasion, replication, and egress is pivotal for designing novel therapeutics.

Apetala2 (AP2) proteins, are a unique and divergent family of transcription factors found only in plants and protozoa including Toxoplasma. Our research focuses on TgAP2X-7, a transcription factor whose function remains undetermined but is hypothesized to be essential for Toxoplasma propagation. Through endogenous tagging approach we validated that AP2X-7 is indeed a nuclear protein in Toxoplasma. To determine the role of AP2X-7, we utilized a conditional knockdown approach using the recently developed AID (Auxin Inducible Degron) system. Accordingly, we successfully inserted the mAID-HA epitope tag at the C-terminus of the gene using CRISPR-Cas9 technology. We further verified that insertion of the mAID tag does not alter its nuclear localization and the protein can be effectively down-regulated using auxin. Plaque assays revealed that loss of AP2X-7 abolishes parasite growth suggesting that the protein is indeed essential for Toxoplasma propagation. Importantly, absence of AP2X-7 results in slower replication and severe defects in host-cell invasion by Toxoplasma. Furthermore, lack of AP2X-7 results in significant alterations in Toxoplasma gene expression profile. Our forthcoming research endeavors include comprehensive dissection of different domains of the protein, identification of its interactome, and elucidation of signaling pathways mediated by this novel transcription factor. Additionally, we aim to dissect its role in both acute and chronic form of the disease in the animal model.

Support: NIAID (National Institute of Allergy and Infectious Diseases)

P42
Characterizing the Glioblastoma "Ablatosome" Treated with High-Frequency Irreversible Electroporation

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Glioblastoma (GBM) is a highly invasive and aggressive brain tumor in humans and dogs with a low post-treatment survival rate. An effective treatment for GBM will require overcoming the difficulties of treating such a neuroinvasive mass without harming the surrounding brain; replacing an immunosuppressive, tumor-promoting microenvironment with an anti-tumor microenvironment; and overcoming the blood-brain-barrier (BBB) to allow for delivery of cancer-targeting drugs to the brain. High-frequency irreversible electroporation (H-FIRE) is a non-thermal tumor ablation method in which an electric field is applied to cells in ultra-short, bipolar pulses to disrupt the cell membrane and transiently permeabilize the BBB. In dog and rodent models GBM, we have shown that HFIRE can simultaneously ablate tumor cells and facilitate drug delivery to the tumor. Treatment effect can be controlled by toggling the strength of the electric field (i.e., “reversible” versus “irreversible” electroporation). Our objective is to transcriptomically and proteomically characterize the HFIRE ablation microenvironment of GBM (the “ablatosome”),” to elucidate mechanisms of action of HFIRE therapy. We hypothesize that the nonthermal nature of the HFIRE will preserve and facilitate presentation of tumor neoantigens to induce immunogenic tumor cell death, and subsequently initiate a robust and sustained anti-tumor immunological response, acting as an in situ tumor vaccine. Transcriptomic data from canine gliomas treated with H-FIRE demonstrated significant upregulation of Type III interferon (IFN-l/IL-28; $p<0.0006$) and related downstream (JAK/STAT) signaling networks, as well antigen processing and presentation of endogenous peptide pathways in post-treatment samples[MOU1]. Proteomic analysis of orthotopic rat gliomas treated with H-FIRE demonstrated significant, but temporally dependent, acute activation of complement mediated cytotoxicity and ferroptosis cellular death pathways, as well enrichment of signaling networks mediated by damage associated molecular patterns via RAGE receptor ligands (Hsp90, Hmgb1) and extracellular matrix proteins (fibrinogen), supporting our hypothesis that H-FIRE causes tumor immunogenic cell death. Ongoing investigations of these mechanisms are being conducted via comparative analyses of rat proteomic to gene expression data, and use of in-vitro hydrogel GBM models to decouple direct electric pulse induced from immunologically mediated cellular effects.

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P43
Nitroxoline: A Promising Candidate for Treating Gonococcal Infections

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Neisseria gonorrhoeae has developed resistance to every class of antibiotics, leaving the medical community with limited treatment options. Currently, ceftriaxone is the only available treatment option, but it faces high-level resistance issues. This raises the alarming prospect of an impending era with untreatable gonorrhea. Therefore, there is an urgent need for the development of novel anti-N. gonorrhoeae therapeutics. Through drug repurposing strategy, we identified nitroxoline, an FDA-approved drug, as a potent anti-gonococcal agent. We evaluated nitroxoline's anti-gonococcal activity against a panel of clinically important multidrug-resistant N. gonorrhoeae strains including ceftriaxone-resistant strains. The drug displayed a potent activity inhibiting 90% of the strains tested (MIC90) at the concentration of 0.5 μg/mL. Time-kill kinetics assays demonstrated that nitroxoline possesses bactericidal properties, eradicating N. gonorrhoeae burden within 12 hours. It displayed a post-antibiotic effect of 6 hours comparable to that of ceftriaxone. Additionally, no N. gonorrhoeae resistant mutants were isolated against nitroxoline, suggesting a low likelihood of N. gonorrhoeae developing resistance to nitroxoline. Nitroxoline also was capable of effectively penetrating the human cervical carcinoma cell line and eradicating the intracellular N. gonorrhoeae. Furthermore, nitroxoline exhibited a minimal inhibitory activity against the representative members of commensal vaginal flora. Based on our mechanistic investigation, nitroxoline's anti-gonococcal action appeared to involve chelation with metal ions, particularly Fe3+ and Fe2+. Iron supplementation has resulted in diminished anti-gonococcal activity of nitroxoline. In conclusion, nitroxoline, with its favorable safety profile and potent activity against multidrug-resistant N. gonorrhoeae strains, offers a promising avenue for tackling N. gonorrhoeae infections.

Support: NIH

P44
Functional Characterization of a Novel Kinase TgTKL1 in Toxoplasma Pathogenesis

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Toxoplasma gondii, the causative agent of toxoplasmosis, is a significant global public health threat, affecting nearly one-third of the human population worldwide. The clinical manifestations of T.gondii vary widely, ranging from asymptomatic to lethal, depending on the host immunity and stage of infection. In pregnant women, Toxoplasma infection can lead to miscarriage, while neonatal exposure may cause blindness and cognitive impairments. Immunocompromised individuals face the risk of fatal encephalitis. Current available treatments have limitations, including toxicities and inefficacy against chronic toxoplasmosis. Consequently, there is a prompt need to develop novel therapeutic strategies. Accordingly, the identification of novel and unique parasite factors crucial for pathogenesis remains a priority in the ongoing battle against toxoplasmosis. Tyrosine Kinase-Like (TKL) family proteins are plant-like kinases that have critical roles in Toxoplasma growth but remain underexplored. In an attempt to identify novel drug targets in T. gondii, we focused on the characterization of T. gondii nuclear kinase (TgTKL1). Our studies revealed that this kinase is important for Toxoplasma growth in vitro and essential for virulence in vivo. TgTKL1 contains four distinct domains: the RNI domain, Enhanced Disease Resistance 1 (EDR1) domain, kinase domain, and Nuclear Localization Signal (NLS) motif, yet the contributions of these domains to TgTKL1 function remain unknown. To characterize the role these domains, we generated different domain mutant strains using CRISPR/Cas9. To define the role of the kinase domain, we generated a kinase mutant strain, which, intriguingly, exhibited defects in growth and virulence similar to the TgTKL1 null mutant. Furthermore, RNA-seq analysis showed downregulation of invasion-related genes, including TgSUB1 (subtilisin 1), a protease required for microneme processing during host-cell invasion. Accordingly, TgTKL1 kinase mutant displayed impaired processing of micronemal proteins suggesting that the kinase activity is crucial for TgTKL1 function. Our future studies will focus on generating NLS, RNI and EDR1 deletion mutants, and undergoing rigorous phenotypic assays including growth, invasion, microneme secretion, and virulence for these mutants. Additionally, multiple approaches including quantitative phosphoproteomics, immunoprecipitation, and proximity ligation assays will be used to unravel TgTKL1-modulated signaling pathways.

Support: NIH, American Heart Association

P45
Comparative Analysis of Equine Encephalitis viruses (EEV), Traumatic Brain Injuries (TBI), and Organophosphorus Nerve Agents (OPNA) as a Path to Neuroprotective Therapeutics

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Disease manifestations of equine encephalitis viruses (EEVs) have a variety of shared symptoms with other commonly occurring neurological injuries including Traumatic brain injuries (TBI), and organophosphorus nerve agents (OPNA) indicating similarities in altered pathways. There are currently no publicly available antiviral treatments for EEVs, or neuroprotective drugs to combat the combined long-term neurological deficits impact of TBI, OPNA, and EEVs which result in significant population health problems, increased health costs, and negative impacts on civilian and military personnel worldwide. We hypothesize that comparative neurological analysis of injuries from EEVs, OPNAs, and TBIs, can be utilized to identify potential medical countermeasures. To this end, we wanted to first characterize the pathology, behavior, and transcriptomic changes caused by Venezuelan equine encephalitis virus (VEEV) infection in C57BL/6 mice. Mice were intranasally infected with a dose of VEEV TC-83 yielding 80-90% survival, weighed, and monitored for clinical scores for 90 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 both acute (Day 2 and 7), and chronic (Day 90) timepoints, mice were sacrificed to look at gross pathology as well as IHC analysis for neuronal, astrocyte, and microglia activity. Preliminary pathology analysis indicates that neurological damage is highly dynamic dependent on time post infection. At days 30, 60, and 90 post infection mice were assessed via Novel object Recognition and Y-Maze (memory), Elevated Plus maze (anxiety), and a modified SHIRPA (neuromuscular). Finally, we performed bulk RNA-seq at acute timepoints (Day 4, 7, 28). The VEEV mouse data was analyzed and compared to both in house and publicly available TBI and OPNA transcriptomic data via a combination of pathway tools including gene ontology and Ingenuity Pathway Analysis. Ultimately, we have identified several potentially druggable pathways to reduce long term neurological injury based on in house and published transcriptomic data. Ongoing experiments are designed to evaluate drug efficacy and target specificity in vitro and in vivo against VEEV infection with future potential evaluation against OPNA and TBI neuropathologies.

Support: Defense Threat Reduction Agency

P46
Three-dimensional echocardiographic determinants of the age of onset of myxomatous mitral valve disease in Cavalier King Charles Spaniels dogs

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Myxomatous mitral valve disease (MMVD) is prevalent in Cavalier King Charles Spaniels (CKCSs) and relative to other canine breeds, the disease becomes clinically evident at a younger age. Previous studies using three-dimensional transthoracic echocardiography (3DTTE) have suggested that some CKCSs have a mitral valve (MV) of different morphology compared to other breeds. It has been hypothesized that a differently shaped mitral valve apparatus may lead to altered mechanical stresses on valve leaflets that can activate signaling pathways contributing to myxomatous degeneration and its progression. This study aimed to determine if morphological variables of the MV in healthy CKCSs could predict the age of onset of MMVD. CKCSs without echocardiographic evidence of MMVD were prospectively enrolled, and rechecks were performed every four months. Endpoints were the development of MMVD or 24 months from enrollment without the development of MMVD. At enrollment and at each recheck, standard echocardiogram and 3DTTE were performed. Dedicated software was used to analyze the 3DTTE datasets and obtain MV morphologic variables. Univariate Cox's proportional hazard models were used to investigate the effect of 3DTTE MV morphologic variables at enrollment on the time to onset of MMVD. A multivariate model was also created with a backward stepwise selection to identify independent predictors of the age of onset of MMVD. MV morphologic variables of 76 CKCSs were analyzed using dedicated software. Non-planar angle (NPA) ($p<0.001$, HR: 1.05), tenting area ($p=0.001$, HR: 0.55), and tenting volume normalized by body weight (nTnV) ($p<0.001$, HR: 0.81) were significant in the univariable models. NPA ($p<0.001$, HR: 1.05) and nTnV ($p=0.009$, HR: 0.82) remained significant in the multivariable model. This study demonstrated that MV variables obtained with 3DTTE can predict the early onset of MMVD in healthy CKCSs. Therefore, 3DTTE could be used in screening and breeding programs aimed at reducing the prevalence of MMVD in CKCSs.

Support: AKC

P47
Comparison of gait characteristics for horses without shoes, with steel shoes, and with aluminum shoes

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Background: Many horses used for athletics wear metal shoes to provide protection, support, and traction. The two most common horseshoe materials are steel and aluminum. In some equine disciplines, shoes are removed (barefoot) or changed to a lighter shoe material (aluminum) during an equestrian event to achieve perceived advantages such as more aesthetic limb flight. It is clear that shoeing has an impact on performance and health of athletic horses. However, objective information regarding the effects of shoeing on gait characteristics of performance and show horses is scant and there is little scientific evidence to guide decisions about shoeing and its impact on competition fairness and animal welfare. Body- and hoof-mounted inertial sensor systems are available that allow analysis of gait characteristics. This technology would be a useful tool to measure changes in gaits of horses under various hoof shoeing conditions.

Objective: To determine differences in gait characteristics of horses under various hoof shoeing conditions (barefoot, aluminum shoes, and steel shoes). We hypothesized that hoof shoeing conditions will not affect symmetry of head and pelvic movements and that lower foot-shoe weight (bare feet or aluminum shoes) results in lower hoof flight height, shorter stride length, and shorter duration of stride swing, stance, and breakover times versus higher weight (steel shoes).

Study Design: Prospective crossover design.

Methods: 12 healthy, adult, client-owned horses without lameness, hoof or limb abnormalities, or positive response to hoof testers were included. Horses were evaluated with body- and hoof-mounted inertial sensors at a trot on firm and soft surfaces. Evaluations were performed with their own shoes (baseline), no shoes (barefoot), and with aluminum and steel shoes. Data collected included Q score (measure of head and pelvic asymmetry), 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.

Results: Body- and hoof-mounted inertial sensors yielded data for all measured variables. Analysis suggests differences in some variables among various hoof-shoeing conditions and surface substrates.

Conclusions: Trimming and shoeing of hooves has measurable effects on gait characteristics of horses. These data will be useful for informing decisions about shoeing during competition.

Support: *ERC*

P48
Development and Characterization of a Sublethal-Sequelae Mouse Model of EEEV Infection

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Eastern equine encephalitis virus (EEEV) is a mosquito-borne that causes disease in both equines and humans resulting in overt encephalitis in a significant percentage of cases. Infection 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. The significant mortality and morbidity associated with EEEV infection underscores the need for useful interventions and currently, there are no therapeutic options available. EEEV pathogenesis has been studied in lethal mouse models, but there is no neurological sequelae model currently available. We aim to address this deficiency by developing and characterizing a novel neurological sequelae model of EEEV infection. We hypothesize that animals that appear to have recovered from EEEV infection still suffer substantial cognitive deficits resulting from neurodegeneration associated with viral infection. A pilot study was designed to measure neurological sequalae through behavioral alterations following EEEV infection, where 10-week-old mice underwent cognitive testing before and after infection, consisting of a modified SHIRPA test, elevated plus maze, and y-maze. All mice with detectible virus in the brain, determined by IVIS imaging, succumbed to infection. Cognitive testing showed uninfected mice and infected mice performed similarly post-infection, suggesting that anxiety and working memory were not affected post-EEEV infection in surviving mice. Because all mice with virus replication in the brain succumbed to infection, attenuated strains of EEEV were developed to enable animal survival following EEEV infection. Ongoing studies will determine the potential lasting cognitive impairments in mice that have recovered from EEEV infection through a battery of behavioral testing followed by pathological analysis of formalin fixed brains in surviving mice. Developing a model of this nature will enable characterization of the host response to EEEV infection and understanding the consequential neuropathology associated with infection.

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P49
Female Sex Hormones Enhance HSV Replication and Disease Severity

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Females are disproportionately affected by herpes simplex virus (HSV) compared to males, with approximately twice as many women infected as men. While this could be due to the ease of transmission from male to female, sex-based differences have not been adequately investigated. During primary genital infection, HSV establishes life-long latency in sensory and autonomic neurons that innervate the genitourinary system. The latent virus can then become reactivated by a variety of stimuli, including menstruation and hormone imbalances, transporting out of the neurons to cause a recurrence of painful lesions or viral shedding. With female animals having been used almost exclusively to study HSV genital infection, any sex-based dichotomy of HSV pathogenesis remains undetermined. Using both males (n= 32) and females (n= 34), we show that guinea pigs accurately correlate HSV pathogenesis differences between sexes as observed in human patients. While females developed more severe acute infection compared to males, males experienced a greater number of cumulative recurrences over time. To better understand effects of individual sex hormones on HSV replication at the neuronal level, we assessed viral DNA during productive infection with the addition of estrogen (17-β-estradiol), progesterone, or testosterone in primary adult neuronal cultures. Estrogen increased HSV-1 replication in autonomic neurons, but not in sensory neurons. In contrast, progesterone increased replication in both types of neurons. Our results demonstrate that female sex hormones have a direct effect at the cellular level to enhance HSV replication within neurons, which likely contributes to more severe primary herpetic disease in females.

Support: NIH NIAID

P50
Canadian hydroelectricity imports to the U.S.; Modeling of hourly carbon emissions reduction in New England

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United States' hydroelectricity imports from Canada have increased by > 1 TWh per year between 2007 and 2021. This occurs as policymakers in the U.S. try to ramp up the deployment of new carbon free electricity generation and transmission infrastructure. Furthermore, recent modeling in the northeast U.S. demonstrates that Canadian hydroelectricity will play a significant role in New England's least-cost decarbonization scenario. Additionally, decarbonization targets are well-defined in all states within the New England region, making it a priority. Consequently, it is anticipated that more hydroelectricity will flow from Canada into New England, resulting in the expansion of transborder electricity interconnections. To characterize the costs and benefits of such projects as compared to alternatives, a high-resolution simulation (i.e., hourly) of the electric grid is needed. In this study, we utilize the U.S. Environmental Protection Agency's dataset on hourly electricity generation and carbon emissions. Using pre-established decarbonization scenarios, we can calculate the precise reduction in greenhouse gas and air pollutant emissions for each scenario. Our preliminary results demonstrate that the scenario projection for 2026–2027 by New England ISO, which involves a combination of Canadian hydroelectric imports (2100 MW summer, 826 MW winter), new wind (308 MW summer and 682 MW), and solar (92 MW summer, 28 MW winter) generation commitments, can effectively offset carbon emissions in New England. These results further support the current decarbonization policy, which relies on a diversified mix of carbon free electricity sources.

Support: U.S. Environmental Protection Agency

P51
Single Dose Pharmacokinetics of Pimobendan and O-Desmethyl-Pimobendan (ODMP) in Healthy Adult Horses

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Background: Few drugs are available to treat congestive heart failure and other cardiac diseases in horses. Pimobendan is an inodilator approved as Vetmedin® for treatment of canine cardiac disease. Previous research shows that pimobendan increases heart rate and contractility following intravenous administration in horses. Oral pimobendan has been used clinically in horses with cardiac disease as Vetmedin tablets, or in compounded formulations, despite the fact that the pharmacokinetics of oral pimobendan have not been investigated in horses.

Hypothesis/Objectives: The hypothesis of this study was that Vetmedin® (V) would be absorbed following oral administration to healthy adult horses and reach concentrations known to be therapeutic in other species. Additional objectives were to determine the bioequivalence of compounded pimobendan capsules (C) and suspension (S) and the effects of sample site on plasma drug concentrations.

Animals: Six privately-owned healthy adult horses (5 mares, 1 gelding).

Methods: All horses received a single 0.5mg/kg dose of pimobendan via oral syringe after being fasted for 8-12 hours. The initial two horses received C, S, or V using a crossover design with a minimum 1-week washout period. Samples were collected simultaneously from lateral thoracic and jugular catheters before and after drug administration at predetermined times. Differences between formulation and sample site were analyzed by one-way ANOVA. Four additional horses received (V) only with jugular samples collected at the same predetermined times. Analysis was done by ultraperformance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) and noncompartmental pharmacokinetics for (V).

Results: No significant differences were noted between formulations ($P = 0.98$) or sample site ($P = 0.62$). Concentrations in compounded formulations were 88%(S) and 90%(C) of label. For V, mean (±SD) maximum plasma concentration (Cmax) was 4.96 ± 2.13ng/mL at 2.17 ± 0.98hr, and area under the curve ($AUC_{0-\infty}$) was 22.1 ± 8.8hr*ng/mL. Concentration of the active metabolite (O-desmethyl-pimobendan) was below the limit of detection (0.07ng/mL) for all samples.

Conclusions and clinical importance: At 0.5mg/kg PO, pimobendan plasma concentrations were considerably lower than reported in dogs. There was no evidence of oral transmucosal absorption. Pimobendan is poorly absorbed in horses, regardless of formulation, and appears unlikely to have clinical effects.

Support: VT Foundation

P52
Dissecting the Role of NF-KB Inducing Kinase as a Possible Therapeutic Target for Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by an overactive immune system and loss of self-tolerance. While there is not currently a cure for SLE, current therapies center around immunosuppressants, anti-malarial drugs, and biologics like Belimumab, a B cell activating factor (BAFF) receptor monoclonal antibody. Along with BAFF, ligands of the Tumor Necrosis Factor Superfamily (TNFSF), including LT-beta, CD40L, and TWEAK activate the non-canonical NF-KB pathway. NF-KB Inducing Kinase (NIK) is a master regulator of the non-canonical NF-KB pathway implicated in promoting pro-inflammatory immune responses and overall immune regulation. We hypothesize that cell specific targeted NIK deletion will alleviate lupus symptoms by reducing expression of pro-inflammatory mediators. J774A mouse macrophages treated with the selective NIK inhibitor, B022, for 5 hours prior to stimulation with the non-canonical NF-KB activator, anti-CD40, for 16 hours. After stimulation, the cells were harvested, and protein was collected for western blot. Cell media was also collected to evaluate the effects of NIK inhibition on cytokine secretion. Western blot quantification using ImageJ demonstrated the critical role NIK plays in the non-canonical NF-KB pathway. Western blot analysis showed that inhibition of NIK lead to decreased p100 processing/p52 maturation, IKK-alpha activation, and a significant reduction in phosphorylated NIK. Additionally, we observed a dose dependent decrease in iNOS production. Secretion of cytokines BAFF and CXCL12 measured by ELISA also exhibited reduced levels when compared to control treatment. In summary, our findings suggest that NIK inhibition plays a key role in decreasing a hyperactive immune profile as seen in SLE. Future studies will focus on expanding in vitro studies to other cell lines which play critical roles in lupus pathogenesis like B cells, dendritic cells, and kidney mesangial cells.

Support: NIH

P53
Pimvanserine, A Promising Adjuvant to Fluconazole Monotherapy Against Cryptococcosis

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Cryptococcal disease poses a significant threat to immunocompromised individuals. Cryptococcus neoformans (C. neoformans) is a key culprit in this illness, primarily affecting the lungs and the central nervous system, causing life-threatening meningitis. Nearly 150,000 new cases of cryptococcal meningitis (CM) are reported every year around the world, emphasizing its substantial impact. The current standard treatment approach includes the administration of amphotericin B alongside other antifungals, mainly flucytosine, to expedite fungal clearance and mitigate potential toxicity. Although fluconazole is recommended as an alternative, its fungistatic nature often results in treatment failure and relapse.

In the present work, we identified pimvanserine (PVT), an atypical antipsychotic, as an adjuvant to enhance fluconazole monotherapy. Here, we found that PVT synergistically interacts in conjugation with fluconazole against 85% of the investigated strains of C. neoformans/gattii complex as evaluated by checkerboard assay. Moreover, PVT, both alone and in combination with fluconazole, significantly impacts the metabolic activity of mature Cryptococcus biofilm. Our investigation also demonstrated that the PVT/fluconazole combination effectively reduces the fungal burden in Caenorhabditis elegans infected with C. neoformans. Delving into the mechanism of PVT's synergy with fluconazole, our study unveiled that PVT disrupts the mitochondrial membrane potential and induces the accumulation of reactive oxygen species (ROS) dose-dependent. Additionally, PVT influences the integrity of the cell membrane, contributing to the loss of cell viability. This work highlights the significance of PVT, with its ability to cross the blood-brain barrier, as a potential adjuvant to improve fluconazole monotherapy. The synergy exhibited by PVT and fluconazole offers a potential avenue to address the challenges posed by Cryptococcus neoformans/gattii complex infections.

Support: NIH

P54
Exploiting capsid-importin interactions to develop novel inhibitors against Venezuelan equine encephalitis virus

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Venezuelan equine encephalitis virus (VEEV) is a mosquito-borne, positive sense, single-stranded RNA virus that belongs to the genus Alphavirus. As a zoonotic pathogen, VEEV can infect both equines, with an associated neurological complications in ~14% of human cases. Due to its low infectious dose, ease of aerosolization and manipulation, this virus is classified as a select agent by both the CDC and USDA. However, there are currently no FDA-approved therapeutics or licensed vaccines against VEEV infection in humans. The VEEV capsid protein is an essential virulence factor of VEEV. The capsid protein can simultaneously bind to the host's nuclear import receptors, importin α/β1, and the host's export receptor, CRM1 to form a tetrameric complex. This complex accumulates at the nuclear pore channel, halting nucleocytoplasmic trafficking and resulting in downregulation of cellular transcription and antiviral response. Moreover, VEEV TC83 Cm, with a mutated non-functional nuclear localization sequence within the capsid, fails to downregulate cellular transcription and antiviral response. This suggests that the nuclear import of VEEV capsid is pertinent for pathogenesis and could be exploited as an attractive target for therapeutic development. We hypothesized that chemical inhibitors capable of disrupting the interaction of capsid with importin α/β1 should increase cellular antiviral response, resulting in reduced viral titers and rescue of cells from VEEV-induced cell death. Two small molecule inhibitors, I2 and 1564, were designed to disrupt the interaction between capsid and importin α. These inhibitors were well tolerated by HMC3 microglia cells with CC50 of >250 μM and >500 μM for I2 and 1564, respectively. Both compounds impacted VEEV TC83 titer with a >1 log10 decrease at 9 hpi. Moreover, I2 displayed an EC50 of 2.96 μM and 1564 an EC50 of 5.38 μM. The antiviral activity of these compounds was MOI-dependent with ~50% viral reduction noted at an MOI of 1 and a >90% reduction at MOIs of 0.1 and 0.01. Interestingly, both compounds also rescued infected cells from VEEV-induced cell death. Next, the contribution of the innate immune response to the antiviral effect and rescue of inhibitor-treated cells from cell death will be delineated by monitoring the localization of antiviral transcription factors as well as the expression of antiviral cytokines and interferon-stimulated genes.

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P55

Potential for a New Supplemental Diagnostic Assay to Identify Horses with Equine Protozoal Myeloencephalitis (EPM)

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Advances in antibody testing, most notably the use of serum: cerebrospinal fluid (CSF) titer ratios to detect intrathecal antibody production, have improved our ability to diagnose horses with EPM. However, multiple factors can influence the accuracy of antibody testing, and some cases are still misdiagnosed, potentially leading to devastating disease. If a supplemental diagnostic test were available, utilizing serum, cerebrospinal fluid (CSF), or both, diagnostic certainty and hence treatment and outcomes would be improved. Our previous study, while small, identified that horses with EPM (n=7) had significantly greater soluble CD14 (sCD14) in their CSF compared to control horses (n=6) (Hay et al. 2019). This study is focused on evaluating sCD14 in a larger group of horses. Horses with both EPM and cervical vertebral instability malformation (CVSM) were analyzed compared to controls and for differences between each group. Serum and CSF samples were obtained from a biobank of well-characterized cases, which met our case definitions. The horses were classified into 4 groups: control (n=21), CVSM (n=44), EPM (n=46) and EPM+CVSM (n=3). Samples were analyzed by the Animal Health Diagnostic Laboratory at Cornell University for the measurement of sCD14 concentrations using a previously validated fluorescent bead-based sCD14 assay (Wagner et al. 2013). Figure 1 illustrates our results. There were no significant differences between the serum sCD14 values between the groups (Figure 1A). When directly compared, a significant difference and <p=0.0001 between the CSF in the EPM and CVSM group (Figure 1B). There was also a significant difference between the sCD14 values in the CSF of control and EPM horses (p=0.0248). These data support the ability to use sCD14 as a supplemental diagnostic biomarker to differentiate between CVSM, horses with EPM, and control horses. Use of this supplemental assay along with gold standard diagnostic tools may allow for quicker and improved diagnostics for horses suffering from these diseases and allow for quicker treatments for their ailments. Continuing research on this assay is required to confirm the assay's efficacy in diagnosing neurologic disease.

Support: IRC, VHIB

P56
NIK influences the course of eosinophilopoiesis dependent on environmental factors

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Hypereosinophilic (HES) syndrome is an umbrella term encompassing several disease subsets, including the myeloid variant, lymphoid variant, familial variant, and the idiopathic variant. Despite the underlying cause, each disease subset ultimately results in >1,500 eosinophils/uL circulating in the blood documented over a six month period with resultant end-organ infiltration and increased morbidity and mortality in affected patients. The disease not only affects humans but has also been implicated in a variety of veterinary species. Despite advances in both medical fields, an overwhelming proportion of patients are still diagnosed with the idiopathic form of the disease. Studies performed in a murine model in which the gene MAP3K14 encoding NF -kB inducing kinase (NIK) is knocked out, a HES-like syndrome develops. Despite previous work assessing the response of eosinophils to Th2 lymphocytes and radioresistant tissues, the mechanisms underlying eosinophil development in conjunction with the bone marrow microenvironment have not been fully elucidated. In the present work, we determined that Nik-/- mice exhibit altered extramedullary hematopoiesis (EMH) and eosinophilopoiesis in the spleen and bone marrow when compared to WT counterparts. We also determined Nik-/- mice contain higher percentages and counts of various eosinophil subsets, eosinophil precursors, and plastic neutrophils that differ between the bone marrow and splenic compartments when compared to WT counterparts. We also determined that the in vivo apoptotic rate of Ly6G+ mature eosinophils is higher in Nik-/- mice despite their abundance. In vitro, we also determined that Nik-/- eosinophils mature faster, are more metabolically active, proliferate slower and have a lower apoptotic rate than WT counterparts at the initiation of eosinophilopoiesis. On days 10, 13, and 15 of this process, we also found that NIK is dispensable for stromal control of eosinophilopoiesis, but Nik-/- environments contained lower TNFR1 levels on day 13 of culture. Additionally, when the microenvironment was disrupted or cytokines such as IL-33 were added to culture, we discovered that Nik-/- eosinophils exhibited altered metabolism, proliferation, and maturation as well as altered BCL-XL levels, suggesting hyperresponsiveness to exogenous factors. Overall, these results provide further insight into the potential mechanisms underlying eosinophilopoiesis in the Nik-/- murine model.

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P57
Phenotypic Characterization of Exhausted Monocytes in Lupus-Prone Mice

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Prolonged exposure to inflammatory signals drives immune cells to a dysregulated ("exhausted") state. Immune exhaustion (IE) is characterized by differential expression of canonical cell-surface proteins, ultimately leading to an alteration in the effector functions of the cell. Historically, IE has been defined in T cells, but recent work has shown that it can also occur in innate immune cells (e.g. monocytes). Notably, while IE is known to occur in T cells in autoimmune conditions (e.g. systemic lupus erythematosus (SLE)), the role of monocyte exhaustion (ME) in this context is still poorly understood. Our objective was to characterize the ME phenotype in a well-characterized murine model of SLE (MRL/lpr). We hypothesized that monocytes from MRL/lpr mice would display a disease-state-dependent exhaustion phenotype. Using flow cytometry, we observed that monocytes from MRL/lpr mice in the earliest part of the active disease stage displayed a downregulation of canonical exhaustion markers compared with pre-disease controls. When monocytes from MRL/lpr mice were cultured in the presence of an inflammatory stimulus using our lab's established ex vivo technique, cells taken from mice in the active disease stage showed a greater upregulation of IE markers than either pre-disease stage or mouse strain controls. Interestingly, the expression of a key macrophage differentiation marker (F4/80) decreased upon exposure to an ex vivo inflammatory stimulus – but only on pre-disease monocytes from MRL/lpr mice relative to active disease. This suggests that disease progression alone can lead to the de-differentiation of exhausted monocytes without an additional inflammatory challenge, and moreover that monocytes from pre-disease MRL/lpr mice are still sensitive to de-differentiation signals ex vivo. Collectively, these data support our hypothesis. Additional experiments are planned to fully characterize this newly described ME phenotype in MRL/lpr mice.

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P58
Regulation of developmental cell death in hypothalamic corticotrophin-releasing hormone neurons through DSCAML1 and cortisol signaling

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The neuroendocrine stress axis, known as the hypothalamic-pituitary-adrenal (HPA) axis, is mediated by the hormones CRH, ACTH, and cortisol. CRH is produced in the neurosecretory area of the hypothalamus and regulates the release of ACTH, which then regulates the release of cortisol. The number of CRH-expressing neuron is precisely regulated during development, but it remains unknown what molecular mechanisms determine cell number and how cell number contributes to HPA axis function. In previous work, we found that Down syndrome cell adhesion molecule like-1 (DSCAML1), a cell adhesion molecule with links to neurodevelopmental disorders, promotes CRH neuron cell death and reduces overall CRH neuron number. In addition, DSCAML1 deficiency also results in abnormal function of the stress axis, notably the overproduction of cortisol at baseline. As cortisol has been shown to affect cell death during development, we hypothesized that DSCAML1 may affect CRH neuron cell death via the regulation of baseline cortisol. To test this hypothesis, we sought to modulate cortisol independently of dscaml1 and determine the effects on developmental CRH neuron cell death. We will use zebrafish as the model organism, as they boast a homologous hypothalamic-pituitary-interrenal (HPI) axis and the ability to easily visualize the brain in early development. To control cortisol levels, CRISPR-mediated genome engineering will be used to delete the Steroidogenic acute regulatory protein (STaR) gene, which is produced in the interrenal glands and is necessary for the synthesis of cortisol. This gene will be targeted using three (3) short-guide RNAs (sgRNAs) to introduce frameshift mutations, inhibiting STaR and consequently cortisol production. Fluorescence in-situ hybridization (FISH) will be used to label CRH neurons and immunostaining with anti-activated caspase 3 will be used to label apoptotic cells. Group-wise comparisons of cell death and CRH neuron cell number will first be made between STaR deficient animals versus sibling controls in the wild-type background. Then, we will examine STaR deficiency in the dscaml1 mutant background to determine whether dscaml1 deficiency affects CRH neuron cell death in the absence of cortisol signaling. These experiments will advance our understanding of stress axis development as well as the role of cortisol in cell death, and provide insights into stress axis-linked human neurodevelopmental and psychiatric disorders.

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P59

Characterization of Upper Respiratory Tract Microbiome of Chickens following Avibacterium paragallinarum Infection

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Avibacterium paragalinarum (AP), is the causative agent of infectious coryza, an upper respiratory tract (URT) disease of chickens that leads to decreased egg production in layers and early marketing of broilers. Pathogens could influence the microbiome of the chicken's respiratory tract leading to more severe diseases in chickens. The impact of AP infection on the microbiome of URT of chicken is unknown and whether different AP strains influence the microbiome differently is also unclear. This study aimed to investigate the impact of AP infection on the URT microbiome and to reveal if specific strains of AP influence the URT microbiome differently. In this study, four different field strains of Avibacterium paragallinarum were used to infect four groups of specific-pathogen-free chickens at four weeks of age, a fifth group was used as control. Pooled choanal swabs were collected from infected chickens and the control group at various time points post-challenge, and DNA extraction was performed and used for microbial 16srRNA gene sequencing. The results showed that the microbiome of the chicken's URT was primarily composed of Firmicutes, followed by Proteobacteria, Actinobacteria, Bacteroidota, and other phyla. Following AP infection, there was a significant decrease in the alpha-diversity of the URT microbiome indicating a decline in microbial diversity. Additionally, beta-diversity analysis revealed significant differences between the infected and non-infected groups, indicating a distinct microbial composition in response to AP infection. In the infected chickens, microbial diversity reached the lowest level on the 4th-day post-infection (DPI) and returned to normal level on the 9th DPI. While there was no significant difference in the alpha diversity between groups infected with different AP strains, the beta diversity analysis indicated distinct microbiome compositions based on the infecting strain. These findings demonstrate that AP infection significantly impacts the URT microbiome composition and diversity in chickens, with variations depending on the specific AP strain. Further research is crucial to understand how respiratory pathogens like AP influence the URT microbiome and its potential role in disease severity and poultry health management.

P60
Characterization of PRRSV nps5 that induces STAT3 degradation

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Porcine respiratory and reproductive syndrome (PRRS) is one of the most economically impacting diseases in swine production. It is caused by the PRRS virus (PRRSV), an enveloped positive-sense RNA virus. The genome of PRRSV is a little over 15 kb in length with more than ten open reading frames (ORFs). Early study showed that PRRSV nsp5 induces degradation of the signal transducer and activator of transcription 3 (STAT3), which plays important role in cell proliferation, inflammation, and immune response. Nsp5 degrades STAT3 via its C-terminal domain. Sequence analysis suggests that nsp5 is has five transmembrane domains, between which are outside-oriented or inside-oriented loops. One of the outside-oriented loops locates in the C-terminal domain and has a potential surface location. In this study, site-directed mutagenesis of this motif in the C-terminal domain was conducted to identify the critical residues for the STAT3 reduction. A series of mutant plasmids encoding nsp5 with substitutions in the highly conserved residues across the PRRSV strains were constructed and used to transfect HEK293 cells to determine their effect on STAT3 protein level. Western blotting results showed that some residues in the loop are essential for inducing STAT3 degradation. Further study is conducted to characterize the unique motif. Data from this project contribute to our understanding of PRRSV-cell interactions and may facilitate development of better vaccine.