

National Veterinary Scholars Symposium

August 2–4, 2018

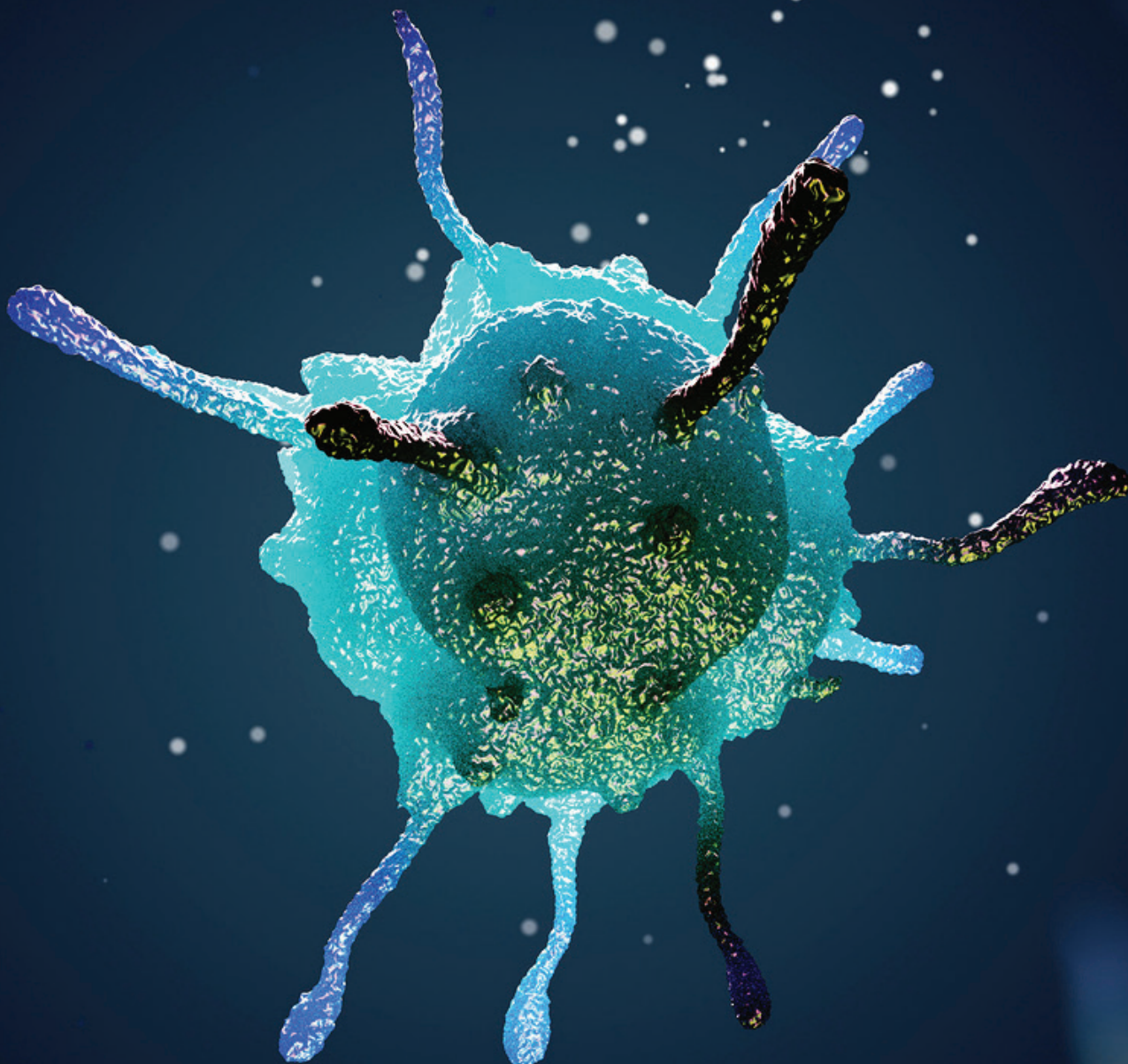


TEXAS A&M UNIVERSITY

Veterinary Medicine
& Biomedical Sciences



VETERINARY SCIENTISTS IN GLOBAL HEALTH RESEARCH



CONTENTS

Welcome	3
Sponsors	4
Acknowledgements	5
Maps	6
Schedule.....	10
Speaker Biographies.....	15
Boehringer Ingelheim (BI) Research Awards	31
AVMA/AVMF Young Investigators Award Finalists	32
AVMA Excellence in Research Awards.....	34
Poster Session Date/Time Assignments	36
Assigned Poster Locations.....	37
Abstracts Listed Alphabetically	38
Symposium Participants by College of Veterinary Medicine	59
Complete Abstracts (listed alphabetically).....	71
Late Abstract Submission	326

Share your symposium experiences on Social Media!
Use the symposium hash tag, #NVSS18,
and tag @tamuvetmed on Facebook, Twitter, and Instagram.

Symposium program edited and designed by CVM Communications
at the Texas A&M College of Veterinary Medicine & Biomedical Sciences.



August 2, 2018

Dear 2018 National Veterinary Scholars Symposium Attendees,

The entire Texas A&M University community welcomes you to Aggieland! We are honored to host this year's National Veterinary Scholars Symposium (NVSS) at the College of Veterinary Medicine & Biomedical Sciences (CVM) on the Texas A&M University campus. This year's attendance is once again projected to be over 600.

The theme of the 2018 NVSS, *Veterinary Scientists in Global Health Research*, will emphasize the role that veterinary medical scientists play in promoting animal, human, and environmental health around the world. We will be taking a global perspective on the contributions of veterinarians in these realms.

All speakers have been invited to discuss their own career paths and development in the context of their science and offering advice to aspiring veterinary medical scientists. In addition, there will be two panels on *Pathways to Research Career Development*, one on Friday and the other on Saturday. Each of these will feature three veterinarians in various career paths and stages, focusing on their paths and answering questions.

Most speakers and award winners have also agreed to meet small groups of students for "Lunch with a Speaker." Hopefully, you have signed up to have an opportunity to meet our distinguished speakers in a casual setting and get to know them better.

We hope that during your visit you will make new friends, meet future colleagues and employers, and be inspired to be part of veterinary medical research and discoveries that will impact the health and well-being of animals and humans.

We hope you have a wonderful experience with us and we look forward to passing the torch to the Cummings School of Veterinary Medicine at Tufts University, who will be hosting the 2019 NVSS.

Sincerely,



Roger Smith III, DVM, PhD
Professor, Veterinary Pathobiology
Texas A&M University



L. Garry Adams, DVM, PhD, DACVP
Senior Professor, Veterinary Pathobiology
Texas A&M University

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**Veterinary Medicine
& Biomedical Sciences**



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**Thank you for your support of the
2018 National Veterinary Scholars Symposium!**

**There are so many to thank for putting together
the 2018 National Veterinary Scholars Symposium.
At the risk of omitting a deserving person, we thank the following:**

From the Texas A&M College of Veterinary Medicine & Biomedical Sciences (CVM):

Overall Organization & Logistics:

Stephanie Hollis

Registration & Student Housing:

Dr. Ashley Seabury & Lauren Pluhar

Finances:

Anila Zaidi, Crystal Trammel, & Belinda Hale

Facilities Management:

Heather Quiram & Michaela Thomas

Technology:

Rick Young, Brice Peterson,
& Mike McConnell

**Our partners at the
Association of American**

Veterinary Medical Colleges (AAVMC):

Ted Mashima, Leslie Wilson,
Dotty Gray, & Andrew Zoeller

NVSS Program Committee:

L. Garry Adams, Chair (Senior Professor,
Veterinary Pathobiology)

Helene Andrews-Polymeris
(Professor, Microbial Pathogenesis
& Immunology, College of Medicine)

Robert Burghardt (Associate Dean
for Research & Graduate Studies)

Noah Cohen (Professor, Veterinary
Large Animal Clinical Sciences)

Sarah Hamer (Richard Schubot
Endowed Chair, & Associate Professor,
Veterinary Integrative Biosciences)

Katrin Hinrichs (Patsy Link Chair in Mare
Reproductive Studies & Regents Professor,
Veterinary Physiology & Pharmacology)

Natalie Johnson (Assistant Professor,
Environmental & Occupational Health,
School of Public Health)

Ann Kier (Senior Professor,
Veterinary Pathobiology)

Jonathan Levine (Helen McWhorter
Endowed Chair, Professor & Head,
Veterinary Small Animal Clinical Sciences)

Peter Nghiem (Assistant Professor,
Veterinary Integrative Biosciences)

Courtney Smith (4th-year veterinary
student & PhD candidate)

James Womack (Distinguished Professor
Emeritus, Veterinary Pathobiology)

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Dr. Megan Palsa

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Tim St. Martin

Tours:

Jennifer Gauntt
& the CVM Ambassadors

Our partners at

Boehringer Ingelheim Animal Health:

Roberto Alva, Cynthia Kahn,
& Melesa Gary

**Our partners at the American
Veterinary Medical Association (AVMA):**

Ed Murphey

Reviewers for the

Young Investigator Award Finalists:

Charles Long, Chair (Texas A&M University)

Angela Arenas (Texas A&M University)

Robert Burghardt (Texas A&M University)

Xinbin Chen (University of California-Davis)

Johanna Elfenbein (NC State University)

Dana Gaddy (Texas A&M University)

L. Garry Adams (Texas A&M University)

Michael Golding (Texas A&M University)

Linda Mansfield (Michigan State University)

Hélène Marquis (Cornell University)

Annie Newell-Fugate (Texas A&M University)

Michael Oglesbee (Ohio State University)

Isaac Pessah (University of California-Davis)

Sanjay Reddy (Texas A&M University)

John Parker (Cornell University)

Susan Sanchez (University of Georgia)

M. Sawkat Anwer (Tufts University)

Jörg Steiner (Texas A&M University)

Larry Suva (Texas A&M University)

L. Tiffany Lyle (Purdue University)

Sue VandeWoude (Colorado State University)

Tracy Vemulapalli (Texas A&M University)

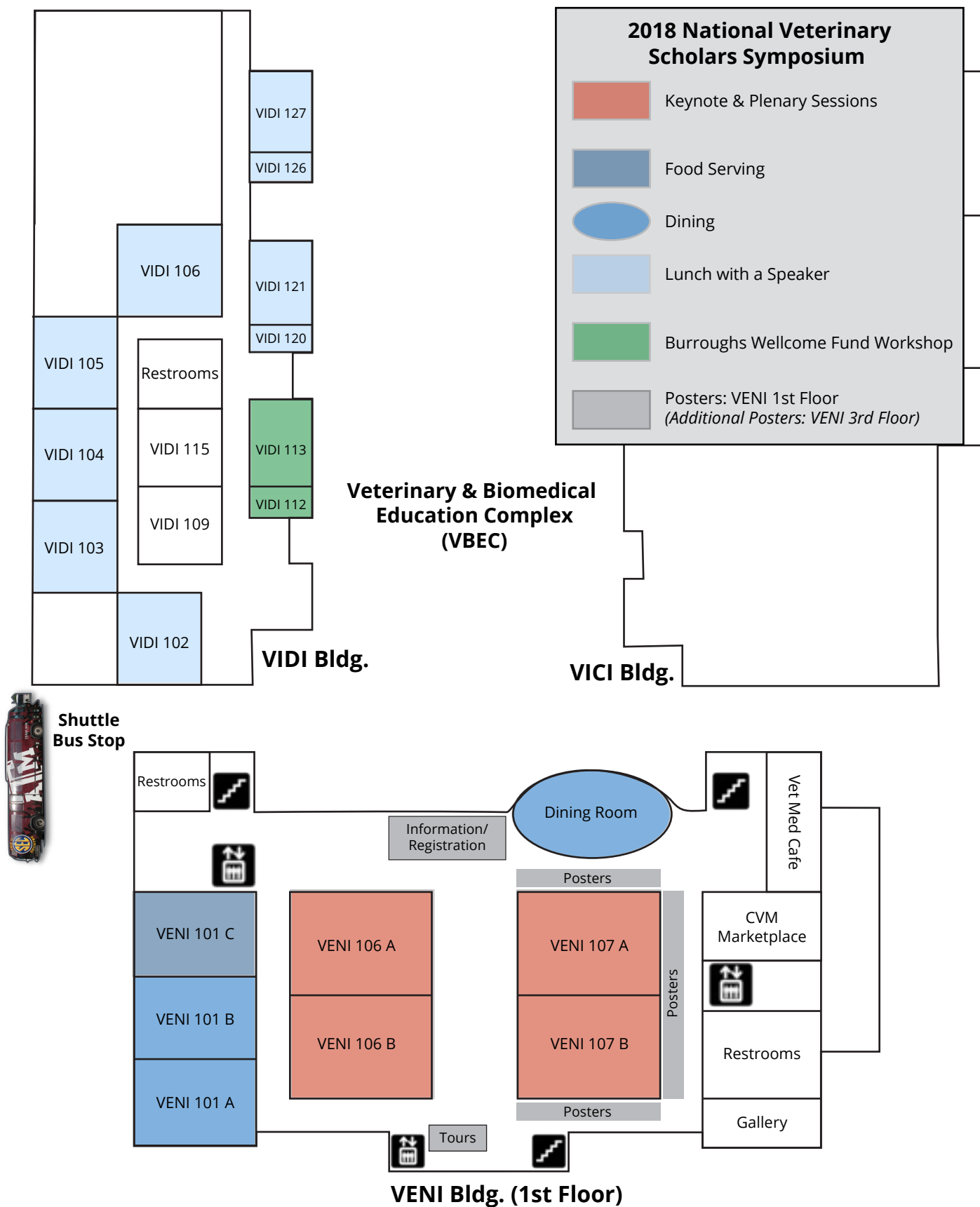
Ashlee Watts (Texas A&M University)

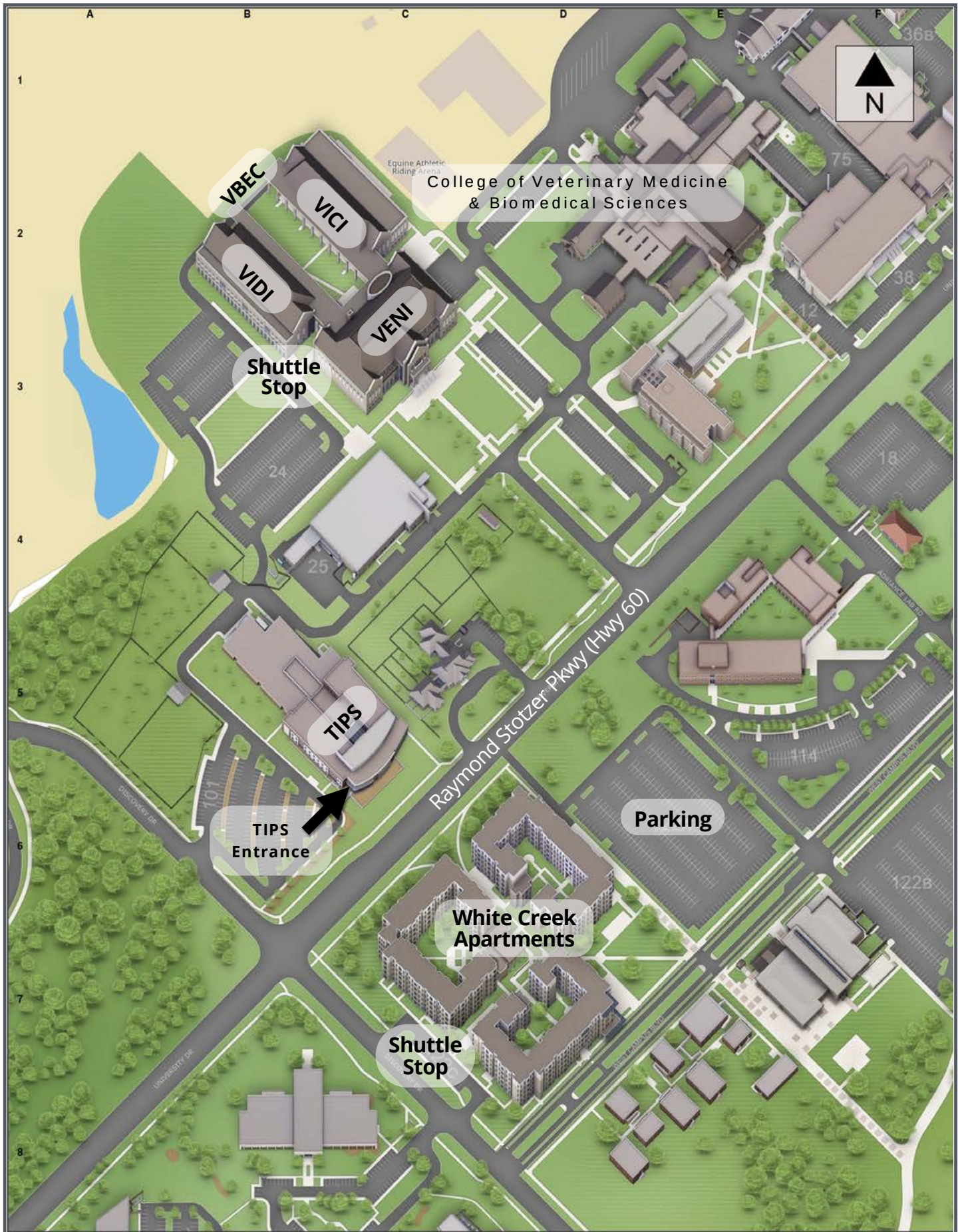
Mark Westhusin (Texas A&M University)

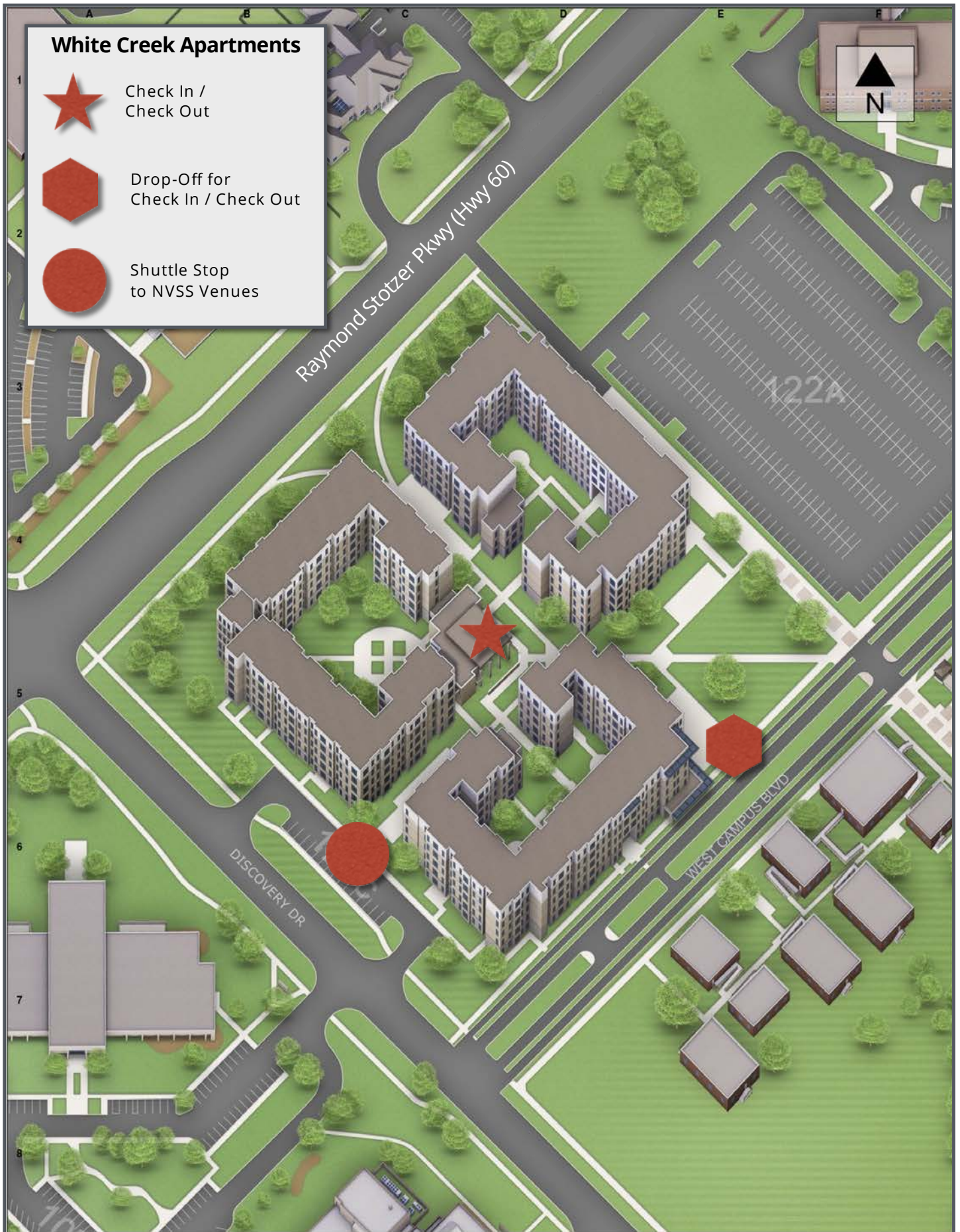
Vilma Yuzbasiyan-Gurkan
(Michigan State University)

ACKNOWLEDGEMENTS

MAPS









2018 National Veterinary Scholars Symposium Schedule

Thursday, August 2

3:00–6:00 pm White Creek Apartments	Arrivals & Registration
4:15–5:30 pm VIDI 106A	T Directors Meeting Harold Watson, PhD Office of Research Infrastructure Programs, Division of Program Coordination, Planning, and Strategic Initiatives, Office of the Director; National Institutes of Health
6:30– 9:00 pm Thomas G. Hildebrand, DVM '56 Equine Complex	Boehringer Ingelheim Welcoming Reception <i>Introduction by Roberto Alva, DVM, MS, PhD</i> Senior Director, Clinical Research & Development, & Executive Director, Boehringer Ingelheim Veterinary Scholars Program <i>Remarks by Paul Fonteyne, MBA, MS</i> Country President, US Animal Health Business Unit, Chairman of Boehringer Ingelheim USA

Friday, August 3

7:00–8:00 am VENI 101	Breakfast Students mount posters for Poster Session A
8:00– 8:30 am VENI 107/106	Welcoming Remarks Eleanor M. Green, DVM, DACVIM, DABVP Carl B. King Dean of Veterinary Medicine, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University Mark A. Barteau, PhD Vice President for Research, Halliburton Chair & Professor, Department of Chemical Engineering, Professor, Department of Chemistry, Texas A&M University Andrew T. Maccabe, DVM, MPH, JD Chief Executive Officer, Association of American Veterinary Medical Colleges Fabian Kausche, med.vet., Dr.med.vet., MS Head of Global Research & Development, Animal Health Business Unit, Boehringer Ingelheim
8:30–9:25 am VENI 107/106	Keynote Address <i>Global Food Security: How will our profession contribute?</i> Corrie Brown, DVM, PhD, DACVP Josiah Meigs Distinguished Teaching Professor, Professor, College of Veterinary Medicine, University of Georgia

9:30–11:00 am VENI Foyers	Poster Session A 9:30–10:15 am: Presenters for Even-Numbered Posters 10:15–11:00 am: Presenters for Odd-Numbered Posters 11:00 am: Students take down posters for Poster Session A
11:15–11:55 am VENI 107/106	Boehringer Ingelheim Research Awards <i>presented by Monica Figueiredo, DVM, PhD (Boehringer Ingelheim)</i> BI Veterinary Research Scholar Award Kathryn C. Taggart DVM Candidate, Class of 2020, University of Florida College of Veterinary Medicine BI Research Award for Graduate Veterinarians Andrew Lewin, BVM&S MRCVS Resident, Comparative Ophthalmology University of Wisconsin-Madison School of Veterinary Medicine
12:00–12:55 pm VENI 101	Lunch Students mount posters for Poster Session B
1:00–2:55 pm VENI 107/106	Frontiers in Veterinary Research: AVMA/AVMF Young Investigators Award Finalists Alix Berglund, DVM, PhD (North Carolina State University) Laura Bryan, DVM, PhD, DACVP (Texas A&M University) Elizabeth Moore, DVM (Cornell University) Amy Stieler Stewart, DVM, DACVIM (North Carolina State University) Marilia Takada, DVM, PhD (Michigan State University) Amanda Ziegler, DVM (North Carolina State University) Moderator: John R. Middleton, DVM, PhD, DACVIM Chair of AVMA Council on Research, Professor, Veterinary Medicine & Surgery, Assistant Director, Agricultural Experiment Station, University of Missouri College of Veterinary Medicine
3:00–4:30 pm VENI Foyers	Poster Session B 3:00–3:45 pm: Presenters for Even-Numbered Posters 3:45–4:30 pm: Presenters for Odd-Numbered Posters 4:30 pm: Students take down posters for Poster Session B

4:45–6:00 pm	Breakout Sessions VENI 106, VENI 107, TIPS Auditorium
Session A: Pathways to Research Career Development I Jonathan Levine, DVM, DACVIM (Texas A&M University) John Parker, BVMS, PhD (Cornell University) Ashlee Watts, DVM, PhD, DACVS (Texas A&M University) <i>Moderator: Kelsey Carroll</i> (4th-Year Student, Texas A&M University)	
Session B: Global Health Research—Focus on Epidemiology Casey Barton Behravesh, MS, DVM, DrPH, DACVPM (CDC) Tom Besser, DVM, PhD (Washington State University) Annette O'Connor, BVSc, MVSc, DVSc, FANZCVS (Iowa State University) <i>Moderator: Michael Manriquez</i> (3rd-Year Student, Texas A&M University)	
Session C: Global Health Research—Focus on Pathogens Helene Andrews-Polymenis, DVM, PhD (Texas A&M University) Voelker Gerds, DVM, PhD (University of Saskatchewan) Andrés Vazquez-Torres, DVM, PhD (University of Colorado) <i>Moderator: Devyn Schultz</i> (3rd-Year Student, Texas A&M University)	
6:30–8:30 pm	Reception The All-American & Legacy Clubs at Kyle Field
Saturday, August 4	
7:30–8:15 am VENI 101	Breakfast Students mount posters for Poster Session A
8:15–8:30 am VENI 107/106	Announcements
8:30–9:25 am VENI 107/106	Plenary Address <i>The Role of Veterinary Medicine in Addressing Global Capacity</i> Wondwossen A. Gebreyes, DVM, PhD DACVPM Hazel C. Youngberg Distinguished Professor of Molecular Epidemiology, Executive Director of the Global One Health Initiative, Veterinary Preventive Medicine, The Ohio State University
9:30–11:00 am VENI Foyers	Poster Session C 9:30–10:15 am: Presenters for Even-Numbered Posters 10:15–11:00 am: Presenters for Odd-Numbered Posters 11:00 am: Students take down posters for Poster Session C

11:15 am–12:10 pm VENI 107/106	Plenary Address <i>Veterinary Medicine as a Driver to Achieve Sustainable Development Goals</i> Guy Palmer, DVM, PhD, DACVP Regents Professor of Pathology & Infectious Diseases, Jan & Jack Creighton Endowed Chair & Senior Director of Global Health, Washington State University
12:15–1:10 pm VENI 101	Lunch Students mount posters for Poster Session D
12:15–1:10 pm VIDI 103	BIVSP Directors Meeting: Roberto Alva (<i>Boehringer Ingelheim</i>)
1:15–2:55 pm VENI 107/106	AVMA Excellence in Research Awards <i>AVMA Career Achievement Award in Canine Research</i> Jörg M Steiner, med.vet., Dr.med.vet., PhD Dr. Mark Morris Chair, Professor of Small Animal Gastroenterology & Nutrition, Director, Gastrointestinal Laboratory, Texas A&M University <i>AVMA Clinical Research Award</i> Brian Gilger, DVM, MS, DACVO, DABT Professor of Ophthalmology, North Carolina State University <i>AVMF/Winn Feline Foundation Research Award</i> Philip Fox, DVM, MS, DACVIM/ECVIM (Cardiology), DACVECC Head of Cardiology, Director of the Caspary Research Institute & Education Outreach, Animal Medical Center, New York <i>AVMA Lifetime Excellence in Research Award</i> Barry T. Rouse, BVSc, MSc, PhD, DSc Lindsay Young Distinguished Professor, University of Tennessee <i>Moderator: John R. Middleton, DVM, PhD, DACVIM</i> Chair of AVMA Council on Research, Professor, Veterinary Medicine & Surgery, Assistant Director, Agricultural Experiment Station, University of Missouri College of Veterinary Medicine
3:00–4:30 pm VENI Foyers	Poster Session D 3:00–3:45 pm: Presenters for Even-Numbered Posters 3:45–4:30 pm: Presenters for Odd-Numbered Posters 4:30 pm: Students take down posters for Poster Session D

4:45–6:00 pm	Breakout Sessions VENI 106, VENI 107, TIPS Auditorium
Session D: Pathways to Research Career Development II Ann Kier, DVM, PhD, DACLAM (Texas A&M University) L. Tiffany Lyle, DVM, PhD, DACVP (Purdue University) Peter Nghiem, DVM, PhD, DACVIM (Texas A&M University) <i>Moderator: Jessica Smith</i> (4th-Year Veterinary Student, Texas A&M University)	
Session E: Global Health Research—Focus on Host Response to Infection Noah Cohen, VMD, MPH, PhD, DACVIM (Texas A&M University) Robert Mealey, DVM, PhD (Washington State University) Jason Osterstock, DVM, PhD (Zoetis) <i>Moderator: Hannah Klein</i> (4th-Year Veterinary Student, Texas A&M University)	
Session F: Global Health Research—Focus on Antimicrobial Resistance Sara Lawhon, DVM, PhD, DACVIM (Texas A&M University) Jeff LeJeune, DVM, PhD, DACVM, DACVPM (The Ohio State University) H. Morgan Scott, DVM, PhD (Texas A&M University) <i>Moderator: Elizabeth Lake</i> (3rd-Year Veterinary Student, Texas A&M University)	
6:30–9:00 pm The Zone at Kyle Field	Closing Banquet <i>Announcement of AVMA/AVMF Young Investigator Award</i>



Opening Reception
Thursday, August 2 • 6:30 pm
Thomas G. Hildebrand, DVM '56 Equine Complex

Paul Fonteyne, MBA, MS

Country President, US Animal Health Business Unit,
Chairman of Boehringer Ingelheim USA

Paul joined Boehringer Ingelheim in 2003, and was responsible for the Human Pharma business in the US from 2003 to 2008. He moved to Germany to lead the global marketing team for the Boehringer Ingelheim global Human Pharma business from 2008 to 2011. During Paul's tenure at Boehringer Ingelheim, the company experienced strong growth in sales, profit, and profitability in the US. Paul is currently on the Board of Directors of Pharmaceutical Research and Manufacturers of America (PhRMA) and is the Chair of the New England Chapter of CEOs Against Cancer (an American Cancer Society Initiative). He previously served as Chairman of the National Pharmaceutical Council (NPC). He is a board member of ResTORbio, and Gelesis, both clinical stage Biotechnology companies headquartered in Boston, MA.



Roberto Alva, DVM, MS, PhD

Senior Director, Clinical Research & Development,
Boehringer Ingelheim
and Executive Director,
Boehringer Ingelheim Veterinary Scholars Program

Dr. Roberto Alva received his DVM degree from the National Autonomous University of Mexico in Mexico City. Following graduation, he worked as a swine practitioner for the Mexican federal government. He moved to the US where he earned an MS in Animal Science and a PhD in Veterinary Pathology from Iowa State University. He conducted postdoctoral research at the University of Bern in Switzerland. He previously worked for Merck on the development of antiparasitic drugs. At Boehringer Ingelheim, he currently leads a group of scientists establishing the safety and efficacy of new pharmaceutical drugs and vaccines. He has been listed as an author on over 60 scientific manuscripts. He also leads the Boehringer Ingelheim Veterinary Scholars Program.



Welcoming Remarks

Friday, August 3 • 8:00 am • VENI 107/106

Fabian Kausche, med.vet., Dr.med.vet., MS

Head of Global Research and Development,
Animal Health Business Unit
Boehringer Ingelheim

In his position as global head of research & development at heritage Merial, Fabian led the company's global research efforts since 2014. He brings many years of experience in the animal and human health industries in both biological and pharmaceutical research and development, as well as companion animal sales. Fabian's expertise covers a unique combination of both science and business, with the leadership and organizational skills to drive growth, spark innovation, and accelerate product approvals.



SPEAKER BIOGRAPHIES

Welcoming Remarks

Friday, August 3 • 8:00 am • VENI 107/106



Eleanor M. Green, DVM, DACVIM, DABVP

Carl B. King Dean of Veterinary Medicine,
College of Veterinary Medicine & Biomedical Sciences,
Texas A&M University

Dr. Eleanor M. Green holds the Carl B. King deanship of the Texas A&M University College of Veterinary Medicine & Biomedical Sciences (CVM) and is a Diplomate of the American College of Veterinary Internal Medicine (ACVIM) and the American Board of Veterinary Practitioners (ABVP).

She received a Bachelor of Science (BS) in Animal Science from the University of Florida and a Doctor of Veterinary Medicine (DVM) from Auburn University. She established a veterinary practice in Mississippi as partner/owner. She became a founding faculty member of the College of Veterinary Medicine at Mississippi State University.

Her academic appointments have included: equine faculty member at University of Missouri; head of the Department of Large Animal Clinical Sciences and director of the large animal hospital at the University of Tennessee; chair of the Department of Large Animal Clinical Sciences and the Chief of Staff of the large animal hospital at the University of Florida.

Dr. Green has found many avenues for service, both in her profession and in her community. She has served as president of four national organizations, the American Association of Equine Practitioners, the American Board of Veterinary Practitioners, the American Association of Veterinary Clinicians, and the Association of American Veterinary Medical Colleges. She is the first woman president of the former three national organizations. She has contributed actively to numerous veterinary and animal industry organizations, including the Boards of Directors of the American Association of Equine Practitioners, American Board of Veterinary Practitioners, American Association of Veterinary Clinicians, the Association of American Veterinary Medical Colleges, North American Veterinary Medical Education Consortium, American Quarter Horse Association, Florida Quarter Horse Association, the National Cowgirl Museum and Hall of Fame, and the NAVC Veterinary Innovation Council. She has been a member of the AQHA Animal Welfare Commission since its inception, currently serving as chair. She just completed a term as chair of the AQHA Public Policy Committee. Presently, she is chair of the AVMA-AAVMC Veterinary Futures Commission. She has been active in her community as a member of Rotary International, having served as President of the Rotary Club of Gainesville. She has volunteered time to various student and youth groups, such as 4-H and gifted student programs.

Her awards include: 2004 Award of Distinction from the University of Florida College of Agriculture and Life Sciences, 2011 Wilford S. Bailey Distinguished Alumni Award from Auburn University, 2012 Women's Progress Award for Administration, induction into the National Cowgirl Hall of Fame in 2013, and 2015 Distinguished Achievement Award for Administration at Texas A&M University.

Dr. Green has a special interest in the future of veterinary medicine and the innovations that will help ensure a thriving profession.

Welcoming Remarks

Friday, August 3 • 8:00 am • VENI 107/106

Mark A. Barteau, PhD

Vice President for Research,
Halliburton Chair & Professor, Department of Chemical Engineering,
Professor, Department of Chemistry,
Texas A&M University



Dr. Mark A. Barteau serves as the Vice President for Research of Texas A&M University. He holds the Halliburton Chair in Engineering, with appointments in the Department of Chemical Engineering and the Department of Chemistry.

He received his BS degree in Chemical Engineering from Washington University in St. Louis, and his MS and PhD from Stanford. He was an NSF Post-doctoral Fellow at the Technische Universität München. He previously served as the Director of the University of Michigan Energy Institute and prior to that as the Senior Vice Provost for Research and Strategic Initiatives at the University of Delaware.

Dr. Barteau brings extensive experience as a researcher, inventor, academic leader, and consultant for both US and international organizations. His research, presented in more than 240 publications and a similar number of invited lectures, focuses on chemical reactions at solid surfaces, and their applications in heterogeneous catalysis and energy processes.

He is a frequent contributor of perspectives on energy and environment to *The Conversation*, *Fortune*, and NPR, among other media outlets. He was elected to the National Academy of Engineering in 2006 and currently serves on the Board on Chemical Sciences and Technology. Barteau was named in 2008 as one of the “100 Engineers of the Modern Era” by the American Institute of Chemical Engineers.

Dr. Barteau is the recipient of numerous awards from the American Chemical Society and national and international catalysis societies. He is a fellow of both the American Institute of Chemical Engineers and the American Association for the Advancement of Science.

Welcoming Remarks

Friday, August 3 • 8:00 am • VENI 107/106



Andrew T. Maccabe, DVM, MPH, JD

Chief Executive Officer,
Association of American Veterinary Medical Colleges (AAVMC)

Dr. Andrew T. Maccabe is the Chief Executive Officer of the Association of American Veterinary Medical Colleges (AAVMC). He received his Bachelor of Science and DVM degrees from The Ohio State University in 1981 and 1985, respectively. Dr. Maccabe began his professional career in Jefferson, Ohio, where he worked in a mixed animal practice with primary emphasis on dairy herd health.

In 1988, he was commissioned as a Public Health Officer in the US Air Force where he managed the preventive medicine activities of several Air Force installations and directed programs in occupational health, communicable disease control, and health promotion.

Dr. Maccabe completed his Master of Public Health degree at Harvard University in 1995. That same year, he became Chief of the Health Risk Assessment Branch of the US Air Force where he directed the health risk assessment program for environmental restoration activities throughout the Air Force.

He completed his Juris Doctor degree, Magna Cum Laude, at the University of Arizona in 2002. He subsequently became the Associate Executive Director at the AAVMC where he led programs to advance veterinary medical education. In 2007, he was appointed as the CDC's Liaison to the US Food & Drug Administration where he coordinated policies and programs between the two agencies before returning to AAVMC in 2012, as the CEO.

Dr. Maccabe holds memberships in many professional organizations including the American Veterinary Medical Association, the District of Columbia Veterinary Medical Association, and the Pride Veterinary Medical Association. He is a member of the State Bar of Arizona and the Bar of the District of Columbia and is a Licensed Patent Attorney. After 24 years of service in the US Air Force, he retired as a Colonel in 2017.

Keynote Address

Global Food Security: How will our profession contribute?

Friday, August 3 • 8:30 am • VENI 107/106



Corrie Brown, DVM, PhD, DACVP

Josiah Meigs Distinguished Teaching Professor,
College of Veterinary Medicine,
University of Georgia

Dr. Corrie Brown is a Josiah Meigs Distinguished Professor and also a University Distinguished Professor at the University of Georgia, where she teaches in the College of Veterinary Medicine. She holds a DVM from the University of Guelph and a PhD in comparative pathology from the University of California–Davis. Dr. Brown has worked internationally in building animal health infrastructure and diagnostics and conducting workshops on basic field necropsy and diagnostic techniques in multiple countries. She has authored four books on transboundary animal diseases and field diagnostics in resource-poor settings.

Dr. Brown has served on many national and international expert panels about animal health and has received numerous awards for her efforts, including the AVMA International Award, a Fulbright Award, and the Student AVMA National Teaching Award. She is happiest when working with the next generation of the veterinary profession.



Plenary Address

The Role of Veterinary Medicine in Addressing Global Capacity

Saturday, August 4 • 8:45 am • VENI 106/107



Wondwossen A. Gebreyes, DVM, PhD, DACVPM

Hazel C. Youngberg Distinguished Professor of Molecular Epidemiology,
Executive Director of the Global One Health Initiative,
Veterinary Preventive Medicine, The Ohio State University

Dr. Wondwossen A. Gebreyes is the Hazel C. Youngberg Distinguished Professor of molecular epidemiology and the executive director of the Global One Health initiative (GOHi) at The Ohio State University. He completed his DVM at Addis Ababa University and his PhD at North Carolina State University. Dr. Gebreyes is the principal investigator of several research and training programs sponsored by the National Institutes of Health (NIH) Fogarty International Center, the Centers for Disease Control & Prevention (CDC) and other agencies.

As the executive director of the Global One Health initiative (GOHi) at Ohio State, he oversees global training, research, and outreach capacity building activities in targeted areas locally, regionally, and globally. Dr. Gebreyes serves as the president of the Ohio State Global One Health, LLC, a non-governmental entity with a branch regional office in eastern Africa. As a professor, he directs the Infectious Diseases Molecular Epidemiology Laboratory (IDMEL), focused on antimicrobial resistance and food safety. He also co-founded and currently serves as president and chair of the International Congress on Pathogens at the Human Animal Interface (ICOPHAI) (<http://icophai.org>). Dr. Gebreyes is engaged in global capacity building efforts including training on global health opportunities to United States students. He also teaches a molecular epidemiology course offered globally in Brazil, Bulgaria, Ethiopia, Kenya, Mexico, Sudan, Tanzania, the United States and Thailand.

He has been active in national professional services including the USDA, NIH, and American Association for the Advancement of Science (AAAS). He also serves in scientific advisory capacities for global partners including the Canada Research Chairs Program, the Austrian Government Competency Center (COMET), the Poland Scientific Advisory (POMOST), the Royal Saudi Arabian Science & Technology Program, and the National Research Foundation (NRF) of South Africa, among others.

Dr. Gebreyes is a recipient of several awards including the Universitas 21 International Award, the Michael P. Malone International Leadership Award from the APLU, the Battelle Endowment for Technology and Human Affairs (BETHA), the Pfizer Award for Veterinary Research Excellence, the Andrew Heiskell Award (honorable mention) by the Institute of International Education (IIE), and the Emerging International Engagement Award.



Plenary Address

Veterinary Medicine as a Driver to Achieve the Sustainable Development Goals

Saturday, August 4 • 11:15 am • VENI 106/107

Guy Palmer, DVM, PhD, DACVP

Regents Professor of Pathology & Infectious Diseases,
Jan & Jack Creighton Endowed Chair & Senior Director of Global Health,
Washington State University



Dr. Guy Palmer holds the Jan & Jack Creighton Endowed Chair at Washington State University (WSU) where he is a Regents Professor of pathology & infectious diseases. As the founding director of WSU's Paul G. Allen School for Global Animal Health, he leads global efforts at the university as the Senior Director of Global Health. He also serves as the President of Global Animal Health-Tanzania and as Chairman of WSU Global Health-Kenya.

Dr. Palmer currently directs global health programs in east and west Africa and Latin America. He heads the NIH Postdoctoral Training Program in Infectious Diseases & Microbial Immunology at WSU and holds a NIH MERIT Award for research on pathogen emergence. He holds a joint appointment as a professor of life sciences & bioengineering at the Nelson Mandela African Institution of Science & Technology in Tanzania and directs the Bill & Melinda Gates Foundation-supported Integrated PhD Program between WSU and the Mandela Institution. Dr. Palmer also holds adjunct appointments with the Center for Emerging & Re-emerging Infectious Diseases in the University of Washington School of Medicine and in the Center for Bioinformatics & Computational Biology.

He was elected to the National Academy of Medicine in 2006, is a Medical Sciences Fellow of the AAAS, and is a founding member of the Washington State Academy of Sciences, where he served as President in 2012–13. He serves on the State of Washington Advisory Board for the US Global Leadership Coalition and the Executive Roundtable of the Washington Global Health Alliance. He also chairs NIH Advisory Groups for COBRE and INBRE Programs and for the Biosecurity Research Institute.

Dr. Palmer earned a BS (Biology, summa cum laude) and a DVM, both from Kansas State University, and a PhD in infectious diseases from WSU. He is board-certified in anatomic pathology. He has been recognized with the Poppensiek Professorship at Cornell, the IBM Professorship at Colby, the Schalm Lectureship at the University of California–Davis, the Distinguished Scientist Lectureship at NIH, the Science in Medicine Lectureship at the University of Washington, and the Merck Award for Creativity.

He has been honored with WSU's Sahlin Award for Research, Scholarship, and the Arts in 2008, the Eminent Faculty Award in 2013, and the Alumni Achievement Award in 2017. He received the Distinguished Alumnus and Alumni Fellow Awards from Kansas State University in 2009 and 2011, respectively, and holds honorary doctorates from the University of Bern (Dr.Med.Vet, 2011) and Kansas State University (PhD, 2016).



Breakout Session
Pathways to Research Career Development I
Friday, August 3 • 4:45 pm • VENI 106



Jonathan Levine, DVM, DACVIM

Helen McWhorter Chair in Small Animal Medicine,
Professor & Head, Small Animal Clinical Sciences,
Texas A&M University

Dr. Jonathan Levine obtained his Bachelor of Science and DVM degrees at Cornell University in Ithaca, New York. He went on to do a rotating internship in small animal medicine and surgery at Colorado State University and residencies in neurology/neurosurgery at Texas A&M University and the University of Missouri. Dr. Levine's research interests include spinal cord injury, neuro-oncology, and magnetic resonance imaging. He has authored over 95 peer-reviewed manuscripts and presented lectures at national and international meetings. Dr. Levine has received funding from the NIH, the National Science Foundation, the US Department of Defense, the Canine Health Foundation, and many

other groups. He is a diplomate of the American College of Veterinary Internal Medicine (Neurology), a professor of neurology/neurosurgery, holder of the Helen McWhorter Chair in Small Animal Medicine, and head of the Department of Small Animal Clinical Sciences at Texas A&M University.



John Parker, BVMS, PhD

Associate Professor, Microbiology & Immunology,
Cornell University

Dr. John Parker received his veterinary degree from the University of Glasgow in 1983 and his PhD in virology from Cornell University in 1999. He worked for nine years in clinical practice as a veterinarian in the UK and Australia. His graduate studies at Cornell focused on the cellular and viral determinants of host range. As a postdoctoral student at Harvard Medical School, Dr. Parker worked with Max Nibert on mammalian reoviruses in the Department of Microbiology & Genetics. He is currently an associate professor of virology in the Department of Microbiology & Immunology within the College of Veterinary Medicine at Cornell University. His lab at the Baker Institute for Animal Health studies the cell biology of viral infection with a particular interest in how viruses usurp normal cellular

processes for virus replication. The lab works with several viruses, but primarily uses mammalian reoviruses (REOV) and feline caliciviruses (FCV). Current areas of investigation include virus entry into cells (FCV), virus interaction with the cellular translational machinery (REOV), virus-induced programmed cell death or apoptosis (REOV), virus assembly and cellular chaperones (REOV), and virus effects on host translation.

Dr. Parker is the recipient of a Burroughs Wellcome Fund Investigatorship in the Pathogenesis of Infectious Disease and a Pfizer Award for Research Excellence. He served on the council of the American Society for Virology as councilor for Veterinary Virology from 2010 to 2013 and he is currently a member of the ASM Council on Microbial Science (COMS). Dr. Parker is the director of the Cornell Leadership Program for Veterinary Students and the director of two NIH training grants.



Ashlee Watts, DVM, PhD, DACVS

Associate Professor, Large Animal Clinical Sciences,
Texas A&M University

Dr. Ashlee Watts graduated veterinary school from Colorado State University and completed an internship at Pioneer Equine Hospital and a surgical residency and PhD at Cornell University. She began as assistant professor of equine orthopedics at Texas A&M University in 2012. She works on the clinic floor seeing equine patients and has a research laboratory where she investigates regenerative medicine in orthopedic applications.

Breakout Session
Global Health Research: Focus on Epidemiology
Friday, August 3 • 4:45 pm • VENI 107

Casey Barton Behravesh, MS, DVM, DrPH, DACVPM

Captain, U.S. Public Health Service; Director, One Health Office,
National Center for Emerging & Zoonotic Infectious Diseases,
Centers for Disease Control & Prevention (CDC)



Dr. Casey Barton Behravesh is the director of the CDC's One Health Office in the National Center for Emerging & Zoonotic Infectious Diseases. She is also a captain in the US Public Health Service. Dr. Barton Behravesh is a veterinary epidemiologist with extensive experience investigating outbreaks and conducting epidemiologic research related to foodborne, zoonotic, and vector-borne diseases. She serves as the CDC lead for implementing an interdisciplinary and multisectoral, One Health approach connecting human, animal, and environmental health to address emerging zoonotic and infectious diseases in order to best protect public health. Dr. Barton Behravesh has extensive experience bridging the gap between human and animal health officials at the local, state, federal, and global level related to emerging zoonotic and infectious diseases and other shared health threats at the human-animal-environment interface.

Dr. Barton Behravesh has a Master of Science degree in veterinary parasitology. She received her DVM from Texas A&M University and a Doctor of Public Health degree from the University of Texas Health Science Center at Houston School of Public Health, both in 2005. She was an Epidemic Intelligence Service (EIS) Officer from 2006–2008 with the Enteric Diseases Epidemiology Branch at the CDC. She is a diplomate of the American College of Veterinary Preventive Medicine, and has been the recipient of national awards, including as the James H. Steele Award from the CDC for outstanding work on veterinary public health issues.

Thomas Besser, DVM, PhD, DACVM

Professor, Veterinary Microbiology & Pathology, Washington State University



Dr. Thomas Besser is a professor at the Washington State University College of Veterinary Medicine in the Department of Veterinary Microbiology & Pathology. He received his BS at the University of Wisconsin (1973), his DVM at the University of Minnesota (1981), and his PhD at Washington State University (WSU, 1986). He is a diplomate of the American College of Veterinary Microbiologists. He has worked at WSU since 1986, initially as a clinician in the Department of Food Animal Medicine & Surgery, but subsequently as a diagnostician in the Washington Animal Disease Diagnostic Laboratory and as a researcher in the Department of Veterinary Microbiology & Pathology. He has spent two sabbatical years abroad, at the University of Edinburgh (Scotland, 2000–2001) and at Massey University (New Zealand, 2008–2009). His work has received several recognitions and awards, including a US-UK Research Fellowship in Food Safety, the WSU Pfizer Award in Research Excellence, the University of Minnesota CVM Distinguished Research Alumnus Award, and the WSU Sahlin Faculty Excellence Award in Research. Dr. Besser is a member of the Washington State Academy of Sciences and a Fellow in the AAAS.

Dr. Besser's career shows that an academic researcher may follow his interests wherever they lead. His research program over time has evolved from passive immunity of neonatal ruminants, through mechanisms of enteric immunity and immunity to enteric pathogens, included side-trips to bovine Marfan syndrome and *Escherichia albertii* in wild birds, and for quite a while, focused on the study of colonization of cattle by zoonotic enteric pathogens such as *E. coli* O157:H7, *Salmonella*, *Campylobacter*, and multi-drug resistant Enterobacteriaceae. That phase of his career resulted in an increasing appreciation of the discipline of veterinary epidemiology and its ability to test real-world hypotheses linking microbiology to real-world impacts. The most recent chapter in Tom's research journey is infectious disease at the wildlife–livestock interface. Beginning in 2005, advising a student summer project on culture-independent microbiology applied to bighorn sheep pneumonia cases, this work has expanded to become Tom's full-time focus since 2016. He has identified a previously unrecognized bacterium, *Mycoplasma ovipneumoniae*, as a key player in bighorn sheep pneumonia outbreaks across the western US and Canada, which in turn has led to a much improved understanding of the microbiology, pathogenesis, and epidemiology of this population-limiting disease. In productive collaborations with wildlife biologists, ecologists, and animal scientists, Dr. Besser is developing novel approaches to both preventing new bighorn sheep pneumonia outbreaks and to managing the disease in previously affected herds.

Breakout Session

Global Health Research: Focus on Epidemiology (continued)

Friday, August 3 • 4:45 pm • VENI 107



Annette O'Connor, BVSc, MVSc, DVSc, FANZCVS

Professor, Veterinary Diagnostic & Production Animal Medicine
Iowa State University

Dr. Annette O'Connor grew up in rural Australia and completed her veterinary training at the University of Sydney. She did her doctoral work in Canada, before moving to the United States and working at Iowa State University. Dr. O'Connor predominately worked with beef and dairy cattle before moving onto a research-focused career path. She is a veterinary epidemiologist who works in the area of research synthesis related to animal health, animal welfare, food production, public health, food safety, and the uses of animals in biomedical research. This work involves combining research in transparent and comprehensive ways that ensure the maximal value of society's investment in research.

Topics evaluated include bovine respiratory disease in feedlot cattle, infectious bovine keratoconjunctivitis in beef calves, anthelmintic protocols for cattle, brucellosis in sheep, salmonellae in pre-harvest pigs, MRSA in swine, pre-harvest food safety interventions, post-harvest interventions, zoonotic pathogens, and the impact of proximity to confined animal operations on community health.

Dr. O'Connor is also very interested in veterinary education after completion of the DVM, and teaches workshops and graduate level classes in epidemiology, meta-analysis, systematic reviews, guideline development, and basic epidemiology. She received a Bachelor of Veterinary Science (BVSc) from the University of Sydney in 1993, a Masters of Veterinary Science (MVSc) from the University of Queensland in 1997, and a Doctor of Veterinary Science (DVSc) from the University of Guelph in 2001. In 2009, Dr. O'Connor was admitted as a Fellow of Epidemiology to the Australian and New Zealand College of Veterinary Scientists. She is a professor of epidemiology at Iowa State University and an adjunct professor in the College of Public Health at the University of Iowa.



Breakout Session
Global Health Research: Focus on Pathogens
Friday, August 2 • 4:45 pm • TIPS Auditorium

Helene Andrews-Polymenis, DVM, PhD

Professor, Medical Microbiology & Immunology, Texas A&M University

Dr. Helene Andrews-Polymenis received her AB in biology from Brown University, her PhD in molecular biology & microbiology from Tufts University in 1999, and her DVM from Texas A&M University in 2001. She joined the faculty in the Department of Microbial & Molecular Pathogenesis at the Texas A&M College of Medicine in 2005, and was promoted to professor in 2017. The main focus of her lab is the identification of genes required for *Salmonella* pathogenesis during gastrointestinal and systemic infections, in mammals and birds. Dr. Andrews-Polymenis participates in teaching medical student and graduate student courses and has trained a number of doctoral students (including veterinarians), postdoctoral fellows, and undergraduate students in her laboratory. She has successfully competed for over \$9 million in external funding for her studies on *Salmonella* from the National Institutes of Health, the US Department of Agriculture, and the US Defense Advanced Research Projects Agency (DARPA).



Dr. Andrews-Polymenis serves regularly on NIH, USDA, AHA, DTRA, Science Foundation of Ireland, Medical Research Council of the UK, and the Canadian Institute for Health Research grant review panels as an expert in bacterial pathogenesis. She is a mini-reviews editor at *Infection & Immunity* (last eight years) and was recently appointed to a four-year term on the communications committee for the American Society for Microbiology. Dr. Andrews-Polymenis is the author of 46 peer-reviewed journal articles and four book chapters. She has more than 20 active collaborations with national and international scientists.

Volker Gerdts, DVM, PhD

Associate Director of Research, Vaccine & Infectious Disease Organization (VIDO-InterVac), University of Saskatchewan

Dr. Volker Gerdts is the associate director of research at the Vaccine & Infectious Disease Organization-International Vaccine Centre (VIDO-InterVac) at the University of Saskatchewan in Saskatoon, Saskatchewan, Canada. He received a DVM, in 1994, from Hanover Veterinary School and a German PhD equivalent from the Federal Research Institute for Animal Health, Island of Riems and Hanover Veterinary School, Germany, in 1997. He has served in various administrative roles and served on various review panels including ones for NSERC, CIHR, CFI, SHRF, NIH, the Gairdner Foundation, and others. Dr. Gerdts is a member of two editorial boards and an ad-hoc reviewer for various journals.



Dr. Gerdts' research interests are in the area of neonatal vaccines for humans and animals, mucosal immunology, and vaccine delivery and formulation. His current research projects are focused on the development of novel adjuvants, the development of vaccines for PEDv and PRRSV, the use of pigs as a model for humans, immune modulation, and regulation of mucosal immune responses.

Andrés Vázquez-Torres, DVM, PhD

Professor, Immunology & Microbiology, University of Colorado

In 1988, Dr. Andrés Vázquez-Torres received his DVM from the University of Córdoba, Spain, and in 1996, he earned a PhD from the University of Wisconsin-Madison. He is a professor of immunology & microbiology at the University of Colorado School of Medicine, and is director of a T32 pre-doctoral training program. He has served on multiple study sections and editorial boards and has been honored for teaching excellence. He has received awards from the Spanish Ministry of Education, NRSA, the Schweppe Foundation, the Burroughs Wellcome Fund, and the ASM Merck Irving S. Sigal Memorial Award. In 2016, Dr. Vázquez-Torres was elected Fellow to the American Academy of Microbiology. His laboratory studies redox active thiol-based sensors in bacterial resistance to the antimicrobial actions of host NADPH phagocyte oxidase and inducible nitric oxide synthase hemoproteins. This research is uncovering novel bacterial mechanisms of redox sensing, antioxidant and antinitrosative defense, antibiotic tolerance, metabolic adaptation, and virulence. VA, and F, K, R and T NIH grants to him or his trainees fund the Vázquez-Torres' lab.



Breakout Session
Pathways to Research Career Development II
Saturday, August 4 • 4:45 pm • VENI 106



Ann Kier, DVM, PhD, DACLAM

Senior Professor, Veterinary Pathobiology, Texas A&M University

Dr. Ann Kier's undergraduate degree was obtained at The University of Texas at Austin, her DVM from Texas A&M University, and her PhD in comparative medicine & pathology from the University of Missouri–Columbia. She directed the first cat veterinary hospital in Texas (Austin) for two years before returning to academia. She has taught courses to veterinary students, lab animal residents, and graduate students in laboratory animal diseases, primate medicine, and animal models of human diseases. Dr. Kier has held faculty positions at the University of Missouri–Columbia, the University of Cincinnati Medical School's Department of Pathology (where she taught medical students), and Texas A&M University. At Texas A&M, she was the Department Head of Veterinary Pathobiology for 12 years, and then stepped back to the faculty for more research and teaching opportunities.

Dr. Kier received the first awarded SERCA grant in comparative medicine from the NIH, followed by NIH R01 grants throughout her career. The main focus of her lab is in understanding the metabolism of lipids, which include cholesterol, fatty acids, and even endocannabinoids, through the use of mouse models. Because lipids are insoluble, they must have carrier proteins, or “chaperones,” to move about the cell, particularly in the liver, for metabolism or storage. Her lab has gene-ablated several of these proteins in mice to determine the effect of absence of these proteins on transport, energy use, metabolism, and pain sensation. Lack of some of these carrier proteins results in differences in pain responses, appetite, obesity, and other conditions. They have recently developed screening assays to test existing and synthesized drugs through varying these carrier protein levels in their ability to alleviate pain, and influence obesity and heart disease. Dr. Kier's lab has also participated in several NIH SBIR grants, which are collaborations between academia and commercial companies, in testing oral vaccines, such as ones used to fight Hepatitis B, Zika, and measles viruses, where the altered virus is over-expressed in transgenic plants and made into wafers. The wafers are fed to mice to determine their ability to raise a significant immune response. The first clinical trial of Hepatitis B wafers is ongoing, and would then be used in underdeveloped countries for vaccinating children where refrigeration and sanitation is limited.

Dr. Kier has been the director of three NIH training grants: an R25 grant for the training of laboratory animal residents, a T32 grant for the development of research-oriented DVM students in pursuing a PhD, and the T35 summer research program to introduce veterinary students to the world of research. She was honored with a Texas A&M Association of Former Students Distinguished Achievement Award in Graduate Mentoring in 2014.

She has never worked a day in her life—well, hardly ever! An old-timer I knew used to tell his students—“Find something you love to do and you will never have to work a day in your life.”—*philosopher Arthur Szathmari, Princeton Alumni Weekly*



L. Tiffany Lyle, DVM, PhD, DACVP

Assistant Professor, Department of Comparative Pathobiology, Purdue University

Dr. L. Tiffany Lyle is an assistant professor of anatomic pathology in the Department of Comparative Pathobiology at the Purdue University College of Veterinary Medicine. She received her DVM from the University of Georgia in 2008, completed a residency in anatomic pathology at Purdue University in 2011, and became a diplomate of the American College of Veterinary Pathologists in 2012. Dr. Lyle received her PhD from Purdue University in 2016, after completing a research fellowship with the Comparative Biomedical Scientists Training Program, a graduate partnership program between Purdue University and the National Cancer Institute. During the fellowship, she conducted her graduate research at the NCI, in Bethesda, Maryland, with a focus on alterations of the blood-brain barrier in brain metastases of breast cancer.



At Purdue University, Dr. Lyle established the Comparative Blood-Brain Barrier Laboratory (CBBBL). The CBBBL currently uses experimental and molecular pathology to elucidate the phenotype of the blood-brain barrier in experimental models of brain metastases of lung cancer and traumatic brain injury. In addition to conducting her primary research, Dr. Lyle is also the director of the Histology Research Laboratory, a division of the Center of Comparative & Translational Research at Purdue. This core laboratory provides experimental pathology support to investigators from a variety of disciplines. Most recently, Dr. Lyle established an anatomic pathology study abroad program in Tokyo, Japan, for veterinary students and pathology residents at Purdue University.

Peter Nghiem, DVM, PhD

Assistant Professor, Veterinary Integrative Biosciences, Texas A&M University

Dr. Peter Nghiem is an assistant professor in the Department of Veterinary Integrative Biosciences and the Texas A&M Institute for Neuroscience at Texas A&M University. He obtained his DVM from Texas A&M and completed a clinical internship after graduation. He went on to complete his PhD with Dr. Eric Hoffman (and Dr. Joe Kornegay as a co-mentor) at the Children's National Medical Center and The George Washington University in Washington, DC. His dissertation evaluated molecular mechanisms of differential muscle involvement in the Golden Retriever muscular dystrophy dog. Dr. Nghiem completed a postdoctoral fellowship under Dr. Hoffman and became a Senior Scientist at Pfizer. Here, he helped create several murine models with the CRISPR/Cas-9 technique.



Since October 2015, Dr. Nghiem has been a tenure-track assistant professor of neuroscience at Texas A&M University. His lab focuses on the molecular, cellular, and phenotypic characterization of the murine (mdx) and canine models of Duchenne muscular dystrophy (DMD, Golden Retriever muscular dystrophy [GRMD], German Short-Haired Pointer muscular dystrophy [GSHPM], and Cavalier King Charles Spaniel muscular dystrophy). His research has focused on molecular characterization with genome-wide mRNA, microRNA and proteomic profiling with mass spectrometry; confirmation of molecular targets with qRT-PCR, western blot, and immunofluorescence microscopy; characterization of novel DMD mutations with whole genome sequencing; cellular characterization of animal models utilizing biopsy extracted muscle stem cells (myoblasts), including evaluation of the molecular and phenotypic effects of various treatments; and phenotypic characterization of the canine models using internationally established functional outcome measures.

Dr. Nghiem's current research focus is on preclinical drug trials, including gene therapy (dystrophin gene replacement) via adeno-associated viral vector delivery; utilizing gene editing techniques such as CRISPR/Cas9 and TALENs for treatments of genetic disease; characterization of genetic modifiers via whole-genome next generation sequencing (discovery approach); evaluation of muscle metabolism in dystrophin deficiency; pre-clinical testing of novel therapeutics in mdx mice; and phenotypic characterization of mdx mice and rat models of Parkinson's disease and Griscelli Syndrome, with grip strength testing, treadmill exercise, and Rotarod, among others.

Breakout Session
Global Health Research: Focus on Host Response to Infection
Saturday, August 4 • 4:45 pm • VENI 107



Noah Cohen, VMD, MPH, PhD, DACVIM

Professor & Associate Head, Large Animal Clinical Sciences, Texas A&M University

Dr. Noah Cohen earned his bachelor's degree and his doctorate in veterinary medicine from the University of Pennsylvania, and a master's of public health and doctorate in epidemiology from Johns Hopkins University. He completed a residency in large animal internal medicine at Texas A&M University. He joined the faculty of the College of Veterinary Medicine & Biomedical Sciences in 1991, and has been employed by Texas A&M University for 29 years. He is a diplomate of the American College of Veterinary Internal Medicine (Large Animal) and of the American Veterinary Epidemiology Society. Dr. Cohen is known as a leading expert in the epidemiology of equine infectious disease, in particular the intracellular pathogen *Rhodococcus equi*. He has made significant contributions to strategies for prevention and management of *Rhodococcus equi* and has co-developed a vaccine that has been effective in initial challenge studies. He has published over 250 peer-reviewed articles and was among the first to influence knowledge of the epidemiology of many equine diseases. Dr. Cohen has been the principal investigator or significant co-investigator for nearly \$2.5 million of funding from extramural granting agencies for research to impact animal disease. He is passionate about training veterinary students and veterinarians for careers in equine practice and veterinary research.



Robert Mealey, DVM, PhD, DACVIM

Robert B. McEachern Distinguished Professor of Equine Medicine,
Professor & Chair, Veterinary Microbiology & Pathology,
College of Veterinary Medicine, Washington State University

Dr. Robert Mealey earned his DVM at Colorado State University in 1990, and completed his large animal internal medicine residency with an equine emphasis at Texas A&M University in 1995. As a board certified equine internist, he held clinical positions in equine medicine at both Texas A&M and Washington State University before earning his PhD in immunology and infectious diseases at Washington State University in 2001. He is currently the Robert B. McEachern Distinguished Professor of Equine Medicine, professor and chair of the Department of Veterinary Microbiology & Pathology at the Washington State University College of Veterinary Medicine. He also serves as the director of the WSU-CVM Animal Health Research Center, has served as the WSU-CVM associate dean for research, and is a founding director of the WSU-CVM Equine Infectious Disease Research Program. Dr. Mealey is a recognized expert in equine infectious diseases, and with support from the NIH, the USDA, the Morris Animal Foundation, and the Grayson Jockey Club Research Foundation, his research program is directed towards understanding how the horse's immune system controls persistent and vector-borne infections, including those caused by viruses, bacteria, and protozoan parasites. In particular, his work has focused on equine infectious anemia virus, *Theileria equi* (the protozoan parasite that causes equine piroplasmiasis), and more recently, equine hepatitis virus, a virus that infects the liver of horses and is the closest known relative to the human Hepatitis C virus.



Jason Osterstock, DVM, PhD

Executive Director, Global Genetics, Zoetis, Inc.

Dr. Jason Osterstock is the executive director of Global Genetics for Zoetis. In this role, he is responsible for managing all commercial, strategic, and operational elements of the genetics business. During his tenure at Zoetis, Jason has led global technical services, quality & product delivery, and global strategic marketing for the genetics business. Prior to joining Pfizer Animal Health in 2010, he held roles as an assistant professor at Texas AgriLife Research, and as a lecturer in the College of Veterinary Medicine & Biomedical Sciences at Texas A&M University. Jason holds a BS in Agriculture (1997) and DVM (2001) from The Ohio State University, completed an internship in food animal medicine & surgery at Colorado State University, and earned a PhD in Epidemiology (2007) from Texas A&M University. He is currently based in Kalamazoo, Michigan.

Breakout Session
Global Health Research: Focus on Antimicrobial Resistance
Saturday, August 4 • 4:45 pm • TIPS Auditorium

Sara Lawhon, DVM, PhD, DACVM

Associate Professor, Veterinary Pathobiology, Texas A&M University

Dr. Sara Lawhon is a clinical veterinary microbiologist and serves as the director of the Clinical Microbiology Laboratory of the Veterinary Medical Teaching Hospital (VMTH) at Texas A&M University. The primary focus of her research has been the study of interactions between zoonotic pathogens and their host species. She received her DVM from Texas A&M University in 1997. She then moved to North Carolina State University where she completed her residency in infectious diseases in 2001, and her PhD in 2003. Her early research interest was in carbon metabolism in *Salmonella enterica* serotype Typhimurium with Dr. Craig Altier.

Dr. Lawhon returned to Texas A&M University as a postdoctoral researcher with Dr. L. Garry Adams and helped to develop an *in silico* model of host response to *Salmonella* Typhimurium using the bovine ligated ileal loop surgical model of intestinal inflammation. She joined the faculty in 2008, and currently studies antimicrobial resistance and the pathogenesis of *Staphylococcus pseudintermedius* in dogs and people. She considers the collaborations and friendships developed with colleagues all over the world as the best part of her work. She credits the Veterinary Medical Scientist Research Training Program (summer research program) and a project on *Streptococcus iniae* in tilapia with Dr. Paul Frelief for getting her started. She says she would (mostly) do it all again if she could, and is lucky to get to work with a great group of veterinary and graduate students every day.



Jeff LeJeune, DVM, PhD, DACVM, DACVPM

Professor, Veterinary Preventive Medicine,
Program Head, Food Animal Health Research Program,
& Food Safety Extension Veterinarian, The Ohio State University

Dr. Jeffrey LeJeune completed his DVM at the University of Prince Edward Island, Canada, and subsequently worked as a food hygiene veterinarian for Agriculture & Agri-Food Canada, prior to completing his PhD and residency in veterinary microbiology at Washington State University. He is board certified by both the American College of Veterinary Microbiologists and the American College of Veterinary Preventive Medicine. Currently, he is professor of food safety, state extension specialist, and head of the Food Animal Health Research Program, a collaborative unit nested within the College of Veterinary Medicine and the College of Food, Agriculture & Environmental Science at The Ohio State University.

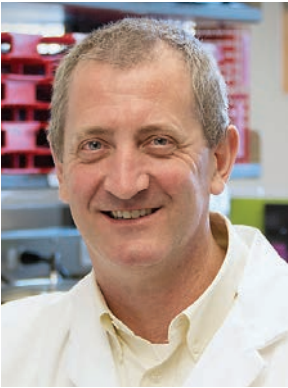
His research has primarily focused on understanding the ecological mechanisms involved with the survival, dissemination, and prevention of foodborne pathogen contamination in reservoir species, and water, wildlife, and edible crops. Special emphasis has been on Shiga toxin-producing *E. coli* and antimicrobial resistant pathogens. Research studies span from the molecular mechanisms of bacterial pathogenesis to the impacts of food safety policy on a global scale. More recently, he has worked extensively with the Food & Agriculture Organization (FAO) of the United Nations on issues related to mitigation of antimicrobial resistance using a One Health approach.



Breakout Session

Global Health Research: Focus on Antimicrobial Resistance (continued)

Saturday, August 4 • 4:45 pm • TIPS Auditorium



H. Morgan Scott, DVM, PhD

Professor, Veterinary Pathobiology, Texas A&M University

Dr. H. Morgan Scott is a graduate veterinarian (University of Saskatchewan, Canada, 1988) holding a PhD in epidemiology (University of Guelph, Canada, 1998) and postdoctoral training in public health (University of Alberta, Canada 1999). In addition to private veterinary practice, he has worked in both government (food safety surveillance) and academic settings. He is currently professor of epidemiology in the Department of Veterinary Pathobiology at Texas A&M University. He was recruited in 2014 as part of the Texas A&M University System Chancellor's Research Initiative and the Texas A&M University President's Initiative on One Health & Infectious Diseases. He relocated from Kansas State University, where he previously held the E.J. Frick Professorship in Veterinary Medicine.

Much of his research emphasis has been on studying factors impacting antimicrobial resistance among commensal and pathogenic enteric bacteria in food animal production systems, with a program spanning the realm from the molecular to the sociological. In particular, he is interested in applying both epidemiological and ecological approaches to quantify the emergence, propagation, dissemination, and persistence of resistant enteric bacterial strains in integrated populations of animals, their food products, and humans. Using this knowledge, he hopes to identify opportunities to prevent and intervene against resistance among enteric pathogens in animal agriculture, preferably by developing readily adoptable and cost-effective management practices suited to modern animal and food production systems. Among his personal interests is an as-yet unfulfilled desire to repeat an around-the-world bicycle odyssey with his wife, preferably before they get too old to remember the first trip.



Boehringer Ingelheim Research Awards
Friday, August 3 • 11:15 am • VENI 106/107

Presenter: Monica Figueiredo, DVM, PhD, DACVIM

Director, Pharmaceutical Lead Sourcing & External Innovation
Boehringer Ingelheim

Dr. Monica Figueiredo earned her veterinary degree at the Universidade Federal Fluminense in Rio de Janeiro, Brazil. Following graduation, she completed a two-year equine internship at the Marion DuPont Scott Equine Medical Center in Virginia, followed by a three-year residency program in large animal medicine at Cornell University in New York. She then moved to the College of Veterinary Medicine at the University of Georgia, where she earned her PhD in the Department of Physiology & Pharmacology. Dr. Figueiredo joined Merial in 2008, as a veterinary scientist in research and development, where she primarily worked in the area of canine vaccine development. For the last five years, she has served as director of external innovation and has been thrilled to work with the latest technology in a variety of fields related to improving animal health. She is based in Athens, Georgia.



Boehringer Ingelheim Research Scholar Award

Role of the pyruvate dehydrogenase kinase 4 (PDK4) gene mutation in mitochondrial metabolism of Doberman Pinschers

Recipient: Kathryn C. Taggart

DVM Candidate, Class of 2020,
University of Florida College of Veterinary Medicine

Kathryn C. Taggart is a third-year veterinary student at the University of Florida (UF) College of Veterinary Medicine from Jacksonville, Florida. She completed her undergraduate degree at UF, where she studied animal science and entrepreneurship. Katy plans to pursue mixed animal medicine and her interests include cardiology, ophthalmology, microbiology, and aquatic animal medicine. In her free time, she enjoys running, playing soccer, and spending time with family and friends.



Boehringer Ingelheim Research Graduate Student Award

Genomic Analysis of Feline Herpesvirus Type 1 Isolates

Recipient: Andrew Lewin, BVM&S, MRCVS

Resident, Comparative Ophthalmology
University of Wisconsin-Madison School of Veterinary Medicine

Dr. Andrew Lewin is a third-year resident in comparative ophthalmology at the University of Wisconsin-Madison. This three-year program encompasses training in medical and surgical veterinary ophthalmology, teaching, and research. His professional interests are ocular infectious disease, glaucoma, and surgery. Previously, he held positions in veterinary private practice, charity/low cost clinics, and animal shelters, as well as completing a 12-month internship in Solihull, England.



Frontiers in Veterinary Research

Friday, August 3 • 1:00 pm • VENI 106/107



TGF- β 2 downregulates MHC I and MHC II surface expression on equine bone marrow-derived mesenchymal stem cells without altering immunomodulatory properties

Alix Berglund, DVM, PhD (North Carolina State University)
Dr. Alix Berglund received her bachelor's and veterinary degrees from Washington State University. Following graduation, she completed her PhD in comparative biomedical sciences at North Carolina State University. Dr. Berglund's research interests include immunology and regenerative medicine with a particular interest in investigating the immunogenicity of allogeneic mesenchymal stem cells. She is currently a postdoctoral researcher at North Carolina State University.



Carriage of exfoliative toxin genes, protein expression, and whole genome sequencing of Staphylococcus pseudintermedius isolates from dogs

Laura Bryan, DVM, PhD, DACVP (Texas A&M University)
Dr. Laura Bryan received her DVM degree from the University of Georgia in 2011, and her PhD from Texas A&M University in 2018, which involved identifying toxin carrying strains of the pathogen *Staphylococcus pseudintermedius*. She completed a residency in anatomic veterinary pathology at Texas A&M University and attained ACVP board certification in 2014. Dr. Bryan is currently a postdoctoral researcher at Texas A&M University investigating non-steroidal drug enteropathy models.



A novel role for the Fanconi Anemia pathway in hepatic bile acid homeostasis and cholesterol metabolism

Elizabeth Moore, DVM (Cornell University)
Dr. Elizabeth Moore graduated with her DVM from Cornell in 2012, and completed the laboratory animal medicine residency with the Cornell Center for Animal Resources & Education in 2015. She began her PhD training in the field of comparative biomedical sciences in the laboratory of Dr. Robert Weiss during her residency, and will be completing her graduate degree at the end of this year. Her PhD work focuses on understanding the mechanisms that maintain genomic integrity. Her projects investigate the role of the Rad9-Hus1-Rad1 DNA repair clamp in double-stranded DNA

break repair, probe the mechanisms behind the unique chemosensitivity of testicular germ cell tumors, and explore the role of the Fanconi Anemia DNA repair pathway in cellular and hepatic metabolism.



Signals arising from the subepithelial niche are critical for intestinal repair following severe ischemic injury

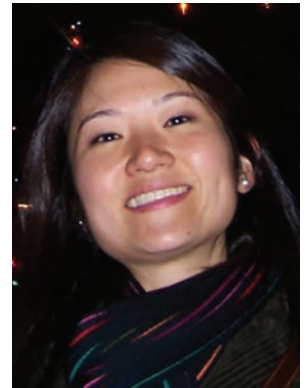
Amy Stieler Stewart, DVM, DACVIM (North Carolina State University)
Dr. Amy Stieler Stewart received her bachelor's and veterinary degrees from the University of Florida. Following graduation, she completed a one-year rotating internship in large animal medicine and surgery at the University of Georgia. She returned to the University of Florida in 2012, completed a three-year residency in large animal medicine and became a diplomate of the American College of Veterinary Internal Medicine in 2015. She then moved to North Carolina State University and is currently working on a PhD

in cellular biology, focusing on the study of intestinal repair following ischemic injury using large animal models. Following completion of her PhD, she hopes to obtain a faculty position at a veterinary teaching hospital continuing her passion for practicing high-quality medicine, educating veterinary students, and conducting research aimed at benefiting her patients and society.

The role of MAPK signaling pathway as a key oncogenic driver in canine histiocytic sarcoma

Marilia Takada, DVM, PhD (Michigan State University)

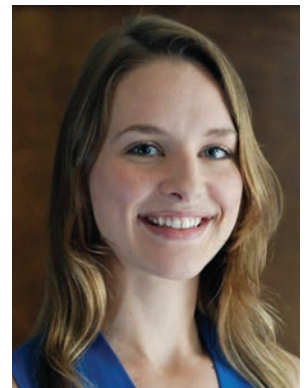
Dr. Marilia Takada holds a DVM from the University of Sao Paulo in Brazil, and she completed a residency in small animal surgery at Anhembi Morumbi University in Brazil in 2008. In 2009, her interest in veterinary oncology led her to pursue her clinical training as an oncology fellow at the University of Tokyo, and later at Michigan State University in 2010. She received both an MS (2012) and a PhD (2018) through the Comparative Medicine and Integrative Biology Program at Michigan State University. Her main research focus has involved the establishment of important model systems of canine histiocytic sarcoma for the identification of promising drug candidates for treatment, and key signaling pathways that are involved in oncogenesis. She will continue her clinical training at the University of Wisconsin–Madison, as a radiation oncology resident. Dr. Takada envisions an academic career where she can contribute to the profession through the advancement of the veterinary oncology field, with the ultimate goal of improving the care for patients.



Neonatal defect in subacute barrier repair is rescued by juvenile mucosal homogenate in a pig model of intestinal ischemia and repair

Amanda Ziegler, DVM (North Carolina State University)

Dr. Amanda Ziegler is a postdoctoral research fellow of the Center for Gastrointestinal Biology & Disease at NC State University in Raleigh, North Carolina. Her research has always centered on epithelial cell biology. Her past work focused on the bovine hoof and the human lung, and she is currently studying postnatal development of epithelial barrier repair in the intestine. She also has experience leading a multi-institutional clinical trial to study postoperative management of equine colic patients. Dr. Ziegler received the National Goldwater Award in 2010, and the Grayson-Jockey Club Research Foundation Elaine Klein Career Development Award in 2016. She has received accolades for her abstract presentations at institutional, national, and international meetings. She is a PhD candidate at NC State University in the comparative biomedical sciences program and received her DVM from the NC State College of Veterinary Medicine in 2015. She initially studied for her BS degree in pre-veterinary medicine at the University of Delaware from 2008–2011.



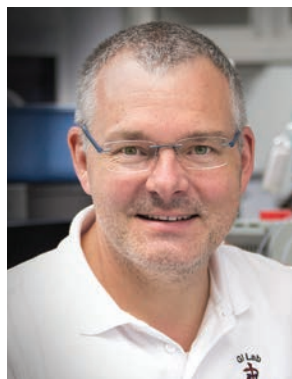
Moderator: John R. Middleton, DVM, PhD, DACVIM

Chair of AVMA Council on Research,
Professor, Veterinary Medicine & Surgery,
Assistant Director, Agricultural Experiment Station,
University of Missouri College of Veterinary Medicine

Dr. John Middleton holds a BS, DVM, and PhD from Washington State University, is a diplomate of the American College of Veterinary Internal Medicine (Large Animal), and an associate member of the European College of Bovine Health Management. He is professor of food animal medicine & surgery and assistant director of the Agricultural Experiment Station at the University of Missouri. He has clinical, teaching, research, administrative, and service responsibilities in the Department of Veterinary Medicine & Surgery. His research is primarily focused on mastitis and milk quality, particularly, staphylococcal mastitis. He is a past president of the National Mastitis Council, and chair of the American Veterinary Medical Association's (AVMA) Council on Research.



AVMA Excellence in Research Awards Saturday, August 4 • 1:15 pm • VENI 106/107



AVMA Career Achievement Award In Canine Research

Jörg M Steiner, med.vet., Dr.med.vet., PhD

Dr. Mark Morris Chair,
Professor of Small Animal Gastroenterology & Nutrition,
Director, Gastrointestinal Laboratory, Texas A&M University

Dr. Jörg Steiner received his veterinary degree from the Ludwig-Maximilians University in Munich, Germany in 1992. He completed an internship in small animal medicine and surgery at the University of Pennsylvania in 1993, and a residency in small animal internal medicine at Purdue University in 1996. He received his Dr.med.vet. degree from the Ludwig-Maximilians University in Munich, Germany in 1995, in recognition of research on feline trypsin and feline trypsin-like immunoreactivity. In 1996, he achieved board certification with the American College of Veterinary Internal Medicine (ACVIM) and the European College of Veterinary Internal Medicine. In 2000, Dr. Steiner received a PhD from Texas A&M University for his work on canine digestive lipases and their use for the diagnosis of gastrointestinal disorders in the dog. In 2012, he was recognized as a Fellow by the American Gastroenterology Association. He currently serves as a professor with the Department of Small Animal Clinical Sciences and the Department of Veterinary Pathobiology at Texas A&M University. In 2016, Dr. Steiner was named as the Dr. Mark Morris Chair in Small Animal Gastroenterology & Nutrition. He also serves as the director of the Gastrointestinal Laboratory at Texas A&M University and is involved in a wide variety of research in small animal and comparative gastroenterology. He has authored or co-authored more than 260 peer-reviewed articles, 90 book chapters, and 410 research abstracts. He has been invited to present his work at many national and international conferences and provides continuing education lectures for veterinarians across the globe. He has also served several professional organizations in various roles and currently serves as the president of Small Animal Internal Medicine for ACVIM.



AVMA Clinical Research Award

Brian Gilger, DVM, MS, DACVO, DABT

Professor of Ophthalmology, North Carolina State University

Dr. Brian Gilger received his veterinary degree from The Ohio State University in Columbus, Ohio. After an ophthalmology residency and masters degree at Auburn University, he returned to The Ohio State University as an assistant professor of ophthalmology in 1992. In October 1995, Dr. Gilger joined the faculty at North Carolina State University as an associate professor of ophthalmology and is now a professor of ophthalmology. He is board certified in Veterinary Ophthalmology and by the American Board of Toxicology. Dr. Gilger has been president of the American College of Veterinary Ophthalmologists, president of the International Equine Ophthalmology Consortium, Chair of the Association for Research in Vision and Ophthalmology (ARVO) Animals in Research Committee, and is a Fellow of ARVO. He was recognized as a Distinguished Alumnus of The Ohio State University College of Veterinary Medicine in 2017, and was awarded the Huffmann Leadership Award by North Carolina State University in 2018. Dr. Gilger's research has focused on innovative ocular drug delivery, immune-mediated ocular diseases, and wound healing. He has much interest in the studying and developing of new treatments for the most common clinical diseases of horse eyes, including equine recurrent uveitis, immune-mediated keratitis, and fungal keratitis. He has authored over 150 peer-reviewed scientific manuscripts, over 30 book chapters, and is the editor of five textbooks, including three editions of *Equine Ophthalmology*.

AVMF/Winn Feline Foundation Research Award

Philip Fox, DVM, MS, DACVIM/ECVIM (Cardiology), DACVECC

Head of Cardiology, Director of the Caspary Research Institute & Education Outreach, Animal Medical Center, New York

Dr. Philip Fox is certified by the American and European Colleges of Veterinary Internal Medicine (Cardiology), the European College of Veterinary Internal Medicine Companion Animal (Cardiology), and the American College of Veterinary Emergency & Critical Care. He is the director of the Caspary Research Institute of the Animal Medical Center, director of educational outreach, and head of cardiology. He received his DVM degree from The Ohio State University, and completed an internship in medicine and surgery, as well as a cardiology residency, at the Animal Medical Center in New York. Dr. Fox has received numerous honors including TNAVC Speaker of the Year, The Ohio State University Distinguished Alumnus award, and an Award of Merit bestowed by the City of New York for work done with search-and-rescue dogs during 9/11. His research interests include congestive heart failure, feline and canine cardiomyopathies, evidence-based medicine, and cardiovascular pathology.

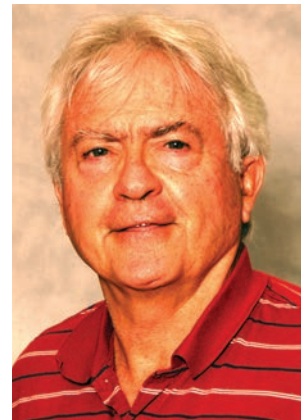


AVMA Lifetime Excellence In Research Award

Barry T. Rouse, BVSc, MSc, PhD, DSc

Lindsay Young Distinguished Professor, University of Tennessee

Dr. Rouse is the Lindsay Young Distinguished Professor in the Department of Biomedical & Diagnostic Sciences at the University of Tennessee's College of Veterinary Medicine. He received his BVSc at the University of Bristol before completing a MSc (Virology) and PhD (Immunology) at the University of Guelph. His postdoctoral study was at the Walter & Eliza Hall Institute of Medical Research in Melbourne, Australia. He also earned a DSc at the University of Bristol. Dr. Rouse is widely recognized for his research in viral immunology and immunopathology, working mainly with herpes simplex virus (HSV) infection in mice both with a view to devising successful vaccines and more particularly to determine how HSV causes tissue damage in critical tissues such as the eye and nervous system. He has received several awards for research accomplishments, most notably the Award for outstanding contributions to vision research (2000) and the Medal of Merit from Warsaw University of Life Sciences (2014). In his distinguished career, he has been extensively involved in reviewing NIH grants, served as the co-organizer of several conferences, and is the member of numerous editorial boards and a reviewer of many journals.



Moderator: John R. Middleton, DVM, PhD, DACVIM

Chair of AVMA Council on Research,
Professor, Veterinary Medicine & Surgery,
Assistant Director, Agricultural Experiment Station,
University of Missouri College of Veterinary Medicine

Dr. John Middleton holds a BS, DVM, and PhD from Washington State University, is a diplomate of the American College of Veterinary Internal Medicine (Large Animal), and an associate member of the European College of Bovine Health Management. He is professor of food animal medicine & surgery and assistant director of the Agricultural Experiment Station at the University of Missouri. He has clinical, teaching, research, administrative, and service responsibilities in the Department of Veterinary Medicine & Surgery. His research is primarily focused on mastitis and milk quality, particularly, staphylococcal mastitis. He is a past president of the National Mastitis Council, and chair of the American Veterinary Medical Association's (AVMA) Council on Research.



Poster Session Date/Time Assignments

Note: Carts will be provided at convenient locations to store posters when not mounted. For example, students presenting in the afternoon may store their posters in the morning until time to mount theirs during lunch. Students presenting posters in the morning may store their posters after taking them down after their sessions.

Session A #1-128

Friday, 9:30am – 11:00pm
7:00AM — Students mount posters for Poster Session A
9:30AM–10:15AM — Even-Numbered Posters
10:15AM–11:00AM — Odd-Numbered Posters
11:00AM — Students take down posters for Poster Session A

Session B #129-256

Friday, 3:00pm – 4:30pm
12:00PM — Students mount posters for Poster Session B
3:00PM–3:45PM — Even-Numbered Posters
3:45PM–4:30PM — Odd-Numbered Posters
4:30PM — Students take down posters for Poster Session B

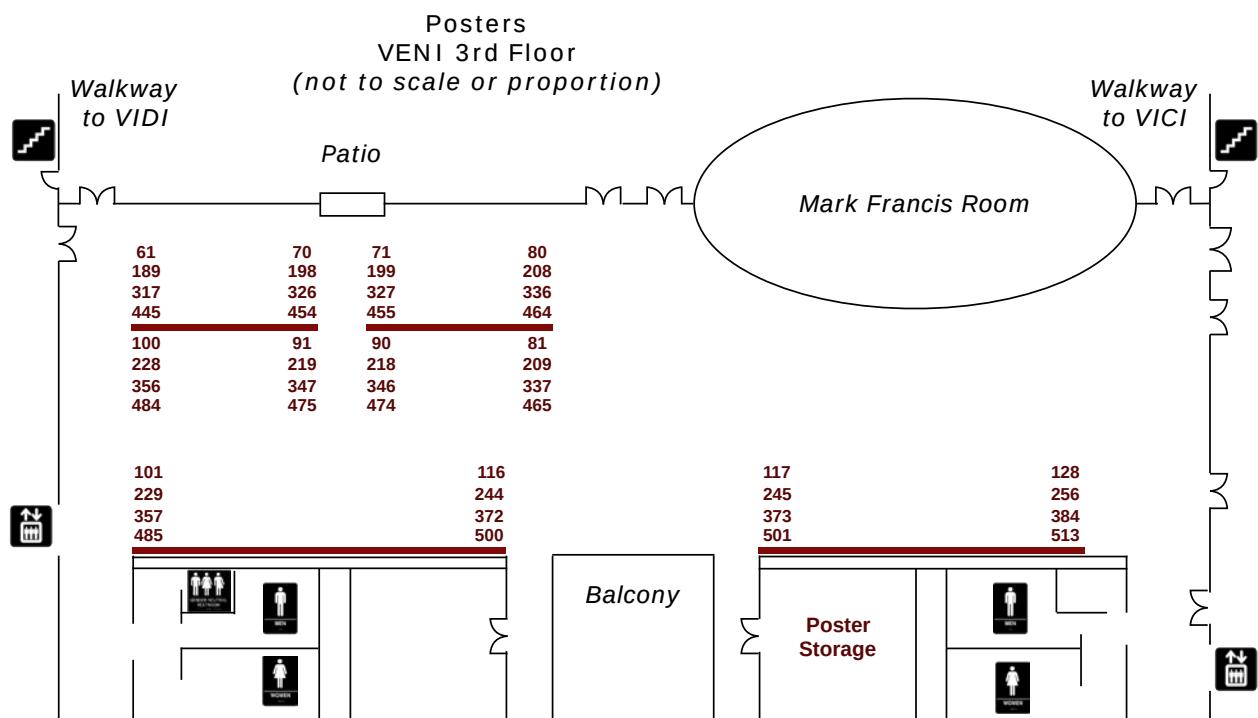
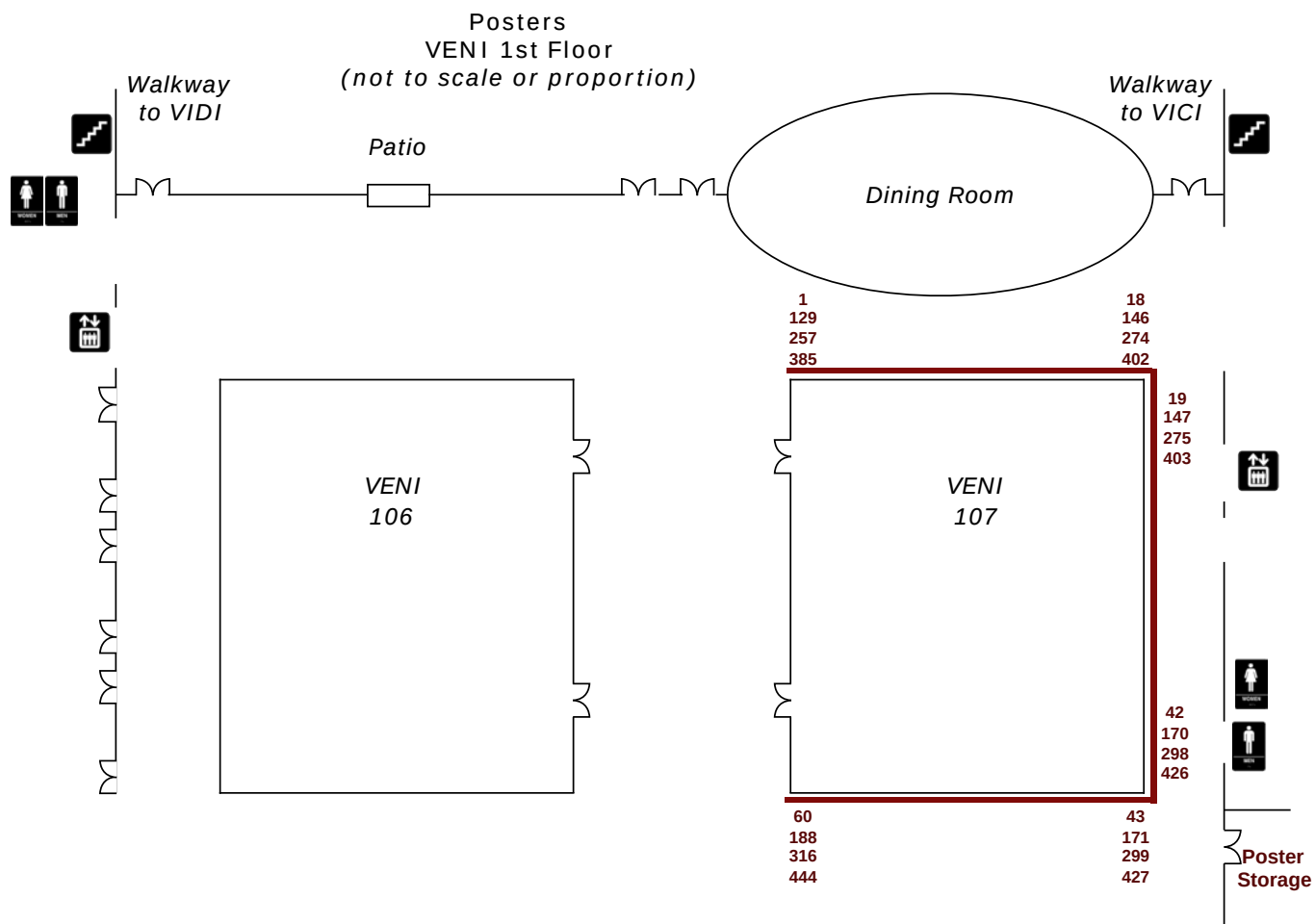
Session C #257-384

Saturday, 9:30am – 11:00pm
7:30AM — Students mount posters for Poster Session C
9:30AM–10:15AM — Even-Numbered Posters
10:15AM–11:00AM — Odd-Numbered Posters
11:00AM — Students take down posters for Poster Session C

Session D #385-511

Saturday, 3:00pm – 4:30pm
12:00PM — Students mount posters for Poster Session D
3:00PM–3:45PM — Even-Numbered Posters
3:45PM–4:30PM — Odd-Numbered Posters
4:30PM — Students take down posters for Poster Session D

Assigned Poster Locations



Abstracts Listed Alphabetically

Abstracts are ordered alphabetically by last name.

Last Name	First Name	Poster #	Abstract Title	Page #
Abedi	Nairi	1	Cloacal microbiome analysis of Turkey Vultures in Southern California	71
Acevedo	Andrew	2	Mechanism by which formate induces invasion gene expression in Salmonella	71
Achtymichuk	Gabrielle	3	Early interpretation of community composition leads to cognitive bias of predation risk in a larval amphibian	72
Ackerman	Leah	4	Detecting interleukin-8 expression in feline oral squamous cell carcinoma	72
Acosta-Bencosme	Joanna	5	Improving neutrophil function in neonatal dairy calves	73
Adams	Rachael	6	Intrauterine antibiotic infusion increases antimicrobial resistance in the reproductive microbiota of mares	73
Addison	Molly	7	Development of an interactive iBook for canine musculoskeletal anatomy dissection	74
Aiello	Taylor	8	Differential expression analysis in feline oral squamous cell carcinoma	74
Aldrich	Kennedy	9	Effect of diet on glycogen repletion in Thoroughbred horses after exercise	75
Anderson	Claretha	10	Evaluation of MMP-9 expression in CSF from canines with intracranial neoplasia and meningoencephalitis	75
Andreatta	Federica	11	Genetic modifiers and the gut microbiota in colorectal cancer development	76
Auld	Claudine	12	Limb dominance in domestic canines according to paw placement and peak vertical force	76
Báez-Ramos	Patricia	13	Use of protein biomarkers to characterize epithelial stem cells in the equine endometrium	77
Bagley	Kristen	14	Evaluating ultrasonic vocalization in a novel rat model that mimics aspects of amyotrophic lateral sclerosis	77
Bakken	Marit	15	Seroprevalence of canine influenza virus (CIV) in wild canid species	78
Barnes	Danielle	16	Pharmacokinetics and pharmacodynamics of grapiprant in a canine ovariohysterectomy pain model	78
Barras	Elise	17	Cyanidin inhibits adipogenic differentiation in 3T3-L1 preadipocytes via a Ca ²⁺ -dependent mechanism	79
Barrett	Erin	18	<i>In vitro</i> evaluation of the forwarder end knot to end a continuous suture line in large gauge suture	79
Bax	Erin	19	Physical activity and the potential metabolic benefits of S-equal in mice fed an obesogenic diet	80
Becerra	Roel	20	A natural murine infection model to delineate the immunobiology and persistence of noroviruses	80
Becker	Karlye	21	Comparison between computed tomography and ultrasonography in staging canine transitional cell carcinoma	81
Beckers	Kalie	22	Association of the uterine microbiome in mares before and during the breeding season	81

Last Name	First Name	Poster #	Abstract Title	Page #
Beckworth	Katie	23	Positive reinforcement training of cats classified as strays or abandoned to an animal control facility	82
Begin	Samantha	24	A pilot study investigating parasite management and egg shedding patterns on Prince Edward Island horse farms	82
Beigel	Brittney	25	Qlp19, a novel infection-associated leptospiral protein	83
Belgrad	Joseph	26	Isolation of <i>Schistosoma mansoni</i> eggs from baboon feces	83
Bell	Christina	27	Performance assessment of current PCR protocols in the detection of <i>Borrelia burgdorferi</i>	84
Belliveau	Patrick	28	Effect of benzoylphenylureas on the moulting process of copepod stage sea lice (<i>Lepeophtheirus salmonis</i>)	84
Berenson	Joshua	29	Investigation of TSG-6-mediated hyaluronic acid modifications using SEC-MALS and a molecular crowding sensor	85
Berg	Catharina	30	Investigation of functional alleles of recurrent exertional rhabdomyolysis in Thoroughbreds and Standardbreds	85
Bersch	Kevin	31	Evaluation of the anabolic WNT pathway response to load in osteocytes using a fluorescence-based mouse model	86
Bertrand	Amanda	32	Death in racehorses at the Fair Grounds Race Course in New Orleans, LA (2009-2018)	86
Bettin	Leonie	33	Characterizing the inflammatory response in a mouse model of Mal de Meleda	87
Bevelock	Thomas	34	Genetic characterization of <i>Strongyloides stercoralis</i> isolates in dogs and cats for assessing zoonotic status	87
Bissinger	David	35	Prions are commonly present in muscle of CWD-infected animals	88
Blake	Jacqueline	36	Investigation of novel prognostic biomarkers in dogs affected with degenerative myelopathy	88
Blakeman	Cody	37	Growing feline regulatory T cells for adoptive immunotherapy	89
Bleicher	Shira	38	Characterization of the guinea pig placental histiostructure	89
Bloodgood	Jennifer	39	Viral RNA titers in kidneys collected from birds experimentally infected with infectious bronchitis virus	90
Boisvert	Natalie	40	Characterizing the biological and molecular consequence of PTEN loss in canine osteosarcoma	90
Bookenberger	Lane	41	A novel technique to quantify spasticity in dogs with spinal cord injury	91
Boone	Eric	42	Hepatic carcinogen metabolizing enzyme activity in dogs with transitional cell carcinoma	91
Bordman	Aryn	43	Evaluating telomere length as a biomarker of reproductive stress and radiation exposure in wild boar	92
Botts	Michaela	44	Seasonal effect on Equine Herpesvirus-1 shedding in Ontario broodmares	92
Bowie	Aleaya	45	Plasma lipopolysaccharide binding protein-1 as a healthspan biomarker in aging vervet monkeys	93
Bradley	Zachary	46	Optimization of the RT-PCR protocol for the detection of turkey arthritis reovirus	93
Bradshaw	Alexander	47	The effects of porcine reproductive and respiratory syndrome virus on T cell stimulation	94

Last Name	First Name	Poster #	Abstract Title	Page #
Brock	Jasmine	48	Neuroinflammation in the context of SIV/Rhesus Macaque Animal Model	94
Brooks	Charles	49	Neuronal migration and GSK3 β levels in the cortex of 129S6/SvEv mice, a genetic model of psychiatric disease	95
Brooks	Michael	50	Differentiation of early and late lytic gene expression using a Marek's disease alphaherpesvirus model	95
Brown	Clare	51	Optimization of methods to study the effect of cannabinoids on immune responses in stimulated canine PBMCs	96
Brown	Shaina	52	Increased expression of galectin-3 and CD25 as pre-neoplastic events in canine T zone lymphoma	96
Brownlee	Michael	53	Hepatocyte growth factor mimetics as a potential therapeutic for muscle wasting diseases	97
Buck	Cameron	54	Evaluating the effect of UVB exposure on protein electrophoresis and biochemistry findings in Leopard Geckos	97
Buckingham	Annie	55	Evaluation of transdermal flunixin and meloxicam in piglets at processing age	98
Buckley	Katherine	56	The effects of hydrocortisone, vitamin C, and thiamine on cytokines in an ex vivo bacterial sepsis model	98
Burdette	Thomas	57	Incidence of non-toxigenic and toxigenic <i>Clostridium difficile</i> isolated from animals	99
Burns	Jamie	58	Annexin V: A possible role in the development of endometrial degenerative fibrosis in the mare?	99
Burns	Madison	59	Bone marrow supernatant is an alternative to fetal bovine serum in MSC isolation and expansion	100
Bussey-Spratling	Jayda	60	Hypertension in vervet monkeys and sodium-lithium countertransport (SLC)	100
Butler	Hailee	61	Endometrial hyperplasia and pyometra in African painted dogs: an overview of the female reproductive pathology	101
Cain	John	62	Molecular dissection of mosquito resistance to heartworm infection	101
Callahan	Shawn	63	Molecular taxonomy of <i>Neorickettsia</i> spp. from <i>Nanophyetus salmincola</i> and dogs with salmon poisoning	102
Callaway	Sabrina	64	Role of microRNAs in the lupus-gut microbiome axis	102
Canada	Sara	65	Dwarf Deerhounds: genetic investigation of the <i>COMP</i> gene in dogs with pseudoachondroplasia	103
Canda	Kaylie	66	Microglial density and morphology following CTB-saporin induced respiratory motor neuron death	103
Cardascio	Alexander	67	The <i>in vitro</i> effects of zoledronic acid and radiation therapy in canine osteosarcoma	104
Carman	Michelle	68	Evaluation of a commercial <i>Haemonchus contortus</i> vaccine in non-domestic hoofstock	104
Carn	Derek	69	Identification of phages binding to epithelial and to mesenchymal (EMT) phenotypic breast cancer cells	105
Carson	Megan	70	Behavioral correlates of canine tail position in a leash-associated aggression model	105
Castro	Andres	71	Influence of <i>Trypanosoma cruzi</i> strain and vector species on selected canine Chagas cardiac disease outcomes	106

Last Name	First Name	Poster #	Abstract Title	Page #
Catarino	Olivia	72	Effect of printing material and parameters on the mechanical properties of 3-dimensionally printed bone models	106
Catini	Pietro	73	Optimization of protocols for RNA, DNA, and chromatin isolation for epigenetic annotation	107
Ceres	Kristina	74	Environmental persistence of MAP as a barrier to Johne's disease elimination	107
Chang	Christina	75	Autism-relevant behavioral outcomes in an antigen-driven rat model of maternal autoantibody-related autism	108
Chang	Diana	76	Assessment of nutritional, clinical and hematological status in dogs fed a plant-based versus meat-based diet	108
Chari	Miriam	77	Cell mediated immunogenicity of TGF- β 2-treated MHC-mismatched equine mesenchymal stem cells	109
Cheatham	Christa	78	Blood-brain barrier pathology in an experimental model of blast-induced neurotrauma	109
Chen	Frances	79	Genome editing at the AF-associated <i>Pitx2</i> Locus: a role for the lncRNA <i>Playrr</i> in cardiac arrhythmias	110
Chen	Kaiwen	80	Optimization of embryo electroporation to enhance genetic modification in rats using CRISPR/Cas9 technology	110
Chen	Kimberly	81	Determining the <i>in vitro</i> effects of extracellular vesicles on peripheral nerve growth	111
Chen	Yue (Polly)	82	Characterizing <i>in vivo</i> and <i>in vitro</i> TLR4 expression and response to LPS in immature and mature mouse intestine	111
Cheung	Hilvy	83	The effect of cryopoor plasma addition to adsorbed plasma to correct prothrombin time: an <i>in vitro</i> study	112
Cigarroa	Annalis	84	Assessing the development of the postnatal canine gastrointestinal microbiome utilizing the dysbiosis index	112
Clark	Jory	85	Genotype assay development for alleles associated with relevant phenotypes in pet and working German Shepherds	113
Clifton	Emily	86	Covariation between personality and immunity in the presence versus absence of parasitism	113
Clontz	Dallas	87	Biofilm formation in commensal and pathogenic <i>Enterococcus cecorum</i> strains isolated from broiler chickens	114
Clouden	Michaela	88	Understanding the effects of oxidative stress on the pyroptotic pathway of programmed cell death	114
Coakley	Chad	89	Evaluation of impacts by probiotics expressing <i>Listeria</i> adhesion protein on porcine intestinal epithelium	115
Cochran	Karlee	90	Effects of S-equol on obesity-associated depression and anxiety in male and female mice	115
Cocke	Natalie	91	Using ImageJ to differentiate histiocytic sarcoma on cytology through automated and manual measures	116
Cockey	James	92	Exosome deficiency leads to an increased T cell-mediated inflammatory response in Rab27dKO mice	116
Cohen	Jonathan	93	Mesenteric lymph node cytology in cats for diagnosing small cell granular lymphocyte intestinal lymphoma	117
Coleman	Kaitlyn	94	Expression and localization of Ezrin in embryonic wild-type kidney and a model of cystic kidney disease	117
Collins	Elizabeth	95	Defining the early stages of skin immunity against a soil-transmitted helminth	118

Last Name	First Name	Poster #	Abstract Title	Page #
Corder	Nicole	96	Mast cells exhibit bone morphogenetic protein signaling in response to inflammation	118
Cordingley	Jessica	97	Effects of temporary dry ice storage on cryopreserved C57BL/6 mouse sperm	119
Cossaboon	Jennifer	98	Contributing effect of organochlorine exposure on Atlantic bottlenose dolphins during a morbillivirus outbreak	119
Cotton	Renee	99	TET2's DNA demethylation in CD8 memory	120
Courtney	Lindsay	100	Comparison of uropathogenic <i>Escherichia coli</i> isolates from human and companion animal patients	120
Covo	Mariel	101	Optimization of canine CAR T Cell production and expansion for re-directed T Cell therapy	121
Cowser	Madalyn	102	Apoptosis in neutrophils and lymphocytes in septic and critically ill dogs	121
Crawford	Barbara	103	Examining the genetic role in <i>Cytauxzoon felis</i> survival and mortality	122
Crawford	Stephanie	104	Assessment of Zika virus tissue tropism and persistence after subcutaneous and sexual infection	122
Crofton	Amanda	105	Identification of genetic markers for familial subvalvular aortic stenosis in Rottweilers	123
Crotty	Sierra	106	Development of a data-driven algorithm from swine to improve management of human cardiovascular disease	123
Cua	Abigail	107	Determining the role of environmental chemicals in Western Pacific Parkinsonism-Dementia Complex (PDC)	124
D'Agostino	Albert	108	Detecting the SRY gene in bone-marrow derived mesenchymal stem cells from <i>Equus caballus</i>	124
Davis	Marina	109	Effects of ovarian hormones on blood pressure regulation in middle aged female rats lacking central serotonin	125
Deadrick	Sheree	110	HDAC inhibitors interfere with meiotic chromosome synapsis in the domestic dog	125
DeProspero	Dylan	111	Identifying a variant associated with cerebellar cortical degeneration in Scottish Terriers	126
DeRouen	Abigail	112	Three-dimensional motion of equine hoof components during axial loading and unloading	126
Dianis	Allison	113	Can IS711 be used as a surrogate for MLST genotyping of <i>Brucella ceti</i> in wild cetaceans?	127
Diaz	Alexa	114	Effects of Gulf War-related chemical exposures on mouse motor behaviors: therapeutic modulation by LNFPIII	127
Dillard	Corinne	115	Effect of antimicrobial administration on respiratory and gastrointestinal microbiota of healthy foals	128
Dlubac	Erin	116	Stem-cell transplantation into stallion testes: Optimization of a method	128
Donohue	Laura	117	Comparison of an internal versus novel external teat sealant for the prevention of mastitis in dairy cattle	129
Drizin	Sienna	118	Psychotropic drug use to combat stress-related disease in animal shelters	129
Dubowitz	Rebecca	119	Developing optimal conditions for 3D <i>in-vitro</i> co-culture of human mesenchymal stem cells and macrophages	130

Last Name	First Name	Poster #	Abstract Title	Page #
Duguay	Marco	120	Genetic testing for progressive rod-cone degeneration and centronuclear myopathy in Labrador Retrievers	130
Dulli	Kelly	121	Subunit vaccine-induced CD8 T-cell immunity to mucosal and systemic viral infection	131
Durfey	Casey	122	Enhancing wild animal semen quality through nanotechnology tools	131
Dwyer	Elizabeth	123	Effect of fecal microbiota transplantation on the fecal microbiome of healthy dogs treated with antibiotics	132
Egers	Amanda	124	Molecular characterization of helminth parasites from North Atlantic wildlife	132
Eidson	Anika	125	Canine gastrointestinal histamine receptor-1 expression in normal and inflammatory bowel disease biopsies	133
Eisemann	Robert	126	Investigating CpG ODN-mediated attenuation of lung metastasis in a preclinical model of osteosarcoma	133
Eldridge	Laura	127	Changes in gonadotropin-like receptor expression in the spawning anemone <i>Aiptasia pallida</i>	134
Ellingson	Lacey	128	Identification of genetic variants associated with two Mendelian diseases in the horse	134
Ellis	Jayne	129	Activation of canine mesenchymal stem cells to enhance control of inflammation, arthritis, and wound healing	135
Ellis	Megan	130	Corticosterone monitoring to assess the effect of transport distance on stress levels in mice	135
Ericksen	Kelsea	131	Laser plume generation and potential associated risks	136
Erickson	Janna	132	Tumor lysate vaccines with immune blockade inhibitors and adverse effects in the treatment of canine glioma	136
Erwood	Eric	133	Detection of IBV using Sanger vs MinION nanopore sequencing	137
Eshelman	Amanda	134	Evaluating the use of deuterium oxide to determine body composition in the big brown bat, <i>Eptesicus fuscus</i>	137
Everett	Terry	135	The cytotoxic effects of iodinated contrast media on renal proximal tubular epithelial cells	138
Faryean	Joe	136	Is pain associated with nerve inflammation in Guillain-Barre Syndrome?	138
Fattori	Chiara	137	Cloning canine CD16 for directed NK cell-immunotherapy	139
Feldner	Mary	138	Variation in cytosine arabinoside protocols based on dosage and route of administration in canine patients	139
Feller	Laine	139	Effects of pharmacological inhibition of RIPK1 in <i>Mycobacterium tuberculosis</i> -infected mice	140
Ferguson	Lindsey	140	Does induction of HIF-1 α in alveolar type II epithelial cells play a role in influenza pathogenesis?	140
Fernandez	Mariana	141	Femoral remodeling in dogs with luxated dysplastic hips. A complicating factor for total hip replacement	141
Fink	Dustin	142	Defining the impact of prenatal Zika Virus infection on neurodevelopment in a novel mouse model	141
Fisher	Corey	143	Chemotherapeutic effects of the tyrosine kinase inhibitor, ponatinib, on canine mast cell tumours <i>in vitro</i>	142
Fitzgerald	Alyson	144	Comparing the pharmacokinetics and analgesic effect of a multi-dose methadone/fluconazole regimen	142

Last Name	First Name	Poster #	Abstract Title	Page #
Floyd	Austin	145	Comparison of the host directed and antimicrobial effects of novel metal-based wound dressings	143
Folmar	Chelsea	146	PNAG hyperimmune plasma is superior to commercial plasmas for opsonizing and killing <i>Rhodococcus equi</i> <i>in vitro</i>	143
Foust	Bethany	147	Antimicrobial-loaded poloxamer 407 hydrogel for the treatment of osteomyelitis	144
Fowler	Peter	148	Ticks & zoos: a subtle inquiry into their conquest	144
Frankovich	Alexandra	149	Detection of <i>Hepatozoon americanum</i> in Gulf Coast ticks from Oktibbeha County, MS	145
Frantz	Brandon	150	Characterizing developmental lung disease related to neonatal mortality in the Norwich Terrier	145
Fray	Leah	151	Whole genome sequencing of colony variants of <i>Burkholderia pseudomallei</i> for use in a chronic infection model	146
Freitas	Nicole	152	Investigating canine cancer-testis antigens to further cancer vaccine development	146
French-Kim	Kristen	153	Testing VSV recombinant vaccines expressing the F, G, M, and N proteins of HRSV in the cotton rat model	147
Frere	Francesca	154	Susceptibility of bird and mosquito cells to African Usutu virus strains	147
Frink	Alexa	155	Identifying the bioactive component of platelet-rich plasma lysate for the treatment of equine osteoarthritis	148
Fuetsch	Stephanie	156	Meloxicam sustained release injection site reactions in male and female mice	148
Gafen	Hannah	157	Decorin modulation of corneal wound healing through autophagy	149
Gagne	Soleil	158	Talin expression markedly increases in myometrium during pregnancy with distinct detection in focal adhesions	149
Galbreth	William	159	Evaluation of recombinant attenuated <i>Salmonella</i> vaccine (RASV) vectors for induction of immune response	150
Gallini	Stefan	160	Evaluation of a commercial probiotic marketed for use in captive hermit crabs (<i>Coenobita</i> spp.)	150
Garcia	Jessica	161	Guiding antimicrobial therapy: prevalence of bacteremia in dairy calves with diarrhea	151
Garcia-Abarca	Nicolas	162	Relationship between endoscopic and histopathologic findings of the gastric glandular mucosa in horses	151
Gartlan	Brina	163	Essentiality and functional analysis of Calcium Dependent Protein Kinase 1 (CDPK1) in <i>Cryptosporidium parvum</i>	152
Geldert	Christina	164	Collaboration between veterinarians and honey bee researchers through exploration of the bee gut microbiome	152
Gerbus	Alyssa	165	Prevalence of <i>Leptospira</i> spp. in mongoose (<i>Herpestes auropunctatus</i>) in Grenada	153
Gerras	Allison	166	Effects of early life stress and biological sex on histamine n-methyltransferase	153
Gibson	Blake	167	Characterization of the canine aural microbiome	154
Gidcumb	Emily	168	Development of a mouse model to study the impact of radiation-induced pain on metastatic propensity of cancer	154

Last Name	First Name	Poster #	Abstract Title	Page #
Gludish	David	169	Novel cellular tools to study HIV replication in tissue reservoirs and improve standard viral outgrowth assays	155
Goldberg	Abby	170	The role of type III collagen in the progression of post-traumatic osteoarthritis	155
Golsen	Bryce	171	Expression analysis of mu, delta and kappa opioid receptor genes in pigeons and cockatiels	156
Gonzalez	Gabriel	172	Analysis of changes in stem cell biomarkers to determine tissue viability in horses with strangulating colic	156
Gooch	Sydney	173	Cryopreservation of body cavity fluids for diagnostic cytology	157
Gorrill	Angelina	174	Molecular characterization of avian influenza virus H9N2 isolated from Algerian poultry flocks	157
Grady	Kylie	175	The olfactory mirror test: testing self-recognition in dogs using anogenital odor	158
Graf	Lynanne	176	Efficacy of Cryptotanshinone against human and canine melanoma	158
Granger Jr	Kyle	177	Polymorphisms in glutathione-S-transferase genes in Boxers with lymphoma and unaffected geriatric controls	159
Gravette	Elizabeth	178	Effect of acupuncture on recovery from general anesthesia in canines	159
Griffin	Katherine	179	An immunohistochemical evaluation of integrin and cadherin expression within mesenchymal stem cell spheroids	160
Groody	Celia	180	The potential of transmission of <i>Ureaplasma diversum</i> by bovine embryo transfer	160
Gruenwald	Samantha	181	The impact of supplemental heat in rodent housing units on behavior, breeding, and anesthetic recovery	161
Guilfoyle	Colleen	182	Pangolins in peril: unraveling reproductive mystery in a race to save a species	161
Guillen	Sara	183	The efficacy of mannosylation of chitosan derivatives as a nanoparticle DNA carrier	162
Gundlach	Carly	184	Assessing differences in the human-animal bond among service dogs and pet dogs	162
Gupta	Ankita	185	Assessment of basal behaviors in genetic mouse models of mild and severe hydrocephalus	163
Han	Susie	186	Use of preference testing to establish reinforcer value in rats and influence perception of handlers	163
Hancock	Kari	187	Investigation of genetic risk factors for squamous cell carcinoma in the equine eye	164
Harris	Cassidy	188	Progression and persistence of nasal turbinate pathology in F344 rats exposed episodically to ozone	164
Harro	Cailin	189	Hepatic concentrations of copper and other metals in dogs with hepatocellular carcinoma	165
Hart	Sarah Niamh	190	Contact networks and season: a longitudinal analysis of the Florida panther population	165
Hartson	Rochelle	191	Role of the OVLT-gut microbiome axis on the development of DOCA-salt hypertension	166
Harver	Dylan	192	Readability as a source of measurement error in veterinary medical education assessments	166

Last Name	First Name	Poster #	Abstract Title	Page #
Hastings	Kaitlyn	193	The role of childhood obesity-associated adipose tissue fibrosis in adulthood cancer development	167
Hawbecker	Tyler	194	Evaluation of antimicrobial resistance to Chlortetracycline in <i>Mannheimia haemolytica</i> and <i>Pasturella multocida</i>	167
Hawks	Meighan	195	Effect of the commercially available probiotic <i>Enterococcus faecium</i> SF-68 on canine giardiasis	168
Hayes	Shantavia	196	Sexual dimorphism in the metabolic response to dietary protein restriction	168
Hendrickx	Robert	197	Development of clinically relevant chronic rotator cuff models in sheep: a pilot study	169
Hengy	Miranda	198	The potential use of MALDI Sepsityper™ technology for rapid diagnosis of bacteremia in dairy calves	169
Hennessy	Elise	199	Understanding the role of avian hosts in the sylvatic transmission of Mayaro virus	170
Herbert	Gillian	200	A selective BCL-2 inhibitor impairs canine B-cell lymphoma cell survival, <i>in vitro</i>	170
Herford	Jacob	201	Non-steroidal anti-inflammatory drugs reduce vas deferens epithelial MRP4 expression and prostaglandin export	171
Hicks	Kelly	202	Surveillance of flaviviruses by use of LAMP and qRT-PCR in insect vectors	171
Hietpas	Margaret	203	Macrophages facilitate angiogenesis in mammary tumors of obese mice	172
Hildreth	Kimberly	204	Increased expression of a marker of endoplasmic reticulum stress in an experimental equine laminitis model	172
Hines	Elizabeth	205	Comparative hematology in a One Health approach - inappropriate metarubricytosis in canine and human diseases	173
Hiscocks	Kenneth	206	CCK-58 prolongs the intermeal interval, whereas CCK-8 reduces this interval	173
Hlavaty	Sabina	207	Phenotypic and transcriptomic characterization of canine myeloid-derived suppressor cells	174
Hogen	Katie	208	Prevalence of <i>Aelurostrongylus abstrusus</i> and <i>Toxoplasma gondii</i> in feral cats in the Willamette Valley	174
Hommel	Caelin	209	Expression and secretion of high mobility group box-1 in lipopolysaccharide-activated canine platelets	175
Hooser	Blair	210	True colors: the frequency of hidden alleles in canine coat color	175
Hopkins	Ashley	211	Safety/efficacy of novel isonitrile compounds as candidates for treatment of resistant <i>Staph. pseudintermedius</i>	176
Horn	Rebecca	212	The role of exocrine pancreatic inflammation in the pathogenesis of canine diabetes mellitus	176
Huang	Patrick	213	Establishment of a drug screen to repurpose drugs targeting the induction of DNMT3B in human breast cancer	177
Hunter	Alexa	214	A retrospective clinicopathological study of myocarditis in dogs: 70 cases (2007-2017)	177
Hurst	Natalie	215	Common practices in cold sterile use in veterinary clinics and prevalence of bacterial contamination	178
Hymel	Paige	216	Meniscal sizing based on morphometric measurements in canines: a cadaveric study	178

Last Name	First Name	Poster #	Abstract Title	Page #
Ineson	Deanna	217	Is cardiac cachexia an independent risk factor for mortality in dogs with congestive heart failure?	179
Jacobs	Julia	218	Interaction between intestinal microbiota, tissue regeneration, and immune response following nerve injury	179
Jenkins	Alyssa	219	Pharmacokinetic profile of insecticide synergist piperonyl butoxide in mice	180
Jindal	Abhilasha	220	Effects of vaccination on chlamydial load in <i>Chlamydia suis</i> -inoculated gilts	180
Johnson	Kaylynn	221	The impact of parasite infection on the canine microbiome	181
Johnson	Kelsey	222	Diversity of marine apicomplexans	181
Johnson	Morgan	223	Effects of short-chain fatty acids on murine intestinal epithelial cell cytokine gene expression <i>in vitro</i>	182
Karklus	Alyssa	224	Antinociception in ball pythons (<i>Python regius</i>) using a clinically relevant model of pain	182
Kastendieck	Emily	225	Investigating the ecology of mobile genetic elements in beef feedlot cattle using high-throughput sequencing	183
Kaufman	Mary	226	Intravenous contrast-enhanced computed tomography: dosing and pharmacokinetics of iopamidol in <i>Cyprinus carpio</i>	183
Kechejian	Sarah	227	Genetic diversity of feline foamy virus (FFV) in Florida panther (<i>Puma concolor coryi</i>)	184
Kendzierski	Jessica	228	Evaluating contributions of amniotic fluid sterility, maternal and infant factors on primate infant mortality	184
Kern	Kimberly	229	Comparison of two serological tests for detection of <i>Leptospira</i> -specific antibodies in dogs from Mexico	185
Kerr	Jessica	230	Hypoglycemia in dogs after ingestion of metformin: a retrospective evaluation (2001 - 2017)	185
Kessler	Jessica	231	The effects of early life stress on diamine oxidase levels in swine and mice	186
Kim	Alan	232	MicroRNA regulation of development of corticospinal motor neurons	186
Kim	Jeffrey	233	Pain assessment via consumption of yogurt treats in rats treated with sustained-release meloxicam	187
Kimes	Austin	234	Using CRISPR-Cas9 to knockout the sterile alpha motif domain in the bicaudal C gene of zebrafish (<i>Danio rerio</i>)	187
King	Emily	235	Computed tomography to evaluate hepatic attenuation in three species of freshwater turtles	188
Kirner	Nicolette	236	Mammalian orthoreovirus as an oncolytic therapy and vaccine candidate for HER2 positive breast cancer	188
Kish	Daniel	237	Leptospirosis in shelter dogs and cats in the tristate area of Kentucky, Tennessee, and Virginia	189
Kitsberg	Rebecca	238	Variability in home cage CO2 euthanasia of the laboratory mouse	189
Klosowski	Marika	239	Lipin-1: a novel target for metabolic reprogramming of triple negative breast cancer cells	190
Koch-Laskowski	Kieran	240	Inducible <i>hGlp-1r</i> knockout in the mouse hypothalamus promotes feeding and body weight gain	190
Kosanovich	DeAnna	241	Acupuncture at Stomach 36/Pericardium 6 does not reduce nausea and sedation during lidocaine infusion in dogs	191

Last Name	First Name	Poster #	Abstract Title	Page #
Kozlov	Shawn	242	Histotripsy effects on primary tumor and metastasis in a 4T1 breast cancer model	191
Krause	Laurel	243	Evaluation for novel causes of chronic kidney disease in cats	192
Krienke	Leslie	244	Quantifying error in measurement of the angle of lateral opening using the cup position assessment device	192
Krishnan	Sneha	245	Amphibians as a possible new reservoir for Dengue virus	193
Ku	Tobi	246	Positive reinforcement of a foundation behavior to reduce perceived anxiety of kennelled dogs: a pilot study	193
Kuerbitz	Ellen	247	Characterization of circulating exosomal microRNA expression in canine osteosarcoma	194
Lachenauer	Erica	248	Investigating the effect of p53 loss on folate metabolism in the development of neural tube defects	194
Lamontagne	Angélique	249	Efficacy of the feline temperament profile in evaluating shelter cats for adoption	195
Lancaster	Meghan	250	Examination of <i>Anaplasma marginale</i> genetic diversity in Kansas beef cattle	195
Lavin	Elizabeth	251	Hmga2 inactivation suppresses the development of a melanoma phenotype in skin exposed to UVB light	196
Lawhon	Tess	252	Modulatory effects of JAK 1,2 inhibitor Oclacitinib on a canine T-cell lymphoma cell line	196
Ledner	Shana	253	Comparison of bacterial DNA found in plant-based and animal-based dry canine diets	197
Lerner	Francesca	254	Verification of feeding tube placement based on tracheal superimposition on thoracic radiographs	197
Leung	Wing Sze	255	Age is a determinant factor of cancer metastasis in mice	198
Levy	Ivana	256	Comparison of axillary and inguinal temperature to rectal temperature in healthy guinea pigs (<i>Cavia porcellus</i>)	198
Lewis	Chaunte	257	Antibody mediated protection against RSV after immunization with a VSV-G and F recombinant	199
Lewis	Lily	258	The effects of acute exposure to di(2-ethylhexyl) phthalate and diisononyl phthalate on hormone levels in mice	199
Lewy	Keith	259	Antibiotic resistance in <i>Escherichia coli</i> in feedlot cattle treated for bovine respiratory disease	200
Lezcano	Victor	260	mRNA expression of Th2 cytokines differs in duodenal mucosa of healthy and diseased dogs	200
Lieberman	Joshua	261	Formation of intestinal epithelial organoids by direct reprogramming of mouse fibroblasts	201
Lilley	Carly	262	MALDI-TOF mass spectrometry as a tool to identify mastitis pathogens and determine antimicrobial resistance	201
Lind	Jenny	263	Multi-drug resistant infections in canine and equine patients: <i>Escherichia Coli</i> and <i>Klebsiella</i>	202
Linder	Jessica	264	Development of a simple method of static body weight distribution in neurologically normal small breed dogs	202
Lisowski	Sasha	265	Assessing the feasibility of canine vaccination to prevent Rocky Mountain spotted fever in Arizona	203
Locker	Seth	266	PDK4 mRNA visualization with fluorescent RNAscope in fibroblasts from Doberman Pinschers	203

Last Name	First Name	Poster #	Abstract Title	Page #
Loehr	Amanda	267	Mammary stem-like cells from mammary cancer resistant and susceptible species differ in response to UV	204
Loftin	Amanda	268	Probiotic therapy in HIV-treated humans improves prognosis-effects on the gut microbiome and immune activation	204
Lonngren	Karalyn	269	Comparison of iSperm to current accepted semen analysis methods	205
Loonsk	Hannah	270	Retrospective and prospective analysis of the effect of serological lead burden on fractures in wild birds	205
Lordi	Nicholas	271	Symmetric dimethylarginine (SDMA) as a marker for renal insufficiency in captive cheetahs (<i>Acinonyx jubatus</i>)	206
Lucius	Sascha	272	Role of gut lactobacillus in priming of neural-immune responses in hypertension	206
Lugardo	Josue	273	Plasma extracellular vesicular miRNA as biomarker for canine osteosarcoma	207
Lukach	Jennifer	274	Investigation of the occurrence of hyperketonemia in subsequent lactations in dairy cows	207
Lund	Lindsey	275	Microsatellite instability in canine tumors	208
Maciejczyk	Kimberly	276	PCR genotyping of a novel FGF4 retrogene in Pit Bull Terriers and mixed breed dogs affected by disc disease	208
Madsen	Virginia	277	Correlating clinical and pathological findings in inflammatory CNS diseases in dogs	209
Majors	Kara	278	Role of catfish B cells in innate and adaptive immune responses to <i>E. ictaluri</i> live attenuated vaccines	209
Makii	Rebecca	279	Investigating the role of WWOX in DNA damage repair and radiosensitivity of canine osteosarcoma cell lines	210
Mancha	Serena	280	Regulation of glutamate receptors trafficking and localization by calcium and reactive oxygen species	210
Maness	Aaron	281	A look into how an energy-dense diet induces inflammation in the feeding centers of the brain	211
Manley	Kristin	282	Quantification of rickettsial load during the acute phase of experimental bovine anaplasmosis	211
Manriquez	Michael	283	Feasibility and position optimization of a wearable smartphone ECG monitor in exercising horses	212
Mapes	Christopher	284	The effect of estrous stage on larynx physiology in rats	212
Mark	Michael	285	Addition of IL-10 response to hyperoxic and normoxic conditions by the Mas agonist AVE-0991	213
Markle	Mariah	286	Developmental competence of ovine and caprine vitrified oocytes using a closed system	213
Marroquin	Priscilla	287	Examination of the <i>Mannheimia haemolytica</i> genotypes in dairy cattle isolated at the WVDL from 2015 to 2018	214
Martin	Hannah	288	Optimizing conditions for non-viral mRNA transfection of bone marrow cells using mineral-coated microparticles	214
Martin	Rachel	289	The effect of irradiated human squamous cell carcinoma cells on microglia activation and survival	215
Martinelli	Laura	290	Sedation protocols in juvenile Blue Poison Dart Frogs (<i>Dendrobates tinctorius azureus</i>)	215
Mathe	Jennifer	291	Determining cryptic speciation in two- and three-toed sloths using molecular phylogenetics	216

Last Name	First Name	Poster #	Abstract Title	Page #
Mayer	Treana	292	Characterizing larval epidemiology of the meningeal worm (<i>Parelaphostrongylus tenuis</i>)	216
McAfee	Erika	293	The role of Tc17 cells in enhancing neutrophil recruitment to the Chlamydia-infected genital tract	217
McAuley	Rachel	294	Effects on equine neonates of exogenous progestogens given to late gestation mares	217
McConnell	Sydney	295	IgE concentrations in bronchoalveolar lavage fluid from horses with severe pasture-associated equine asthma	218
McDaniel	Sarah	296	Pre-operative dermatological findings for dogs undergoing total hip replacement: a retrospective study	218
McDowell	Makenzie	297	The role of gut microbiome in susceptibility to diarrhea associated with enteric pathogens in shelter kittens	219
McNally	Rachel	298	Exploiting biological priors to enhance cruciate ligament rupture candidate locus discovery in dogs by GWAS	219
McNew	James	299	Purification of murine pancreatic lipase - Initial findings	220
Meier	Elisa	300	Colon-targeted delivery of a novel treatment for <i>Tritrichomonas foetus</i> infection in cats	220
Mendieta Calle	Jorge	301	Assessment of AAVR contribution to AAV8-luciferase <i>in vivo</i> transduction post retro-orbital delivery	221
Merry	Hillary	302	Triple negative breast cancers are highly sensitive to ferroptosis	221
Meyerhoeffer	Mitchell	303	Immune response to high frequency irreversible electroporation treatment of canine hepatocellular carcinoma	222
Michalski	Hannah	304	Evaluation of canine cognitive function and exclusion criteria in a phase II rapamycin clinical trial	222
Michel	Jeanne	305	Comparing the significance of animal shelter parasite control programs on heartworm infection in LA mosquitos	223
Mikes	Alison	306	Effect of acupuncture on recovery from general anesthesia in shelter cats	223
Miller	Jonathan	307	Low pH endocytic entry of pseudorabies virus into multiple cell lines	224
Miller	Lillian	308	Herpes Simplex Virus 1 (HSV-1)-mediated oncolytic virotherapy for treating ovarian cancer	224
Mills	Benigno Benjamin	309	Development of a miniature pig model of Juvenile Osteochondrosis Dissecans (JOCD)	225
Minich	David	310	Examining routes of prion entry into the gut of white-tailed deer, <i>Odocoileus virginianus</i>	225
Mishkin	Noah	311	Potential of macrophage response to <i>M. tuberculosis</i> via vitamin A-dependent nuclear receptor activation	226
Moley	Charles	312	Improving the IVF efficacy of porcine embryos through the alteration of IVF protocol and medium selection	226
Molind	Katie	313	The germination and sporulation of <i>Bacillus anthracis</i> in topsoil	227
Monje	Daniela	314	Assessment of <i>Haemonchus contortus</i> burden in a captive pronghorn population at a zoo	227
Montin	Karin Helena	315	A survey of veterinarian and owner attitudes, beliefs and perceptions regarding pain in dogs	228

Last Name	First Name	Poster #	Abstract Title	Page #
Montoya	Sarah	316	Evaluating systemic inflammation as a contributor to the development of canine idiopathic epilepsy	228
Moore	Trusten	317	ECGesy: A mobile application to help with ECG interpretation in dogs and cats	229
Mora	Kevin	318	Eosinophil-mediated clearance is blocked by a <i>Bordetella</i> blood-responsive regulon	229
Morales Coll	Valerie	319	The prevalence of exercise-induced pulmonary hemorrhage in high goal polo horses	230
Morris	Zoe	320	Antimicrobial resistance risk factors in pig slaughterhouse workers, meat products, and environmental samples	230
Moss	Kaitlyn	321	Intra-articular AAV-mediated overexpression of interleukin-10, for the prevention of equine osteoarthritis	231
Mueller	Marissa	322	Carbon dioxide surgical laser versus scalpel excision of soft tissue sarcomas and mastocytomas in dogs	231
Murray	Katherine	323	Whole-genome sequencing to identify AMR determinants in <i>Salmonella</i> obtained from the dairy worker environment	232
Myers	Adam	324	Assessing medical comorbidity burden in a rhesus macaque model of Autism Spectrum Disorder	232
Narvaez	Juan	325	Multi-parameter analysis of skin lesions in MRL/ <i>lpr</i> mice model of chronic cutaneous lupus erythematosus	233
Negron Camacho	Marie	326	Expanding our knowledge of fat: Is stretch-activated Piezo1 found in perivascular adipose tissue?	233
Nelson	Andrew	327	Asthma - not just an airway disease	234
Nelson	Gaberail	328	A comparative study of hepatic abscesses in antibiotic-free cattle and conventional cattle	234
Nelson	Katelynn	329	CRISPR/Cas9 phage and antibiotic combination therapy against <i>Staphylococcus aureus</i> biofilm	235
Nelson	Valerie	330	Molecular characterization of <i>Salmonella</i> Typhimurium isolated from pigs, humans, and the environment	235
Neupauer	Katherine	331	Influenza induces robust vascular regeneration mediated by both canonical and non-canonical progenitor cells	236
Neverson	Jade	332	Differential activation of hepatocyte TF procoagulant activity by apoptotic and necrotic cell death	236
Ngene	Chioma	333	Determining the function and regulation of genes selectively induced by acidic pH in the presence of glycerol	237
Ngo	Ying	334	Synovial response to bone marrow mononuclear cells as an equine joint therapy	237
Nightengale	Marlie	335	Serum vitamin D levels in Hispaniolan Amazon parrots (<i>Amazona ventralis</i>) housed indoors over time	238
Nijveldt	Eline	336	Effects of leptin on equine lamellar keratinocytes: potential role in endocrinopathic laminitis	238
Oates	Andrew	337	Impaired expression of AP Endonuclease-1 impairs fetal development and leads to embryonic loss	239
Olivencia	Pedro	338	Excisional wound healing in diabetic and nondiabetic mice treated with amnion-derived matrix- Pilot Study	239
Olson	Michaela	339	Investigating the respiratory virome of backyard poultry and need for owner education about poultry pathogens	240

Last Name	First Name	Poster #	Abstract Title	Page #
Oppler Jr	Scott	340	Cytokine expression after porcine islet xenotransplantation in immunosuppressed and tolerant nonhuman primates	240
Oswald	Meghan	341	The effect of transdermal flunixin meglumine on resolution of otitis media in pre-weaned Holstein heifers	241
Overcast	Macon	342	Surveillance and genomic characterization of AMR <i>E. coli</i> and from an ovine colony on Navajo Nation	241
Padilla Velez	Lyan	343	Genomics of methicillin-resistant <i>Staphylococcus pseudintermedius</i>	242
Palillo	Michael	344	Anti-inflammatory cytokine profile of synovium constructs	242
Paneitz	Tyler	345	Establishing healthy baseline blood chemistry values in native Missouri bat species	243
Parker	Paxton	346	Effects of a kisspeptin analog on serum concentrations of LH, FSH, and testosterone in prepubertal bulls	243
Parker	Tracy	347	Travelers' diarrhea: <i>Providencia alcalifaciens</i> can infect humans and calves, but what about mice?	244
Peace	Michaela	348	Nutritional practices, knowledge, attitudes and perceptions of clients at a small animal teaching hospital	244
Peagler	Kristen	349	Effects of developmental programming with EDCs on hypothalamic monoamines and neuroendocrine functions	245
Pearson	Morgan	350	Correlates of mucosal immune control of feline enteric coronavirus replication	245
Peck	Chanera	351	Breed association of natural killer (NK) cell number and function in dogs	246
Perdew	Irina	352	Evaluation of the BRAF mutation in canine gliomas	246
Peters	Jordan	353	Establishing hematologic and biochemistry reference values in common leopard geckos (<i>Eublepharis macularius</i>)	247
Peterson	Marisa	354	Isolation of <i>Staphylococcus pseudintermedius</i> bacteriophage	247
Phillips	Robert	355	Detection of the invasive fish parasite <i>B. acheilognathi</i> using loop-mediated isothermal amplification (LAMP)	248
Pickett	Monica	356	Intestinal dysbiosis in dogs with atopic dermatitis	248
Piechowski	Brianna	357	Using multiplex assays to screen for tick-borne pathogens in animals and ticks of Grenada, West Indies	249
Pieczynski	Jordan	358	Canine endometrial and ovarian histology throughout the estrous cycle	249
Pierce	Jack	359	Radiographic quantification of tibial long axis shift using the anatomical-mechanical axis angle	250
Pollard	Anna	360	Peroxisome proliferator-activated receptors pathway utilized to control lipid metabolism in Dengue infection	250
Pollema	Jodi	361	Test method validation for detection of vitamin D (25OHD) in the black rhinoceros (<i>Diceros bicornis spp.</i>)	251
Polniaszek	Mika	362	Effects of routine hoof trimming on hindhoof force distribution biomechanics at midstance in horses	251
Pope	Emily	363	RNA-Seq identifies mutually exclusive driver insertions in PI3K-hyperactive mammary carcinomas	252
Prabhakar	Sumana	364	Evaluating accuracy of AliveCor Vet ECG device for measuring heart rate of dogs with atrial fibrillation	252
Pratt	Haley	365	Expansion of biliary tree stem cells through culture in conditions for intestinal stem cell expansion	253

Last Name	First Name	Poster #	Abstract Title	Page #
Price	Anna	366	Discovery of inhibitors against the <i>Cryptosporidium parvum</i> lactate dehydrogenase (CpLDH)	253
Pyrdek	Dorothy	367	Comparison of injectable and transdermal flunixin meglumine effects on pregnancy after embryo transfer in cows	254
Queffeuilou	Alexis	368	3D imaging of lineage tracing in the mouse prostate	254
Quinn	TJ	369	Purification of mouse embryonic stem cell derived cardiomyocytes (mESC-CMs) by metabolic selection	255
Rajeev	Meenakshi	370	Utilizing microsatellites to track genetic diversity in <i>Cytauxzoon felis</i>	255
Ramos	Alexandra	371	Improvement of bioink accommodating stem cells for bio-fabrication of cartilage	256
Ray	Victoria	372	Pilot project to determine benefit of BVD testing along with Iowa Green tag preconditioning program	256
Redd	Katherine	373	The effect of cannabidiol on seizure activity in uncontrolled canine epilepsy	257
Reed	Leslie	374	Transfection of bovine kidney cells with mRNA for the human cathelicidin, LL-37	257
Regas	April	375	Testing the detection of DNA barcoded microcrystals as a novel mosquito marking method	258
Reinke	Haley	376	Determining the effect of obesity on stem cell growth, proliferation, and the incidence of breast cancer	258
Remerowski	Shannon	377	Anti-ELTD1 monoclonal antibody therapy improves survival times and tumor volumes in mice with glioblastoma	259
Repella	Melanie	378	Ticking all the boxes: bureaucracy, research, and reality in reports of tick-borne diseases	259
Rice	Diana	379	Transmission reduction of Marek's disease by HVT vaccination	260
Richter	Sonja	380	Creation of anatomical framework for gene expressions in brain areas implicated in language and communication	260
Ridenour	Caitlyn	381	Dysregulation of the microRNAs let-7a and let-7b in canine urothelial carcinoma	261
Rieland	Katelyn	382	Biosecurity: Effect of UV germicidal chambers	261
Riker	Jesse	383	Investigation of bubr1 expression in zebrafish cancers	262
Risalvato	Jacqueline	384	Mump virus nucleoprotein domain "top" affects defective interfering particle production	262
Roati	Valeria	385	Tumor suppressor gene copy number loss in horse lymphomas	263
Robbins	Gabrielle	386	<i>Sos1</i> as a novel oncogene in canine leukemia	263
Rodriguez	Emerald	387	Investigating miRNA-29a as a biomarker for increased intestinal permeability in dogs	264
Rodriguez	Trevor	388	Mechanism and cross-resistance patterns of methicillin resistance in <i>Staphylococcus</i> in companion animals	264
Roeder	Megan	389	Towards safer and more effective NSAID dosage regimens for birds	265
Rogers	Callie	390	Evaluation of the feline ocular microbiome before and after treatment with topical antibiotic therapy	265

Last Name	First Name	Poster #	Abstract Title	Page #
Rogich	Allison	391	Evaluation of cytokine production by human natural killer cells expressing engineered Fc receptors	266
Rosen	Sydney	392	Prospective evaluation of orthoses in canine patients	266
Roveto	Jennifer	393	Evaluation of the role of IFN- γ , IL-10 and TNF- α in treatment of heartworm-infected dogs	267
Rowe	John	394	An intranasal vaccination strategy to improve protection against respiratory pathogens	267
Rudnick	Meredith	395	Safe method for digital enlargement of the epiploic foramen	268
Sagot	Coline	396	The role of the dorsal hippocampus in early stage memory reconsolidation	268
Saldinger	Laurel	397	Adipose-derived mesenchymal stem cells as a potential therapy for equine recurrent uveitis	269
Sannajust	Kimberley	398	The influence of engineered nanomaterials on corneal epithelial and fibroblast cell viability and migration	269
Schmidt	Robert	399	Epidemiology of mastitis pathogens on Amish dairy farms in Missouri	270
Schroeder	Anastasia	400	Effect of Enrofloxacin treatment on the prevalence of fluoroquinolone resistant <i>Campylobacter</i> in cattle	270
Schuber	Rebecca	401	Evidence for use of end-expiratory CT in dogs with chronic coughing	271
Schultz	Devyn	402	Comparison of survival among different categories of chronic kidney disease in dogs	271
Schwartz	Katherine	403	Internal parasite loads of wild black bears (<i>Ursus americanus</i>) via fecal testing	272
Schwirian	Aumbriel	404	Effects of liver cell surface glycoconjugates on invasion by <i>Plasmodium berghei</i>	272
Scott	Sarah	405	Bovine antibody response to immunization against <i>Dermacenter andersoni</i> midgut and salivary gland	273
Seelman	Amanda	406	Do California sea lions with temporal lobe epilepsy have fewer CB1 ⁺ inhibitory inputs onto granule cells?	273
Sekhar	Maya	407	Delineating the enzymatic versus non-enzymatic roles of fibroblast activation protein in collagen remodeling	274
Shaffer	Samantha	408	Equine intestinal microbiome; the future of enteric disease prognostics	274
Shaver	Kaylie	409	Insulin resistance and its impact on circadian rhythms of glucose metabolism in hibernating grizzly bears	275
Shelton	Layne	410	Transstadial transmission of <i>Mycobacterium leprae</i> in experimentally infected <i>Amblyomma maculatum</i> ticks	275
Shepley-McTaggart	Ariel	411	Genetic characterization of <i>Cryptosporidium</i> in wild chimpanzees	276
Shroff	Kavita	412	Calcium regulation of NF- κ B signaling in lymphocytes	276
Simmons	Taylor	413	Association between dysregulated non-canonical NF- κ B signaling and eosinophilic esophagitis pathogenesis	277
Singh	Benjamin	414	Cross-sectional second harmonic generation (SHG) histology of healing equine flexor tendons	277
Singh	Maegha	415	Detecting early cartilage degradation by nuclear magnetic resonance spectroscopy in a mechanical injury model	278

Last Name	First Name	Poster #	Abstract Title	Page #
Skolnik	Hannah	416	Immunoprofile of a novel soluble PD-L1 and PD-L2 inhibitor for use in cancer	278
Smith	Anthony	417	TolC and other <i>in vivo</i> virulence factors implicated using <i>Ehrlichia</i> HF transposon mutants	279
Smith	Elysha	418	Preliminary retrospective review of important pathogens in small poultry flocks in Ontario, Canada	279
Smith	Levi	419	Idly infected: a review of infectious agents in populations of two-toed sloths (<i>Choloepus</i> spp.)	280
Smith	Natalie	420	Evaluating biomarkers for pharmacodynamic monitoring of glucocorticoid therapy	280
Smith	Skylare	421	Loss of transcription factor FoxJ1 alters basal behavior of mice in a novel environment	281
Smith	Veronica	422	Risk of respiratory and parasitic disease in shelter dogs that participate in playgroups	281
Sneed	Skye	423	High vector-borne <i>Haemosporidia</i> prevalence in eastern Sandhill Cranes (<i>Antigone canadensis</i>) over two decades	282
Snyder	Kristin	424	Enhancing human NK Fc receptors to improve tumor cell conjugation and killing	282
Sobolewski	Josey	425	Assessing the readability of veterinary clinical trial consent forms	283
Sommerkamp	Ashley	426	Phytocannabinoid metabolism by canine cytochrome P450s	283
Spears	Corwin	427	Phage display for identification of peptides that bind zika virus surface exposed protein e	284
Special	Samantha	428	Identification of pathogenic bacteria derived from natural water sources	284
Speelman	Marguerite	429	Effect of medium-chain triglycerides compared to long-chain triglycerides on enterohormones in healthy dogs	285
Stahl	Rachel	430	Role of HTLV-1 HBZ in transformation and disease	285
Stapley	Emma	431	Turning the oligonucleotide on equine osteoarthritis: an evaluation of a novel anti-DEK aptamer	286
Stella	Stephanie	432	Effects of Nerve Growth Factor- β on ovulatory cascade factors from follicular fluid of pre-ovulatory follicles	286
Stempien	Kaitlyn	433	Impact of dosing regimen of danofloxacin on intestinal pharmacokinetics and the fecal microbiota of steers	287
Stoneburner	Regan	434	Comparison of stress abatement strategies for feline friendly housing	287
Stuebing	Sarah	435	Assessing the likelihood of epizootic hemorrhagic disease virus-2 infection in <i>Culicoides sonorensis</i>	288
Stutzman	Brady	436	Effects of therapeutic ultrasound on passive range of motion and lameness in osteoarthritic horses	288
Sumano	Daniela	437	The use of a head camera for video self-assessment of surgical performance of veterinary students	289
Szafranski	Nicole	438	Investigation of Haemosporida parasites of wild turkeys	289
Sztukowski	Keira	439	HIV-induced neuronal hyperactivity and neuropathology in the FIV infection model	290
Tarpalechee	Layna	440	Survey study to estimate <i>Trypanosoma cruzi</i> infection prevalence in domestic dogs and kissing bugs in Oklahoma	290

Last Name	First Name	Poster #	Abstract Title	Page #
te Riele	Gigi	441	Role of estrogen receptor in the gestational upregulation of AT ₂ R in rat uterine arteries	291
Therriault	Lauren	442	Genomic analysis of the <i>SLC3A1/SLC7A9</i> renal transporter in androgen-dependent cystinuria across canine breeds	291
Thomas	Liam	443	Development of a rapid, affordable system to identify breed-specific progressive retinal atrophy genes in dogs	292
Thornton	Jude	444	Effects of prenatal programming on hypertension related genes in the brainstem	292
Thulson	Emily	445	Analysis of cardiopulmonary values in chemically immobilized white rhinoceros in Kruger National Park	293
Tiessen	Angela	446	Molecular prevalence of <i>Neorickettsia risticii</i> in aquatic invertebrates in Ontario	293
Tocco	Natalie	447	Total cell counts of equine bronchoalveolar lavage samples using ImageJ software	294
Tomasino	Allison	448	Effect of cocoa flavanols on endothelial nitric oxide synthase (eNOS) expression in brains of aged mice	294
Torres	Angelo	449	<i>Chlamydia</i> -activated B cells prime CTL activity that controls tumor growth in mice with HPV-associated cancer	295
Toth	Darby	450	Effects on mitochondrial respiration of rat gut epithelium chronically exposed to angiotensin II	295
Tran	Danielle	451	Effects of cells and proteins on cerebrospinal fluid MRI FLAIR signal	296
Tran Hoang	Christine	452	The effect of simvastatin on CD44 shedding in canine osteosarcoma	296
Travis	Sierrah	453	Impact of gut microbiota on oligodendrocyte development and maturation	297
Tribble	Jack	454	Cloning and expression of feline calicivirus P domain protein	297
Trutwin	Victoria	455	Development of novel vaccines and immunological reagents for pathogenic <i>Brucella</i> species	298
Tu	Darleen	456	BONSAI 2: Effect of non-steroidal anti-inflammatory drugs on bone infection and response to antibiotic therapy	298
Tufts	Sara	457	Intestinal stem cell reparative response to severe ischemic injury	299
Turcios	Rebecca	458	Quantitative PCR assay development for the detection of <i>Ambystoma tigrinum tigrinum</i> in eDNA samples	299
Turnbull	Katherine	459	The transcriptional response of endothelial cells to statin treatment <i>in vivo</i>	300
Unger	Linn Meret	460	The Role of the Golgi Complex in <i>Trypanosoma cruzi</i> Calcium Homeostasis	300
Valente	Monica	461	Indirect impacts of invasive Burmese python introduction in South Florida on the spread of Everglades virus	301
Valomy	Mia	462	Is parenting associated with changes in structural and synaptic plasticity in the prairie vole ?	301
Van	Victoria	463	Correlation of anti-canine parvovirus serum immunoglobulin G and fecal immunoglobulin A in vaccinated dogs	302
van den Brink	Lianne	464	Validating Cr-EDTA as an <i>in vivo</i> measure of barrier function in feed-restricted lactating dairy cows	302

Last Name	First Name	Poster #	Abstract Title	Page #
Van Hesteren	Matthew	465	MLN4924 (pevonedistat) inhibits growth and induces apoptosis in canine oral melanoma cell lines	303
van Kan	Tess	466	Monitoring for arbovirus in mid-Missouri by local tick sampling and viremia in domestic and wild animals	303
Vander Wiel	Caleb	467	Effect of intravenous Maropitant on blood pressure in critically ill dogs and cats; preliminary results	304
Varela-Ortiz	Alexandra	468	Comparison of in vitro susceptibility of <i>Tritrichomonas foetus</i> to metronidazole and fenbendazole	304
Varkey	Jane	469	Reducing captive bird stress using videos and reward-based positive reinforcement	305
Varner	Jennifer	470	Anti-inflammatory properties of firocoxib in equine pregnancy and placentitis	305
Vaughan	Lyndsi	471	Use of intradermal syringe adapter on bovine skin samples	306
Verdoorn	Kristen	472	Protein conservation: urea nitrogen salvage by gut microbiota in the hibernating 13-lined ground squirrel	306
Vogel	Helena	473	Molecular analysis of <i>Cryptosporidium</i> spp., <i>Giardia lamblia</i> , and <i>Entamoeba</i> spp. in asymptomatic humans	307
Voudren	Caylie	474	Engineering 3D model to study metastasis of breast cancer to bone	307
Vrana	Morgan	475	Role of gut metabolome in the colonization of <i>Clostridium perfringens</i> in black-footed ferrets	308
Walden	Brian	476	Cultivation and maturation of feline enteroids utilizing trans-well inserts and WRN-conditioned media	308
Walker	Grayson	477	The role of <i>Enterococcus faecalis</i> in the modulation of avian pathogenic <i>E. coli</i> infections of poultry	309
Wallace	Amy	478	PCR identification of specific BRD pathogens and their integrative conjugative elements	309
Wallace	Miranda	479	Validity and reliability of heart rate variability measurement to assess stress in horses	310
Wallis	Michael	480	Blood coagulation assessment of captive Asian Elephants (<i>Elephas maximus</i>): a pilot study	310
Walton	Julia	481	Developing a vaccine against <i>Brucella</i>	311
Wanderer	Mara	482	Clinical utility of obtaining both right and left lateral abdominal radiographs in cats	311
Wang	Christine	483	Molecular epidemiology of enteric pathogens among livestock in small-scale Sri Lankan farms	312
Wang	Mengyue	484	Developing a point-of-care diagnostic test for viral hemorrhagic septicemia	312
Wattenburger	Kenzi	485	Evaluation of aseptic techniques used to collect bovine milk samples	313
Weatherford	Samantha	486	A case of necrotizing purulent pleuropneumonia in a White-tailed Deer	313
Weaver	Phoebe	487	Determination of famciclovir concentrations in compounded formulations	314
Weston	Corinne	488	Effects of long-acting GLP-1 receptor agonist on glucose and insulin metabolism in alpacas	314
Weyand	Logan	489	Fatal pneumonia in bighorn sheep lambs: the critical role of <i>Mycoplasma ovipneumoniae</i> carrier ewes	315

Last Name	First Name	Poster #	Abstract Title	Page #
White	Anne	490	Genomic characterization of canine squamous cell carcinomas	315
White	Jaquia	491	Role of annexin A2 in plasmin induced macrophage activation	316
White	Joel	492	The clinical utility of a targeted smart phone application in a veterinary setting	316
White	Melissa	493	Validation of PFA-100 to measure platelet function in equine whole blood and platelet-rich plasma	317
Wijetunge	Nadeeshani	494	Role of HMGB1 in angiotensin II induced inflammation: in-vitro study using murine neuronal cells	317
Williams	Donte	495	Diamond microelectrodes for electroanalytical measurements of catechol amine neurotransmitters and metabolites	318
Williams	Zoe	496	Proteomic profiling of Warmblood horses with myofibrillar myopathy	318
Willis	Elinor	497	A genetic-based vaccine overcomes maternal antibody inhibition of immune responses	319
Wise	Elisabeth	498	Development of a clinical electrophysiological protocol to separate cone subclass function in dogs	319
Wolff	Stephanie	499	Comparison of bupivacaine HCl and liposomal bupivacaine for post-operative pain with dental extractions	320
Womble	William	500	Blood parameters and oxidative damage in the aging zebra finch	320
Wood	Kellie	501	Non-immune binding of <i>Staphylococcus pseudintermedius</i> and <i>Staphylococcus schleiferi</i> to IgG of various species	321
Wright	Sarah	502	miR-375 controls intestinal proliferative capacity	321
Wronkowski	Ann Marie	503	Dose escalation study of subcutaneous sustained-release meloxicam in mice	322
Yan	Yuqi	504	Tear fluid pharmacokinetics following oral prednisone administration in dogs with or without conjunctivitis	322
Yates	Bari	505	The evaluation of microcirculatory perfusion in healthy horses treated for hypotension	323
Ye	Danling	506	Investigating the role of cyclin N-terminal domain-containing I in regulating meiotic prophase I	323
Yu	Serene	507	Prevalence of common tick-borne diseases in White-tailed deer and coyotes in south Texas	324
Zakers	Damali	508	Association of canine malignancy with complete blood count and serum biochemistry abnormalities	324
Zalar	Sarah	509	Murine intestinal enteroid cultures and detection of norovirus infection	325
Zanetti	Alexander	510	Effects of a manipulated gut microbiota on lung histopathology in a murine model of allergen mediated asthma	325
Zheng	Junxian	511	Evaluation of olfactory learning in mice with perinatal differentiation defects in the olfactory bulb	326

Symposium Participants by College of Veterinary Medicine

Vet School	Last Name	First Name	Poster #
Atlantic Veterinary College	Begin	Samantha	24
Atlantic Veterinary College	Bell	Christina	27
Atlantic Veterinary College	Belliveau	Patrick	28
Atlantic Veterinary College	Duguay	Marco	120
Atlantic Veterinary College	Egers	Amanda	124
Atlantic Veterinary College	Gorrill	Angelina	174
Atlantic Veterinary College	Groody	Celia	180
Atlantic Veterinary College	Lilley	Carly	262
Atlantic Veterinary College	Peace	Michaela	348
Atlantic Veterinary College	Tocco	Natalie	447
Auburn University CVM	Hennessy	Elise	199
Auburn University CVM	Weatherford	Samantha	446
Colorado State University CVM	Bissinger	David	35
Colorado State University CVM	Bleicher	Shira	38
Colorado State University CVM	Bordman	Aryn	43
Colorado State University CVM	Brown	Shaina	52
Colorado State University CVM	Drizin	Sienna	118
Colorado State University CVM	Ellis	Jayne	129
Colorado State University CVM	Ellis	Megan	130
Colorado State University CVM	Ericksen	Kelsea	131
Colorado State University CVM	Geldert	Christina	164
Colorado State University CVM	Hawks	Meighan	195
Colorado State University CVM	Kastendieck	Emily	225
Colorado State University CVM	Kechejian	Sarah	227
Colorado State University CVM	Krause	Laurel	243
Colorado State University CVM	Krishnan	Sneha	245
Colorado State University CVM	Mancha	Serena	280
Colorado State University CVM	Mishkin	Noah	311
Colorado State University CVM	Pierce	Jack	359
Colorado State University CVM	Pollard	Anna	360
Colorado State University CVM	Price	Anna	366
Colorado State University CVM	Redd	Katherine	373
Colorado State University CVM	Regas	April	375
Colorado State University CVM	Roeder	Megan	389
Colorado State University CVM	Rosen	Sydney	392
Colorado State University CVM	Sztukowski	Keira	439
Colorado State University CVM	Thulson	Emily	445
Cornell University CVM	Acevedo	Andrew	2
Cornell University CVM	Berenson	Joshua	29

Vet School	Last Name	First Name	Poster #
Cornell University CVM	Ceres	Kristina	74
Cornell University CVM	Chen	Frances	79
Cornell University CVM	Chen	Kimberly	81
Cornell University CVM	Cockey	James	92
Cornell University CVM	D Agostino	Albert	108
Cornell University CVM	Donohue	Laura	117
Cornell University CVM	Feller	Laine	139
Cornell University CVM	Gludish	David	169
Cornell University CVM	Jacobs	Julia	218
Cornell University CVM	Koch-Laskowski	Kieran	240
Cornell University CVM	Lachenauer	Erica	248
Cornell University CVM	Lavin	Elizabeth	251
Cornell University CVM	Loehr	Amanda	267
Cornell University CVM	Merry	Hillary	302
Cornell University CVM	Oswald	Meghan	341
Cornell University CVM	Turcios	Rebecca	458
Cornell University CVM	Wright	Sarah	502
Cornell University CVM	Ye	Danling	506
Cummings SVM at Tufts University	Belgrad	Joseph	26
Cummings SVM at Tufts University	Ineson	Deanna	217
Cummings SVM at Tufts University	Krienke	Leslie	244
Cummings SVM at Tufts University	Lugardo	Josue	273
Cummings SVM at Tufts University	Smith	Natalie	420
École Nationale Vétérinaire d'Alfort	Sagot	Coline	396
École Nationale Vétérinaire d'Alfort	Valomy	Mia	462
Georgetown College - Undergraduate Student	Sobolewski	Josey	425
Iowa State University CVM	Barrett	Erin	18
Iowa State University CVM	Hawbecker	Tyler	194
Iowa State University CVM	Hines	Elizabeth	205
Iowa State University CVM	Kirner	Nicolette	236
Iowa State University CVM	Pearson	Morgan	350
Iowa State University CVM	Pollema	Jodi	361
Iowa State University CVM	Ray	Victoria	372
Iowa State University CVM	Schroeder	Anastasia	400
Iowa State University CVM	Turnbull	Katherine	459
Iowa State University CVM	Vander Wiel	Caleb	467
Iowa State University CVM	Yan	Yuqi	504
Kansas State University	Chen	Kaiwen	80
Kansas State University	Fitzgerald	Alyson	144
Kansas State University	Hastings	Kaitlyn	193
Kansas State University	Herford	Jacob	201
Kansas State University	Lancaster	Meghan	250
Kansas State University	Lewy	Keith	259
Kansas State University	Stuebing	Sarah	435

Vet School	Last Name	First Name	Poster #
Lincoln Memorial University CVM	Addison	Molly	7
Lincoln Memorial University CVM	Beigel	Brittney	25
Lincoln Memorial University CVM	Kish	Daniel	237
Lincoln Memorial University CVM	Maness	Aaron	281
Louisiana State University SVM	Barras	Elise	17
Louisiana State University SVM	Beckers	Kalie	22
Louisiana State University SVM	Bertrand	Amanda	32
Louisiana State University SVM	Brooks	Charles	49
Louisiana State University SVM	DeRouen	Abigail	112
Louisiana State University SVM	Dwyer	Elizabeth	123
Louisiana State University SVM	Garcia-Abarca	Nicolas	162
Louisiana State University SVM	Gravette	Elizabeth	178
Louisiana State University SVM	Guillen	Sara	183
Louisiana State University SVM	Hymel	Paige	216
Louisiana State University SVM	Markle	Mariah	286
Louisiana State University SVM	Michel	Jeanne	305
Louisiana State University SVM	Mikes	Alison	306
Louisiana State University SVM	Miller	Lillian	308
Louisiana State University SVM	Nightengale	Marlie	335
Louisiana State University SVM	Peters	Jordan	353
Louisiana State University SVM	Ramos	Alexandra	371
Louisiana State University SVM	Schuber	Rebecca	401
Louisiana State University SVM	Shelton	Layne	410
Louisiana State University SVM	Walden	Brian	426
Louisiana State University SVM	Wanderer	Mara	482
Louisiana State University SVM	Wijetunge	Nadeeshani	494
Michigan State University CVM	White	Jaquia	491
Michigan State University CVM	Acosta-Bencosme	Joanna	5
Michigan State University CVM	Aiello	Taylor	8
Michigan State University CVM	Aldrich	Kennedy	9
Michigan State University CVM	Butler	Hailee	61
Michigan State University CVM	Chen	Yue (Polly)	82
Michigan State University CVM	Everett	Terry	135
Michigan State University CVM	Faryean	Joe	136
Michigan State University CVM	Fowler	Peter	148
Michigan State University CVM	Frantz	Brandon	150
Michigan State University CVM	Gerras	Allison	166
Michigan State University CVM	Gruenwald	Samantha	181
Michigan State University CVM	Harris	Cassidy	188
Michigan State University CVM	Harro	Cailin	189
Michigan State University CVM	Kessler	Jessica	231
Michigan State University CVM	Lund	Lindsey	275
Michigan State University CVM	Mark	Michael	285
Michigan State University CVM	McDowell	Makenzie	297

Vet School	Last Name	First Name	Poster #
Michigan State University CVM	Negron Camacho	Marie	326
Michigan State University CVM	Ngene	Chioma	333
Michigan State University CVM	Pieczynski	Jordan	358
Michigan State University CVM	Prabhakar	Sumana	364
Michigan State University CVM	Rice	Diana	379
Michigan State University CVM	Stapley	Emma	431
Michigan State University CVM	Stutzman	Brady	436
Michigan State University CVM	Thomas	Liam	443
Michigan State University CVM	Williams	Donte	495
Michigan State University CVM	Williams	Zoe	496
Michigan State University CVM	Zanetti	Alexander	510
Midwestern University CVM	Blakeman	Cody	37
Midwestern University CVM	Eisemann	Robert	126
Midwestern University CVM	Lisowski	Sasha	265
Midwestern University CVM	McAfee	Erika	293
Midwestern University CVM	Nelson	Gaberrail	328
Midwestern University CVM	Parker	Tracy	347
Midwestern University CVM	Smith	Veronica	422
Midwestern University CVM	Torres	Angelo	449
Midwestern University CVM	Vrana	Morgan	475
Midwestern University CVM	Wolff	Stephanie	499
Midwestern University CVM	Wronkowski	Ann Marie	503
Mississippi State University CVM	Brown	Clare	51
Mississippi State University CVM	Durfey	Casey	122
Mississippi State University CVM	Eidson	Anika	125
Mississippi State University CVM	Foust	Bethany	147
Mississippi State University CVM	Frankovich	Alexandra	149
Mississippi State University CVM	Ku	Tobi	246
Mississippi State University CVM	Majors	Kara	278
Mississippi State University CVM	McConnell	Sydney	295
Mississippi State University CVM	Nelson	Katelynn	329
Mississippi State University CVM	Reed	Leslie	374
Mississippi State University CVM	Schwirian	Aumbriel	404
Mississippi State University CVM	Wallace	Amy	478
North Carolina State University CVM	Anderson	Claretha	10
North Carolina State University CVM	Báez-Ramos	Patricia	13
North Carolina State University CVM	Bagley	Kristen	14
North Carolina State University CVM	Barnes	Danielle	16
North Carolina State University CVM	Blake	Jacqueline	36
North Carolina State University CVM	Bowie	Aleaya	45
North Carolina State University CVM	Buckingham	Annie	55
North Carolina State University CVM	Burdette	Thomas	57
North Carolina State University CVM	Bussey- Spratling	Jayda	60
North Carolina State University CVM	Carson	Megan	70

Vet School	Last Name	First Name	Poster #
North Carolina State University CVM	Chari	Miriam	77
North Carolina State University CVM	Clontz	Dallas	87
North Carolina State University CVM	DeProspero	Dylan	111
North Carolina State University CVM	Freitas	Nicole	152
North Carolina State University CVM	Gidcumb	Emily	168
North Carolina State University CVM	Gonzalez	Gabriel	172
North Carolina State University CVM	Gupta	Ankita	185
North Carolina State University CVM	Harver	Dylan	192
North Carolina State University CVM	Jindal	Abhilasha	220
North Carolina State University CVM	Meier	Elisa	300
North Carolina State University CVM	Montoya	Sarah	316
North Carolina State University CVM	Nelson	Valerie	330
North Carolina State University CVM	Perdew	Irina	352
North Carolina State University CVM	Pratt	Haley	365
North Carolina State University CVM	Riker	Jesse	383
North Carolina State University CVM	Smith	Skylare	421
North Carolina State University CVM	Stempien	Kaitlyn	433
North Carolina State University CVM	Stoneburner	Regan	434
North Carolina State University CVM	Tufts	Sara	457
North Carolina State University CVM	Walker	Grayson	477
North Carolina State University CVM	Wang	Christine	483
North Carolina State University CVM	White	Anne	490
North Carolina State University CVM	Wise	Elisabeth	498
North Carolina State University CVM	Zheng	Junxian	511
Ohio State University CVM	Adams	Rachael	6
Ohio State University CVM	Boisvert	Natalie	40
Ohio State University CVM	Bookenberger	Lane	41
Ohio State University CVM	Carman	Michelle	68
Ohio State University CVM	Courtney	Lindsay	100
Ohio State University CVM	Ferguson	Lindsey	140
Ohio State University CVM	Fernandez	Mariana	141
Ohio State University CVM	French-Kim	Kristen	153
Ohio State University CVM	Garcia	Jessica	161
Ohio State University CVM	Hengy	Miranda	198
Ohio State University CVM	Kendziorski	Jessica	228
Ohio State University CVM	Kim	Alan	232
Ohio State University CVM	Kim	Jeffrey	233
Ohio State University CVM	King	Emily	235
Ohio State University CVM	Kitsberg	Rebecca	238
Ohio State University CVM	Kuerbitz	Ellen	247
Ohio State University CVM	Lewis	Chaunte	257
Ohio State University CVM	Lordi	Nicholas	271
Ohio State University CVM	Makii	Rebecca	279
Ohio State University CVM	McAuley	Rachel	294

Vet School	Last Name	First Name	Poster #
Ohio State University CVM	Minich	David	310
Ohio State University CVM	Monje	Daniela	314
Ohio State University CVM	Nelson	Andrew	327
Ohio State University CVM	Nijveldt	Eline	336
Ohio State University CVM	Overcast	Macon	342
Ohio State University CVM	Palillo	Michael	344
Ohio State University CVM	Peck	Chanera	351
Ohio State University CVM	Rowe	John	394
Ohio State University CVM	Singh	Benjamin	414
Ohio State University CVM	Smith	Anthony	417
Ohio State University CVM	Stahl	Rachel	430
Oklahoma State University CVM	Hunter	Alexa	214
Oklahoma State University CVM	Johnson	Morgan	223
Oklahoma State University CVM	Kern	Kimberly	229
Oklahoma State University CVM	Remerowski	Shannon	377
Oklahoma State University CVM	Schwartz	Katherine	403
Oklahoma State University CVM	Tarpalechee	Layna	440
Oklahoma State University CVM	Vaughan	Lyndsi	471
Oklahoma State University CVM	Wallis	Michael	480
Oklahoma State University CVM	Womble	William	500
Ontario Veterinary College	Botts	Michaela	44
Ontario Veterinary College	Burns	Jamie	58
Ontario Veterinary College	Madsen	Virginia	277
Ontario Veterinary College	Roati	Valeria	385
Ontario Veterinary College	Smith	Elysha	418
Ontario Veterinary College	Tiessen	Angela	446
Oregon State University CVM	Callahan	Shawn	63
Oregon State University CVM	Eldridge	Laura	127
Oregon State University CVM	Hogen	Katie	208
Oregon State University CVM	Weston	Corinne	489
Purdue University CVM	Auld	Claudine	12
Purdue University CVM	Becerra	Roel	20
Purdue University CVM	Bersch	Kevin	31
Purdue University CVM	Boone	Eric	42
Purdue University CVM	Canada	Sara	65
Purdue University CVM	Cheatham	Christa	78
Purdue University CVM	Coakley	Chad	89
Purdue University CVM	Gundlach	Carly	184
Purdue University CVM	Hooser	Blair	210
Purdue University CVM	Hopkins	Ashley	211
Purdue University CVM	Linder	Jessica	264
Purdue University CVM	Mapes	Christopher	284
Purdue University CVM	Ridenour	Caitlyn	381
Purdue University CVM	Smith	Levi	419

Vet School	Last Name	First Name	Poster #
Ross University SVM	Johnson	Kelsey	222
Ross University SVM	Morales Coll	Valerie	319
Royal (Dick) School of Veterinary Studies	Dubowitz	Rebecca	119
St Georges University SVM	Gerbus	Alyssa	165
St Georges University SVM	Hicks	Kelly	202
St Georges University SVM	Murray	Katherine	323
St Georges University SVM	Piechowski	Brianna	357
Texas A&M University CVM	Burns	Madison	59
Texas A&M University CVM	Castro	Andres	71
Texas A&M University CVM	Cigarroa	Annalis	84
Texas A&M University CVM	Cocke	Natalie	91
Texas A&M University CVM	Floyd	Austin	145
Texas A&M University CVM	Folmar	Chelsea	146
Texas A&M University CVM	Graf	Lynanne	176
Texas A&M University CVM	Manriquez	Michael	283
Texas A&M University CVM	McDaniel	Sarah	296
Texas A&M University CVM	McNew	James	299
Texas A&M University CVM	Michalski	Hannah	304
Texas A&M University CVM	Neverson	Jade	332
Texas A&M University CVM	Peterson	Marisa	354
Texas A&M University CVM	Pickett	Monica	356
Texas A&M University CVM	Rodriguez	Emerald	387
Texas A&M University CVM	Rogers	Callie	390
Texas A&M University CVM	Schultz	Devyn	402
Texas A&M University CVM	Sneed	Skye	423
Texas A&M University CVM	Varkey	Jane	469
Texas A&M University CVM	Voudren	Caylie	474
Texas A&M University CVM	White	Joel	492
Texas A&M University CVM	Yu	Serene	507
Tuskegee University SVM	Brock	Jasmine	48
Tuskegee University SVM	Buck	Cameron	54
Tuskegee University SVM	Carn	Derek	69
Tuskegee University SVM	Coleman	Kaitlyn	94
Tuskegee University SVM	Deadrick	Sheree	110
Tuskegee University SVM	Galbreth	William	159
Tuskegee University SVM	Hayes	Shantavia	196
Tuskegee University SVM	Hiscocks	Kenneth	206
Tuskegee University SVM	Lezcano	Victor	260
Tuskegee University SVM	Olivencia	Pedro	338
Tuskegee University SVM	Spears	Corwin	427
Tuskegee University SVM	Special	Samantha	428
Tuskegee University SVM	Varela-Ortiz	Alexandra	468
Tuskegee University SVM	Zakers	Damali	508
University of California - Davis SVM	Chang	Christina	75

Vet School	Last Name	First Name	Poster #
University of California - Davis SVM	Cheung	Hilvy	83
University of California - Davis SVM	Cohen	Jonathan	93
University of California - Davis SVM	Cossaboon	Jennifer	98
University of California - Davis SVM	Crofton	Amanda	105
University of California - Davis SVM	Fuetsch	Stephanie	156
University of California - Davis SVM	Griffin	Katherine	179
University of California - Davis SVM	Hancock	Kari	187
University of California - Davis SVM	Hommel	Caelin	209
University of California - Davis SVM	Horn	Rebecca	212
University of California - Davis SVM	Huang	Patrick	213
University of California - Davis SVM	Kaufman	Mary	226
University of California - Davis SVM	Loftin	Amanda	268
University of California - Davis SVM	Maciejczyk	Kimberly	276
University of California - Davis SVM	Oates	Andrew	337
University of California - Davis SVM	Saldinger	Laurel	397
University of California - Davis SVM	Sannajust	Kimberley	398
University of California - Davis SVM	Singh	Maegha	415
University of California - Davis SVM	Skolnik	Hannah	416
University of California - Davis SVM	Tran	Danielle	451
University of California - Davis SVM	Vogel	Helena	473
University of California - Davis SVM	Weaver	Phoebe	487
University of Florida CVM	Ackerman	Leah	4
University of Florida CVM	Becker	Karlye	21
University of Florida CVM	Catarino	Olivia	72
University of Florida CVM	Dlubac	Erin	116
University of Florida CVM	Fisher	Corey	143
University of Florida CVM	Lerner	Francesca	254
University of Florida CVM	Lind	Jenny	263
University of Florida CVM	Locker	Seth	266
University of Florida CVM	Lucius	Sascha	272
University of Florida CVM	Mendieta Calle	Jorge	301
University of Florida CVM	Rudnick	Meredith	395
University of Florida CVM	Toth	Darby	450
University of Florida CVM	Valente	Monica	461
University of Florida CVM	White	Melissa	493
University of Georgia CVM	Beckworth	Katie	23
University of Georgia CVM	Bevelock	Thomas	34
University of Georgia CVM	Bloodgood	Jennifer	39
University of Georgia CVM	Buckley	Katherine	56
University of Georgia CVM	Clifton	Emily	86
University of Georgia CVM	Diaz	Alexa	114
University of Georgia CVM	Dillard	Corinne	115
University of Georgia CVM	Erwood	Eric	133
University of Georgia CVM	Golsen	Bryce	171

Vet School	Last Name	First Name	Poster #
University of Georgia CVM	Herbert	Gillian	200
University of Georgia CVM	Lawhon	Tess	252
University of Georgia CVM	Mora	Kevin	318
University of Georgia CVM	Narvaez	Juan	325
University of Georgia CVM	Peagler	Kristen	349
University of Georgia CVM	Rajeev	Meenakshi	370
University of Georgia CVM	Risalvato	Jacqueline	384
University of Georgia CVM	Roveto	Jennifer	393
University of Georgia CVM	Thornton	Jude	444
University of Georgia CVM	Trutwin	Victoria	455
University of Georgia CVM	Varner	Jennifer	470
University of Georgia CVM	Walton	Julia	481
University of Georgia CVM	Yates	Bari	505
University of Illinois CVM	Brooks	Michael	50
University of Illinois CVM	Dianis	Allison	113
University of Illinois CVM	Feldner	Mary	138
University of Illinois CVM	Gartlan	Brina	163
University of Illinois CVM	Kerr	Jessica	230
University of Illinois CVM	Levy	Ivana	256
University of Illinois CVM	Lewis	Lily	258
University of Illinois CVM	Morris	Zoe	320
University of Illinois CVM	Padilla Velez	Lyan	343
University of Illinois CVM	Repella	Melanie	378
University of Illinois CVM	Sommerkamp	Ashley	426
University of Illinois CVM	Stella	Stephanie	432
University of Illinois CVM	Tomasino	Allison	448
University of Illinois CVM	Tran Hoang	Christine	452
University of Illinois CVM	Tribble	Jack	454
University of Illinois CVM	Zalar	Sarah	509
University of Minnesota CVM	Berg	Catharina	30
University of Minnesota CVM	Bradley	Zachary	46
University of Minnesota CVM	Ellingson	Lacey	128
University of Minnesota CVM	Erickson	Janna	132
University of Minnesota CVM	Fattori	Chiara	137
University of Minnesota CVM	Han	Susie	186
University of Minnesota CVM	Hart	Sarah Niamh	190
University of Minnesota CVM	Hartson	Rochelle	191
University of Minnesota CVM	Klosowski	Marika	239
University of Minnesota CVM	Lukach	Jennifer	274
University of Minnesota CVM	Martin	Rachel	289
University of Minnesota CVM	Mayer	Treana	292
University of Minnesota CVM	Mills	Benigno Benjamin	309
University of Minnesota CVM	Montin	Karin Helena	315
University of Minnesota CVM	Olson	Michaela	339

Vet School	Last Name	First Name	Poster #
University of Minnesota CVM	Oppler Jr	Scott	340
University of Minnesota CVM	Pope	Emily	363
University of Minnesota CVM	Rieland	Katelyn	382
University of Minnesota CVM	Robbins	Gabrielle	386
University of Minnesota CVM	Rogich	Allison	391
University of Minnesota CVM	Snyder	Kristin	424
University of Minnesota CVM	Wang	Mengyue	484
University of Missouri CVM	Bax	Erin	19
University of Missouri CVM	Canda	Kaylie	66
University of Missouri CVM	Cardascio	Alexander	67
University of Missouri CVM	Clouden	Michaela	88
University of Missouri CVM	Cochran	Karlee	90
University of Missouri CVM	Cordingley	Jessica	97
University of Missouri CVM	Cowser	Madalyn	102
University of Missouri CVM	Crawford	Barbara	103
University of Missouri CVM	Crotty	Sierra	106
University of Missouri CVM	Davis	Marina	109
University of Missouri CVM	Eshelman	Amanda	134
University of Missouri CVM	Fray	Leah	151
University of Missouri CVM	Gafen	Hannah	157
University of Missouri CVM	Gibson	Blake	167
University of Missouri CVM	Gooch	Sydney	173
University of Missouri CVM	Kimes	Austin	234
University of Missouri CVM	Lamontagne	Angélique	249
University of Missouri CVM	Manley	Kristin	282
University of Missouri CVM	Moley	Charles	312
University of Missouri CVM	Molind	Katie	313
University of Missouri CVM	Mueller	Marissa	322
University of Missouri CVM	Paneitz	Tyler	345
University of Missouri CVM	Schmidt	Robert	399
University of Missouri CVM	Scott	Sarah	405
University of Missouri CVM	Shaffer	Samantha	408
University of Missouri CVM	van Kan	Tess	467
University of Missouri CVM	Wallace	Miranda	479
University of Missouri CVM	Wattenburger	Kenzi	485
University of Padova	Andreatta	Federica	11
University of Pennsylvania SVM	Cain	John	62
University of Pennsylvania SVM	Collins	Elizabeth	95
University of Pennsylvania SVM	Cotton	Renee	99
University of Pennsylvania SVM	Covo	Mariel	101
University of Pennsylvania SVM	Frink	Alexa	155
University of Pennsylvania SVM	Gallini	Stefan	160
University of Pennsylvania SVM	Goldberg	Abby	170
University of Pennsylvania SVM	Hildreth	Kimberly	204

Vet School	Last Name	First Name	Poster #
University of Pennsylvania SVM	Hlavaty	Sabina	207
University of Pennsylvania SVM	Johnson	Kaylynn	221
University of Pennsylvania SVM	Lieberman	Joshua	261
University of Pennsylvania SVM	Lonngren	Karalyn	269
University of Pennsylvania SVM	Loonsk	Hannah	270
University of Pennsylvania SVM	Moss	Kaitlyn	321
University of Pennsylvania SVM	Neupauer	Katherine	331
University of Pennsylvania SVM	Sekhar	Maya	407
University of Pennsylvania SVM	Shepley-Mctaggart	Ariel	411
University of Pennsylvania SVM	Shroff	Kavita	412
University of Pennsylvania SVM	Therriault	Lauren	442
University of Pennsylvania SVM	Willis	Elinor	497
University of Saskatchewan	Speelman	Marguerite	429
University of Tennessee CVM	Corder	Nicole	96
University of Tennessee CVM	Parker	Paxton	346
University of Tennessee CVM	Szafranski	Nicole	438
University of Tennessee CVM	Wood	Kellie	501
University of Veterinary Medicine Hannover, Foundation	Unger	Linn Meret	460
University of Veterinary Medicine Hannover, Foundation	Bettin	Leonie	33
University of Wisconsin SVM	Bakken	Marit	15
University of Wisconsin SVM	Dulli	Kelly	121
University of Wisconsin SVM	Fink	Dustin	142
University of Wisconsin SVM	Grady	Kylie	175
University of Wisconsin SVM	Granger Jr	Kyle	177
University of Wisconsin SVM	Hietpas	Margaret	203
University of Wisconsin SVM	Jenkins	Alyssa	219
University of Wisconsin SVM	Karklus	Alyssa	224
University of Wisconsin SVM	Kosanovich	DeAnna	241
University of Wisconsin SVM	Marroquin	Priscilla	287
University of Wisconsin SVM	Martin	Hannah	288
University of Wisconsin SVM	Martinelli	Laura	290
University of Wisconsin SVM	Mathe	Jennifer	291
University of Wisconsin SVM	McNally	Rachel	298
University of Wisconsin SVM	Phillips	Robert	355
University of Wisconsin SVM	Reinke	Haley	376
University of Wisconsin SVM	Van Hesteren	Matthew	465
University of Wisconsin SVM	Verdoorn	Kristen	472
Utrecht University	Hendrickx	Robert	197
Utrecht University	te Riele	Gigi	441
Utrecht University	van den Brink	Lianne	464
VetAgro Sup Lyon	Queffeulou	Alexis	368
Virginia-Maryland Regional CVM	Bradshaw	Alexander	47
Virginia-Maryland Regional CVM	Callaway	Sabrina	64
Virginia-Maryland Regional CVM	Crawford	Stephanie	104

Vet School	Last Name	First Name	Poster #
Virginia-Maryland Regional CVM	Frere	Francesca	154
Virginia-Maryland Regional CVM	Kozlov	Shawn	242
Virginia-Maryland Regional CVM	Meyerhoeffer	Mitchell	303
Virginia-Maryland Regional CVM	Ngo	Ying	334
Virginia-Maryland Regional CVM	Quinn	TJ	369
Virginia-Maryland Regional CVM	Simmons	Taylor	413
Virginia-Maryland Regional CVM	Travis	Sierrah	453
Washington State University CVM	Brownlee	Michael	53
Washington State University CVM	Hurst	Natalie	215
Washington State University CVM	Leung	Wing Sze	255
Washington State University CVM	Miller	Jonathan	307
Washington State University CVM	Pyrdek	Dorothy	367
Washington State University CVM	Shaver	Kaylie	409
Washington State University CVM	Weyand	Logan	489
Western College of Veterinary Medicine	Achtymichuk	Gabrielle	3
Western College of Veterinary Medicine	Gagne	Soleil	158
Western University of Health Sciences	Abedi	Nairi	1
Western University of Health Sciences	Catini	Pietro	73
Western University of Health Sciences	Chang	Diana	76
Western University of Health Sciences	Clark	Jory	85
Western University of Health Sciences	Cua	Abigail	107
Western University of Health Sciences	Guilfoyle	Colleen	182
Western University of Health Sciences	Ledner	Shana	253
Western University of Health Sciences	Moore	Trusten	317
Western University of Health Sciences	Myers	Adam	324
Western University of Health Sciences	Polniaszek	Mika	362
Western University of Health Sciences	Richter	Sonja	380
Western University of Health Sciences	Rodriguez	Trevor	388
Western University of Health Sciences	Seelman	Amanda	406
Western University of Health Sciences	Sumano	Daniela	437
Western University of Health Sciences	Tu	Darleen	456
Western University of Health Sciences	Van	Victoria	463

Complete Abstracts

Cloacal microbiome analysis of Turkey Vultures in Southern California

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Although Turkey vultures' (*C. aura*) strong immune system allows them to feed on decaying flesh, Turkey vultures (TV) may have a unique resident bacterial population which can potentially interact with host cells to modify gene expression and aid host resistance against pathogens in decaying animals. This study was conducted to characterize the bacterial community (microbiome) of the TV oral cavity and cloaca. To date, only cloacal samples from 6 vultures were collected. PCR was used to amplify DNA16S rRNA genes using universal primers to later conduct next generation sequencing on the Illumina MiSeq platform using standard methodology, and Bioinformatics to identify microorganisms' taxonomy. The most abundant bacterial populations found in the TVs' cloaca were presumed anaerobes belonging to genera in the Clostridiales: *Fusobacterium*, *Clostridium XI*, *Clostridium XIX*, *Clostridium sensu stricto*, *Anaerotruncus*, *Peptostreptococcus*, consistent with former research on TV's gut microbiota. The genus *Fusobacterium* is closely related to taxa previously placed in *Clostridium* Cluster XIX known to contain pathogenic species. Further, *Clostridium* Cluster XI contains possible pathogenic species such as *C. difficile*. In one of the TVs, approximately 20% of the sequences were classified as *Wohlfahrtiimonas* or *Ignatzschineria*, aerobic γ -Proteobacteria proposed as emerging infectious disease agents, with high relative abundance of this genus linked to its ability to replicate within the TV's GI tract. Further sample collection and comparison of the cloacal microbiome to the oral microbiome will allow us to better characterize TV microbiota to understand how the host and microbiome may interact to potentially modulate pathogen resistance.

Research Grant: WUHS, College of Veterinary Medicine intramural funds

Student Support: Morris Animal Foundation Veterinary Research Scholars Program

Mechanism by which formate induces invasion gene expression in Salmonella

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In order to infect a host, *Salmonella enterica* serovar Typhimurium invades the intestinal epithelium. It does this through a type III secretion system encoded by *Salmonella* Pathogenicity Island 1 (SPI-1). In previous studies, it was shown that a deletion of acetate kinase and phosphotransacetylase genes (*ackA-pta*) reduced invasion gene expression, but the addition of formate to the growth medium for these mutants restored or elevated invasion gene expression past that of the wild type. The purpose of this study is to find the pathway by which formate is able to induce invasion gene expression in these *ackA-pta* deficient mutants. A Δ *ackA-pta* mutation was transduced into various other strains. We used *lacZ* reporter fusions to *sipC*, *invF*, *prgH*, *hilD*, and *hilA*, which are all genes at different points in the invasion cascade, to assess the effects of a Δ *ackA-pta* mutation on invasion gene expression. Galactosidase assays were then performed on these strains to evaluate the level of gene expression. Results showed that *hilD* expression was unchanged with the addition of formate to growth medium. Also, it was confirmed that a slightly acidic pH (6.7) is needed for formate to have its effect on Δ *ackA-pta* mutants. Currently, other genes further upstream in the invasion cascade are being investigated since *hilD*, a major regulator for SPI-1 has been found to be unaffected by the addition of formate.

Research Grant: Ruth L. Kirschstein NRSA Short-Term Institutional Research Training Grant

Student Support: Cornell Veterinary Investigators Program

Early interpretation of community composition leads to cognitive bias of predation risk in a larval amphibian

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The vast majority of species can learn to identify predators and non-predators, a skill crucial to their survival. Yet, when faced with altered environmental conditions, it becomes obvious that many are unable to cope with novel predator invaders, while others even maintain fearful responses towards harmless species. To understand why some species thrive while others do not in the face of environmental change, we investigated the existence of factors that could limit their cognitive plasticity - an individual's ability to change the cues used to make decisions. One such factor is the development of cognitive biases - a deviation or error in thinking based on one's subjective interpretation of reality, which will affect one's decision making. Using woodfrogs, *Lithobates sylvaticus*, we tested whether early community composition (i.e., living in an environment containing at least 4 non-threatening species, 1 non-threatening species or a neutral environment) and the ontogeny of that experience (i.e., the information about the community was learned either as an embryo or later on as a larvae) would subsequently affect the responses of tadpoles to cues related to predation risk. We found that early positive (i.e., non-threatening) community experience created a cognitive bias in woodfrog tadpoles, which led them to underestimate risk-related cues later on. This safety bias prevented adaptive behavioural responses to novel predators, and this impact was more pronounced when the community experience occurred as embryos. This study highlights the role of early life experience in shaping an individual's ability to respond to environmental change.

Research Grant: NSERC

Student Support: Boehringer Ingelheim Student Veterinary Scholars Program, NSERC USRA

Detecting interleukin-8 expression in feline oral squamous cell carcinoma

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Squamous cell carcinoma is the most common malignant oral tumor in felines. Despite treatment, which may include surgery, radiation therapy, and/or chemotherapy, the prognosis is usually very grave since these treatments, even when used in combination, often fail to significantly improve quality of life. Interleukin 8 (IL-8), a pro-inflammatory chemokine, is produced by neutrophils, macrophages, and sometimes cancer cells. IL-8 promotes angiogenesis, tumor invasion, and contributes to tumor microenvironment. Human oral squamous cell carcinomas express high levels of IL-8, when compared to unaffected oral squamous cells. Thus IL-8 can be a target of investigation for future therapeutic treatment of feline oral squamous cell carcinoma. The current study evaluated and compared the gene expression of IL-8 in two feline oral squamous cell carcinoma cell lines to unaffected feline oral mucosa using quantitative PCR.

Research Grant: University of Florida College of Veterinary Medicine

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Improving neutrophil function in neonatal dairy calves

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Background: Between 7 to 15% of dairy calves die within the first 60 days of life in the US, mainly due to infections. Calves are born with a naive immune system that compromises their ability to fight infections. Part of this increased disease susceptibility is due to decreased functionality of polymorphonuclear neutrophils (PMN). PMN kills phagocytosed microbes by oxygen-dependent and oxygen-independent mechanisms. They are also involved in the recruitment of other immune effector cells by the release of chemoattractant factors. Therefore, new strategies are needed to increase PMN function in neonatal calves. A drug containing granulocyte colony-stimulating factor (G-CSF) has been shown to enhance PMN microbiocidal capabilities in periparturient cattle. However, neonatal PMN are immature and use different pathways to achieve effective killing of pathogens than PMN from adult cattle. Hence, the effect of G-CSF on neonatal PMN microbiocidal function remains unexplored. The aim of this study is to investigate the extent to which PMN mechanisms for pathogen killing are affected by G-CSF supplementation in newborn calves. Blood samples were collected from 6 newborn calves (2 to 3 days old); their PMN isolated, and incubated with three different concentrations (0.005, 0.05, or 0.5 $\mu\text{g/mL}$) of bovine recombinant G-CSF. Following incubation, two immunology tests were used to assess PMN pathogen-killing function: phagocytic activity and myeloperoxidase release. PMN from six adult cows will serve as controls to provide an indication of the PMN function observed in adult animals. We anticipate an increase in all measured variables, leading to an enhanced pathogen killing capability of neonatal PMN.

Research Grant: Boehringer Ingelheim

Student Support: Boehringer Ingelheim

Intrauterine antibiotic infusion increases antimicrobial resistance in the reproductive microbiota of mares

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In the thoroughbred racing industry, prophylactic intrauterine infusion with extended-spectrum cephalosporin (ESC) antibiotics after breeding to improve conception is a common practice, though efficacy has not been established. There is also anecdotal evidence of carbapenem antibiotics used for this purpose. We measured the impact of intrauterine infusion of ESC and carbapenem antibiotics on the recovery of antimicrobial resistant bacteria from the reproductive and fecal microbiota of mares. We hypothesized that intrauterine infusion would increase the recovery of β -lactam resistant uterine, vaginal, and fecal bacteria. Seven mares were infused after estrous cycle synchronization; 4 received 1 gram of meropenem and 3 received 2 grams of ceftiofur sodium. Resistant bacteria were not recovered from vaginal or uterine samples prior to infusion. An increase in the recovery of fecal microbiota resistant to both antimicrobials occurred at 24 hrs post-infusion and remained elevated until 18 days post-infusion. In vaginal samples, ESC and carbapenem resistant organisms were first observed 24 hrs and 48 hrs post-infusion, respectively; in uterine samples, resistance was observed at 72 hrs post-infusion. The highest prevalence of resistant bacteria from uterine and vaginal samples occurred at 72 hrs post-infusion. These data suggest that intrauterine antibiotic infusion transiently selects for the recovery of resistant bacteria from the enteric and reproductive systems of mares. Our preliminary data support the need for additional field studies to evaluate post-breeding antibiotic infusion efficacy and its impact on antimicrobial resistant bacteria to improve the welfare and reproductive success of thoroughbred mares.

Research Grant: USDA NIFA award no. 2013-68003-21282

Student Support: NIH T35 Training Grant T35OD010977

Development of an interactive iBook for Canine Musculoskeletal Anatomy Dissection

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In recent years, many veterinary anatomy students have demonstrated difficulty when translating written dissection instructions into action when utilizing traditional dissection resources. That said, contemporary learners are often particularly familiar and adept at utilizing technology to learn new skills. In an effort to meet these needs, Lincoln Memorial University - College of Veterinary Medicine (LMU-CVM) hopes to capitalize on the preferences and skill sets of modern students to address student concerns and observed deficiencies with traditional resources. Accordingly, interactive educational resources, specifically downloadable, non-web-based modules, have been created to reinforce students' training. These modules are being created as EBooks, developed for the Apple iBooks platform (iBooks). The iBooks can be downloaded onto various Apple products such as tablets, phones, and laptops, allowing users to select a device of their choosing and further increase their access to instructional materials. The iBooks have been developed to include videos and interactive components, in addition to text. They will function like an application, or app, providing easy-to-use features and include tools for veterinary instructors who require grading and screen-sharing features in the classroom and laboratory. Users will be able to customize their interface in order to suit their study preferences and facilitate multiple avenues for learning. As an initial step toward our ultimate goal, we are developing an iBook for canine musculoskeletal dissection, and we hypothesize that students will benefit from the use of this iBook and will demonstrate a preference for electronic media to supplement and guide their learning.

Research Grant: None

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Differential expression analysis in feline oral squamous cell carcinoma

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Feline oral squamous cell carcinoma (FOSCC) is the most common oral cancer in cats. The disease is highly invasive and has a poor prognosis despite intensive treatment. A thorough characterization of the molecular landscape of the disease would provide valuable insight into the pathobiology of FOSCC and point to novel treatment options. The goal of this study was to identify differentially expressed genes in FOSCC to deduce pathways involved in FOSCC oncogenesis. Total RNA was isolated from three FOSCC cell lines and from primary normal feline respiratory epithelial (FRE) cells and sequenced using 150 bp paired-end next-generation sequencing with Illumina HiSeq 2500. Affected FOSCC transcriptomes were compared to that of the normal FRE cells. Transcripts were annotated and analyzed for differential gene expression and pathway characteristics using HISAT2, featureCounts, and DESeq2 programs. Highly expressed genes averaged over the three cell lines included WNT7A, HOXC6, PAX2, and KLK10 with log2 fold changes of 7.8, 7.8, 6.6, and 6.5, respectively. Gene set enrichment analyses through the WEBgestalt server using the Gene Ontology database and Reactome pathway revealed cell cycle checkpoint and chromosome segregation pathways to be significantly up-regulated. Cell cycle, separation of sister chromatids, and regulation of PLK1 activity at G2/M transition were significant Reactome pathways identified, further reinforcing the importance of dysregulation of cell cycle checkpoints in FOSCC. Our data provide a foundation for future studies to improve our understanding of the pathogenesis of this disease. Additional analysis of RNAseq data may also identify key markers for targeted treatment of SCC in cats and other species.

Research Grant: Michigan State University (MSU) Center for Feline Health and Well-being and MSU-CVM Endowed Research Funds.

Student Support: NIH Grant T35OD016477 to Michigan State University

Effect of diet on glycogen repletion in Thoroughbred horses after exercise

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When muscle glycogen stores in horses are depleted after exercise, re-synthesis of glycogen proceeds at a rate 2-3 times slower than that of other mammals. The reason for slow repletion is unknown. Humans and other mammals can speed glycogen replenishment by consuming carbohydrates after exercise, however, similar dietary manipulations to enhance repletion rates in horses has met with little success. We hypothesize that transcriptomic (RNAseq) and proteomic analysis of equine muscle during glycogen depletion and repletion will reveal the underlying mechanism responsible for slow glycogen repletion in horses. A cross over glycogen depletion and repletion trial using isocaloric high starch (HS) and low starch-high fat (LS) diets was performed in partnership with Kentucky Equine Research. HS and LS diets were fed to 5 Thoroughbred geldings for 3 weeks which was followed by 3 days of intense, glycogen depleting exercise and 3 days of repletion. Percutaneous gluteus medius muscle biopsies were obtained before and after exercise and during repletion. Muscle glycogen concentrations were measured fluorometrically as glucose residues. Muscle glycogen percent depletion was 30 ± 9 (SEM) following exercise on HS and 35 ± 7 following LS diets. On Day 3 of repletion, muscle glycogen was 79 ± 7 percent repleted on HS, which was not significantly greater than 69 ± 7 percent repletion for LS. Analysis of alterations in the skeletal muscle transcriptome and proteome during depletion and repletion is underway. Elucidating the underlying mechanism limiting glycogen repletion rate in horses may lead to nutritional methods to improve performance.

Research Grant: Michigan Alliance for Animal Agriculture and Kentucky Equine Research

Student Support: Boehringer Ingelheim Veterinary Scholars Grant to Michigan State University

Evaluation of MMP-9 expression in CSF from canines with intracranial neoplasia and meningoencephalitis

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Intracranial tumors and meningoencephalitis are common canine central nervous system (CNS) diseases that can be challenging to definitively diagnose even when advanced diagnostics such as cerebrospinal fluid (CSF) analysis and cross-sectional imaging are employed. Evaluation of biomarkers in the CSF including matrix metalloproteinases (MMPs), may prove useful in improving diagnostic utility. MMPs are a family of zinc-dependent enzymes that degrade extracellular matrix (ECM) proteins, which are thought to be important for tumor cell invasion and metastasis but also for inflammatory cell entry into the brain. MMP-9 degrades type IV collagen in addition to certain other ECM components. The purpose of this study was to evaluate the expression of MMP-9 in CSF samples from canine patients with neoplastic or inflammatory CNS diseases. We hypothesized that expression of MMP-9 would be expressed in a subset of dogs with brain tumors and meningoencephalitis and that expression would vary depending on the tumor or meningoencephalitis subtype. CSF from canine patients was analyzed using gelatin zymography to detect the presence of MMP-9. A total of 119 CSF samples have been analyzed thus far- including those from 30 dogs with intracranial neoplasia, 80 with encephalitis, and 9 with other CNS diseases. MMP-9 was expressed in 10/30 tumor samples, 40/80 meningoencephalitis samples, and 4/9 samples with other CNS diseases with the most robust expression in meningoencephalitis samples. MMP-9 appears to be important for inflammatory cell invasion into the CNS, which may assist in shaping future therapies for this condition.

Research Grant: NCSU CVM Veterinary Scholars Program

Student Support: NCSU CVM Veterinary Scholars Program

Genetic modifiers and the gut microbiota in colorectal cancer development

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Colorectal cancer (CRC), a major cause of mortality worldwide, is a multifactorial disease induced both by multiple genetic factors and environmental factors. Recent evidence suggests that the gut microbiota (GM) is a significant mediator of the environment that substantially impacts CRC pathogenesis. Though some genetic and environmental modifiers of the C57BL/6-*Apc*^{+/Min} (*Apc*^{+/Min}) mouse model of CRC have been characterized, these factors remain poorly understood. The purpose of the present study is to test whether genetic modifiers are present in the C57BL/6NHsd (B6NHsd) inbred mouse strain and how these modifiers interact with complex GM populations to modulate the *Apc*^{+/Min} mouse tumor phenotype. We hypothesized that dominant genetic modifiers present in the wild-type (WT) B6NHsd genome will reduce the tumor number compared with C57BL/6JD-*Apc*^{+/Min} (B6D^{+/Min}) inbred strain. To assess this, we crossed WT B6NHsd females with B6D^{+/Min} to generate B6NB6DF1 hybrid embryos, and rederived these embryos into two distinct complex GMs, such that we obtained ten B6NB6DF1_{GMJAX} and eight B6NB6DF1_{GMHSD} *Apc*^{+/Min} mice. Fecal samples were collected at months 1, 2 and 3, and sequencing of the microbial 16S rRNA V4 region was performed to detect the relative abundance of all Operational Taxonomic Units (OTUs). Mice were sacrificed at 100 days of age for tumor assessment. We expect lower tumor number in B6NB6DF1 compared to B6D^{+/Min} due to genetic modifiers introduced by B6NHsd and additional modifier effect of the GM. These data will provide important information for future mechanistic studies, elucidating the role of genetic and environmental modifiers of CRC risk.

Research Grant: Mutant Mouse Resource and Research Center

Student Support: Endowment established by IDEXX-BioResearch

Limb dominance in domestic canines according to paw placement and peak vertical force

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During a normal walk or trot there is an asymmetry between the left and right thoracic limb (LTL and RTL) and the left and right pelvic limb (LPL and RPL) that is reflected in differences in kinetic and kinematic gait variables. The physiological and clinical significance of this asymmetry is poorly understood. The goal of this study is to determine whether the asymmetry is due to true dominance or a random event. Eight dogs were started in a square stance and then moved forward on a Tekscan Walkway, while peak vertical force (PVF) and sequence of limb movement were recorded. Twelve valid trials were analyzed per dog. First, we tested the hypothesis of dominance in terms of sequence of limb movement. Overall, the RTL was used most as first limb (34%), followed by LPL (31%), LTL (19%) and RPL (16%). Limb preference, that is, the most frequently used limb for walk initiation, was 57% (ranging from 33 to 92%), which was significantly different from 25% (i.e., a random event; $P=0.027$). Next, we tested the hypothesis that the limb to take the first step had the lower PVF during stance. Overall, the first limb used had the lowest PVF in 67% (ranging from 25 to 92%; $P=0.289$) of trials. The last hypothesis was that during stand and initial step the TL and PL with the highest PVF would be contralaterally related. The results, however, showed that higher PVFs were more ipsilaterally distributed during standing (52%, ranging from 33 to 75%; $P > 0.05$) and initial step (67%, ranging from 50 to 92%; $P=0.289$). The findings suggest that dogs use a preferred limb for initiating walk. The preferred limb often has the lower PVF, and the limbs with the highest PVF tend to be ipsilaterally related; however, the results are not significant.

Research Grant: None

Student Support: Purdue Veterinary Medicine

Use of protein biomarkers to characterize epithelial stem cells in the equine endometrium

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Endometritis is a major cause of infertility in mares. Chemical or mechanical curettage is used as a last resort when traditional treatments fail to resolve this condition. Paradoxically, curettage results in epithelial injury. Epithelial regeneration is mediated by stem cells in multiple organs. Epithelial stem cells have been identified in both mouse and human endometrium. However, stem cell populations have not been identified in the horse and the mechanism by which epithelial regeneration occurs in the equine endometrium is unknown. In other species, several antibodies are used to mark progenitor cells (Sox9) and cells that are actively cycling (Ki67, PH3 and MCM2) using immunofluorescence. We hypothesized that immunofluorescence would aid in the characterization of epithelial stem cells in the equine endometrium. We aimed to determine if antibodies used in other tissues can cross-react with the equine endometrium in order to characterize stem cells in the luminal and glandular epithelium. Endometrial samples from five mares were collected and used for immunofluorescence. The antibodies Ki67, Sox9, PH3, and MCM2 were tested. Preliminary results show Ki67 and Sox9 positive cells in luminal and glandular epithelium. MCM2 and PH3 positive cells were found predominantly in the luminal epithelium. This study showed that antibodies used to identify epithelial stem cells in other tissues cross-react with equine endometrium and can be used to identify the epithelial stem cell population. These antibodies can be applied to the study of the role of epithelial stem cells in tissue regeneration following endometrial injury.

Research Grant: None

Student Support: Boehringer Ingelheim Veterinary Scholars Program and College of Veterinary Medicine, NCSU

Evaluating ultrasonic vocalization in a novel rat model that mimics aspects of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a progressive neurological disorder characterized by the death of motor neurons, which causes patients to lose voluntary movement. ALS patients will also gradually experience global muscle atrophy, including paresis and paralysis of the tongue resulting in dysarthria (speech dysfunction) and dysphagia (swallowing dysfunction), which can lead to aspiration pneumonia and associated respiratory failure. Despite these severe impacts, there are currently no effective treatments to preserve or restore tongue function. Although transgenic rodent models of ALS are available for research, they take months to show symptoms and the type of impairment exhibited can be highly variable. Nichols et al. (2015) previously developed a rat model to specifically study respiratory dysfunction *via* intrapleural injections of cholera toxin B conjugated to saporin (CTB-SAP). We recently adapted this approach to develop a rat model of dysphagia using intralingual injections of CTB-SAP. These rats quickly display neural and behavioral deficits as a result of hypoglossal motor neuron death. However, it remains unknown if our CTB-SAP model develops dysarthria. In this study, we will use Avisoft recording software to capture and analyze ultrasonic vocalization (USV) in CTB-SAP treated and control rats. We hypothesize that the CTB-SAP treated rats will not have a change in the number of calls (compared to controls), but will have impaired call features that mimic dysarthric speech in human ALS. By developing rat models that replicate specific symptoms, such as dysarthria, we will be able to ultimately develop targeted treatments to prevent symptoms, slow the progression, or restore function in patients with ALS.

Research Grant: School of Medicine Bridge funding for USV research equipment, MU Research Board Grant, private funding

Student Support: The American Society of Laboratory Animal Practitioners (ASLAP) Foundation

Seroprevalence of canine influenza virus (CIV) in wild canid species

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Canine influenza virus (CIV), also known as dog flu, is a highly contagious viral disease that can infect both domestic dogs and cats. Although canine influenza has been well documented in dogs and cats, there are few studies determining if canine influenza has infected close relatives of dogs, such as the coyote or the fox. The point of our study was to see if coyotes from either rural or urban environments in the United States have been exposed to either the H3N8 or the H3N2 strains of CIV, or a strain novel to the family Canidae. We hypothesized that traces of canine flu antibodies would be found in serum samples from coyotes and foxes in areas that have had previous reported canine flu outbreaks in domestic dogs. We obtained 109 serum samples from coyotes, grey foxes and red foxes from Madison, WI, Atlanta, GA, Albany, NY, Logan, UT and rural South Carolina. We tested the wild canid sera for antibodies to CIV through IDEXX Enzyme-Linked Immunosorbent Assays (ELISAs). Besides one previously tested sample from rural Texas, all sera samples were negative for CIV antibodies. This leads us to believe that while canine influenza appears to be able to affect wild canid populations, CIV is not very prevalent in the rural or urban wild canid community and is probably not endemic to wild canid populations.

Research Grant: NIAID, NIH, Department of Health and Human Services contract HHSN272201400006C

Student Support: Boehringer Ingelheim Veterinary Scholars Program Grant (PI: Bjorling)

Pharmacokinetics and pharmacodynamics of grapiprant in a canine ovariohysterectomy pain model

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Grapiprant, a novel drug belonging to the piroxicam class of compounds, is a highly selective EP4 receptor antagonist. The objective of this study was to describe the plasma and incisional interstitial fluid (ISF) pharmacokinetics and pharmacodynamics of grapiprant in healthy dogs undergoing an ovariohysterectomy (OVH). We hypothesized that the PGE2 concentrations would be higher in dogs receiving grapiprant versus carprofen, but pain scores would not differ between the two treatments. Twelve adult female Beagle dogs were randomly assigned to one of two drug groups: oral grapiprant, 2 mg/kg ($n = 6$) and oral carprofen, 4.4 mg/kg ($n = 6$). All dogs received treatment 30-45 minutes before surgery, then every 24 hours for 72 hours postoperatively. Routine OVH was performed. An ultrafiltration probe was placed within the incision after surgery to collect ISF. Plasma and ISF samples were obtained at baseline ($T = 0$), and multiple time points for 72 hours postoperatively. Pain scores, which were assessed by 2 blinded observers, were performed using the short-form of the Glasgow Composite Pain Scale (GCPS) and were obtained at similar time points as plasma samples. Grapiprant plasma and ISF concentrations will be quantified via HPLC, and PGE2 in ISF was quantified via ELISA. Data was analyzed using ANOVA. PGE2 was significantly higher in grapiprant-treated dogs ($P < 0.001$), and was decreased significantly over time ($P < 0.007$). There was also a significant difference in GCPS over time ($P < 0.05$), but no difference between the two treatment groups. This study supports the use of grapiprant for post-operative pain, but not inflammation, following ovariohysterectomy in dogs; however, larger studies in clinical patients are warranted.

Research Grant: NCSU CVM Department of Clinical Sciences Firestone Canine Research Endowment Award

Student Support: Boehringer Ingelheim-NIH National Veterinary Scholars Program

Cyanidin inhibits adipogenic differentiation in 3T3-L1 preadipocytes via a Ca²⁺-dependent mechanism

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Cyanidin is a pigment found in the skin of many fruits and vegetables (like blueberries, cherries, and blackberries) giving the food its red, blue, or purple color. Recently, cyanidin has received attention for its anti-diabetic effects. Preliminary studies by our laboratory have found that cyanidin increased intracellular Ca²⁺ signals in mouse 3T3-L1 cells. Since obesity can often lead to type 2 diabetes mellitus, it is possible that another anti-diabetic effect of cyanidin includes inhibition of adipogenesis. We hypothesized that cyanidin supplementation to the adipogenic differentiation medium would inhibit adipogenesis via a Ca²⁺-dependent mechanism. Treatment of 3T3-L1 cells with cyanidin 0.01-100 µM reduced cell survival in a concentration-dependent manner during culture. Based on this observation, we selected cyanidin concentrations that did not compromise cell survival to examine cyanidin's effects on adipogenesis. Cyanidin 0.01-30 µM supplementation to the adipogenic differentiation medium inhibited adipocyte formation. Furthermore, stimulation of cells with increasing cyanidin concentrations induced intracellular Ca²⁺ signals in a concentration-dependent manner. Under extracellular Ca²⁺-free conditions, cyanidin still increased Ca²⁺ signals, but not after depletion of endoplasmic reticulum Ca²⁺ with thapsigargin. Our data suggests that another anti-diabetic effect of cyanidin is to control adipocyte formation. Its mechanism of action in 3T3-L1 cells appears to be via Ca²⁺ release from the endoplasmic reticulum rather than influx from the extracellular space.

Research Grant: None

Student Support: NIH T35 Training Grant 5T35OD011151-15

***In vitro* evaluation of the forwarder end knot to end a continuous suture line in large gauge suture**

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The forwarder end (FE) knot is a self-locking knot that, once tied, can be tightened to prevent loss of tension in a continuous suture line. The objective of this study was to evaluate, *in vitro*, the strength and size of the FE knot in comparison to the Aberdeen (A), surgeon's (Su), and square (Sq) knots to end a continuous suture line using large gauge suture. The four knot types were tied using 2 USP polydioxanone, 2 and 3 USP polyglactin 910 and tested on a universal testing machine under linear tension. Mode of failure and knot holding capacity (KHC) were recorded, and KHC was used to calculate relative knot security (RKS) as a percentage of suture tensile strength. Knot volume and weight were determined by digital micrometer and balance. Knot strength, size, and weight between knot type, number of throws, and suture type and size were compared using ANOVA testing, with $P < 0.05$ as significant. In all suture types and number of throws, the FE knots had significantly greater KHC and RKS than Su and Sq knots ($P < 0.0001$). For 2 USP polydioxanone, FE knots had significantly greater KHC and RKS than A knots ($P=0.035$). However, in both 2 and 3 USP polyglactin 910, FE knots had significantly lower KHC and RKS than A knots ($P=0.002$; $P < 0.001$). All FE and A knots failed by suture breakage at the knot. FE knots tied with 2 and 3 USP polyglactin 910 were significantly smaller than Su knots ($P=0.001$; $P=0.009$). In all other comparisons, FE knots showed no size or weight advantage over other knot types. Based on these results, the FE knot may provide increased strength compared to Su or Sq knots, but provides little strength or size advantage over the A knot. Additional *ex vivo* and *in vivo* testing are needed before clinical use.

Research Grant: Suture material donated by Ethicon, US, LLC

Student Support: Boehringer Ingelheim Vetmedica, Inc

Physical activity and the potential metabolic benefits of S-equal in mice fed an obesogenic diet

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In the U.S. and 35 other recorded countries, obesity rates continue to rise dramatically, with the U.S. and Mexico projected for 47% and 39% obesity by 2030. It is imperative to find options to combat obesity, and S-equal may be an adjuvant treatment. In 25% of Western individuals and 80% of Asians, soy is naturally converted by gut bacteria to a metabolite called S-equal. S-equal has shown beneficial effects in those who produce it—like reduced prostate disease in men and alleviation of menopausal symptoms in women. While select research has been conducted on S-equal, sex effects, exogenous dosing, and ability to negate obesogenic diet and promote activity have not been addressed. To bridge the gap, this study used 28 5-week-old C57 mice placed on HFD and assigned them to four groups: seven females on S-equal, seven females on control, seven males on S-equal, and seven males on control. The control solution contained 2.5%DMSO/0.5%CMC while experimental solution contained 4mg/mL S-equal in 2.5%DMSO/0.5%CMC. After a month on HFD and oral dosing of solutions, the mice were placed in an indirect calorimetry unit to evaluate physical activity, sleep, and food and water intake. Next, body fat and water content were measured using an Echo MRI. The mice then underwent glucose tolerance tests. Serum concentrations of leptin, adiponectin, insulin, glucose, and corticosterone were assessed, and fat content weighed. Mice treated with S-equal are predicted to demonstrate metabolic resistance to an obesogenic diet, with outcomes including increased activity, lower fat content, and faster glucose clearing times. If so, these results could advocate S-equal as a suitable supplement in combating obesity and other metabolic disorders.

Research Grant: NIEHS 1R01ES025547-A1 to C.S.R.

Student Support: The University of Missouri College of Veterinary Medicine Office of Research

A natural murine infection model to delineate the immunobiology and persistence of noroviruses

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Human norovirus (HuNoV) is the major cause of epidemic acute gastroenteritis worldwide. Murine norovirus (MNV) infection in laboratory mice has been used by research community worldwide as an excellent alternative model for investigating the pathobiology of HuNoV. The aim of this project was to define the significance of host innate and adaptive immunity in the pathogenesis and persistence of MNV infection using immunodeficient mice, including IFN- γ knockout (IFN- $\gamma^{-/-}$), Rag $^{-/-}$, and IL-17 $^{-/-}$ mice. A portion of neonatal mice from these groups and a wild-type group were previously reported as MNV-positive, presumably resulted from natural infection. We sacrificed these mice later, collected fecal pellets and tissue samples such as spleen, liver, ileum, colon, and mesenteric lymph nodes (MLN), and performed qRT-PCR to quantify the MNV titers. Our results showed that fecal pellets from all groups were MNV-negative, tissue samples from wild-type, Rag $^{-/-}$, and IL-17 $^{-/-}$ mice were MNV-negative. However, three out of six IFN- $\gamma^{-/-}$ mice were MNV-positive in ileum, colon, and MLN. In addition, we perform ELISA to detect MNV-specific IgG antibody. Our results showed that three IFN- $\gamma^{-/-}$ mice and one out of five IL17 $^{-/-}$ mice were positive, indicating MNV infection in those mice earlier. To conclude, our data shows that natural MNV infection is more likely to persist in IFN- $\gamma^{-/-}$ mice; wild-type and Rag $^{-/-}$ mice were negative for MNV in feces and tissues, as well as for MNV-specific antibody, it is likely that these mice were not naturally infected. Our natural MNV infection study and following MNV challenge studies pave the way to delineate the pathogenesis of MNV.

Research Grant: Unknown

Student Support: T35OD011887 NIH Office of Director

Comparison between computed tomography and ultrasonography in staging canine transitional cell carcinoma

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Transitional cell carcinoma (TCC) is the most prevalent neoplasia of the canine urinary tract and is typically aggressive with invasive metastatic features. Accurately determining the extent and degree of invasion of TCC facilitates prognosis and informs treatment recommendations. Imaging studies in humans have found computed tomography (CT) to be a more sensitive and specific technique than ultrasonography (US) in characterizing bladder neoplasms. In this retrospective study, we directly compared the efficacy of these two imaging modalities in accurately determining the features of canine TCC. Thirteen dogs with urinary bladder TCC confirmed by cytology or histology had abdominal US and CT, as well as cystoscopy or abdominal surgery that allowed visualization of the primary mass. Accuracy in identifying factors such as location, ureteral, prostatic or urethral involvement, and potential distant metastasis was compared between the modalities. The impact of these factors on surgical recommendations was also assessed. We anticipate that this information will inform selection of imaging modalities for initial and pre-surgical staging of TCC. Results will be presented in poster form at the event.

Research Grant: None

Student Support: Florida Veterinary Scholars Program and Boehringer Ingelheim Veterinary Scholars Program

Association of the uterine microbiome in mares before and during the breeding season

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Endometritis is the leading cause of decreased fertility in mares. It contributes to major economic losses for the equine breeding industry. The traditional approach for diagnosis of endometritis in the mare is by endometrial cytology and culture, which confirms the presence of inflammatory cells and bacteria in the uterus, respectively. Next generation sequencing is now being used to study the uterine microbiome in many species including humans. The once thought 'sterile' pregnant uterus may have a set of core bacteria that is needed for pregnancy success. There is a need to characterize beneficial bacteria from pathologic. The goal of this study is two-fold. First, we will compare the resident uterine microbiome in the healthy mare during estrus and anestrus by metagenomics to identify resident bacteria during and prior to the breeding season. Second, we will determine if stallion semen harbors resident bacterial communities, using metagenomics, that may impact the uterine microbiome of the female prior to breeding and the establishment of pregnancy. We hypothesize that the bacterial communities within the equine uterus are much more complex than previously thought. Furthermore, we believe metagenomics will reveal distinct communities and specific taxa that aid in healthy cyclicity and maintenance of pregnancy in the horse.

Research Grant: LSU Equine Health Studies Program

Student Support: Boehringer Ingelheim Summer Scholars Program

Positive reinforcement training of cats classified as strays or abandoned to an animal control facility

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Positive reinforcement training (PRT) has been used as a way to assess learning in cats by teaching them to respond to cues. The purpose of this study was to use PRT to teach cats to respond to cues in order to assess their ability to learn while in the high-stress situation of living in an animal control facility. If they can learn in a high-stress environment, this may affect their future adoptability, as well as give us valuable information about cats' ability to learn and the timeframe in which this can be accomplished. All cats participating in this study were first habituated to the room in which the sessions were held as well as to the trainer before moving into the training group. Cats eligible for training were taught the cues "come" and "sit" using PRT. Once these cues were learned, further behaviors were taught such as "up," "nose," and "high-five." Subsequent cues selected were dependent upon the cats' preferred movement. Because every cat in the training group was able to learn to respond to several cues with the appropriately taught behavior, and retained those cues for the duration of the experiment, we were able to determine that cats are capable of not only learning in the high-stress environment of the shelter but are also capable of remembering what they have been taught over a period of time. There was no significant difference between single and group housed cats as well as stray and owner surrender cats in the amount of time it took to habituate or learn a cue. Further research can assess if implementing a PRT program within a shelter can help increase adoptability, decrease length of stay; and increase retention in the cat's adoptive home.

Research Grant: None

Student Support: PetSmart Charities Summer Research Stipend, UGA College of Veterinary Medicine

A pilot study investigating parasite management and egg shedding patterns on Prince Edward Island horse farms

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Widespread over use of anthelmintics in North America has produced a growing population of cyathostomes in horses that are resistant to anthelmintics. This poses a serious threat to the welfare of horses and future effectiveness of parasite control programs. In order to prevent anthelmintic resistance development, selective parasite control programs are currently recommended. These programs depend on egg shedding patterns with 80% of eggs being shed by 20% of adult horses. By using fecal egg counts (FEC), we can select only these horses for treatment and reduce the selective pressure for resistance. Currently, the strongyle egg shedding patterns and parasite control programs in use on Prince Edward Island (PEI) horse farms are unknown. The study aims were to investigate the strongyle egg shedding patterns and parasite control programs on PEI horse farms. Modified McMaster technique was used to estimate strongyle egg shedding. In person surveys were administered to characterize current parasite control programs. Results from 309 horses revealed 18.4% were shedding 79.8% of the strongyle type eggs. Of the 56 survey responses, 73.2% of owners considered the use of FEC to be very important for a deworming program. However, only 4.2% of horses were regularly treated with an anthelmintic based on FEC surveillance on the 35 farms evaluated. These findings indicate the need to educate clients on the importance of selective parasite control programs for the prevention of anthelmintic resistance development. Future research will be aimed at estimating the prevalence of anthelmintic resistant cyathostomes on Prince Edward Island horse farms.

Research Grant: Startup package to Dr. W. Ben Stoughton

Student Support: Veterinary Student Research Award, Boehringer Ingelheim Veterinary Scholars Program

Qlp19, a novel infection-associated leptospiral protein

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The pathogenic mechanisms in leptospirosis are not well understood though proteins produced by *Leptospira interrogans* appear to be important. This study describes a novel, infection-associated leptospiral protein, Qlp19. Through screening of a lambda phage expression library of *L. interrogans*, we identified a novel protein, Qlp19. Triton-X extraction of *Leptospira interrogans* was utilized for cellular fractionation and localization studies. Recombinant Qlp19 was used to develop an ELISA to assess the host response to the protein by screening for the presence of Qlp19-specific antibodies in eye fluids and sera of infected horses. The gene encoding Qlp19 was detected in all pathogenic *L. interrogans* but not in nonpathogenic *L. biflexa*. Cellular localization studies indicate that Qlp19 is located in the leptospiral periplasm or outer membrane. Qlp19-specific antibodies were present in the eye fluids and sera of significant number of infected horses, but absent in the sera of vaccinated animals. Qlp19 is an immunogenic protein conserved amongst pathogenic *Leptospira* spp. These qualities make Qlp19 an attractive candidate for use in diagnosis of leptospirosis. The presence of anti-Qlp19 antibodies in naturally infected equines suggest that Qlp19 could be useful in the diagnosis of leptospirosis. Further analysis is necessary to evaluate the correlation between a Qlp19-based ELISA and standard serological assays.

Research Grant: Lincoln Memorial University-College of Veterinary Medicine Intramural Grant

Student Support: Lincoln Memorial University-College of Veterinary Medicine Summer Research Scholars Program

Isolation of *Schistosoma mansoni* eggs from baboon feces

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Schistosomiasis is a devastating disease of developing countries caused by blood flukes. At least 207 million individuals required prophylactic treatment in 2016. Schistosomiasis is zoonotic. Researchers in Tanzania have reported *Schistosoma* eggs in feces from a wild troop of baboons via fecal smear analysis. Our long-term aim is to determine how related the baboon schistosomes are to schistosomes infecting humans in the area. The current project's goal is to develop laboratory methods at CSVM to recover schistosome eggs from baboon feces to, later, perform molecular analyses using field recovered material. *S. mansoni* eggs from homogenized livers of infected laboratory mice were added to baboon feces from a local wildlife facility. The feces were spiked with eggs from a low (10), intermediate (100), or high (1000) number (per gram fecal material). The materials were mixed and passed through a series of sieves with decreasing pore sizes of 425 μ m, 180 μ m, 106 μ m, and 45 μ m. Eggs were successfully recovered via this method at a rate of 71 $\pm$ 9.6%, 61 $\pm$ 12.3%, and 74 $\pm$ 6.6%, respectively (n=3). An alternative method involved adding the egg/fecal mixture (100 eggs/g and 1000 eggs/g) in a large volume (1L) of fresh water to promote parasite hatching. Larval parasites (miracidia) were recovered in low numbers using this method. In later work, recovered eggs or miracidia will be placed on Whatman FTA filter paper and subjected to PCR analysis using *S. mansoni* cytochrome oxidase 1 (cox1) primers. PCR products will be resolved by agarose gel electrophoresis, purified, and sequenced. Overall, our findings demonstrate that the filtration method offers a viable procedure for isolating *S. mansoni* eggs from baboon feces applicable in the field.

Research Grant: None

Student Support: NIH Summer Research Training Grant

Performance assessment of current PCR protocols in the detection of *Borrelia burgdorferi*

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Borrelia burgdorferi sensu stricto is the principal etiological species responsible for Lyme disease in North America, with the objective of this study being to identify the most robust testing available of those in current use by laboratories and Public Health Agencies across Canada and the Eastern United States. Tick (*Ixodes scapularis*) surveillance is a powerful epidemiological tool influencing professional and public awarenesses, although there is an evident absence in fundamental access to testing in some regions, in addition to standardization, where variants in polymerase chain reaction (PCR) techniques and primers are utilized to detect the spirochaete in a binary convention. PCR is considered a gold-standard technique in microbial diagnostics, efficacious in relation to time and cost, analytical sensitivity and specificity, with overall low limits of detection. PCR is not without its limitations however, which are a consequence of the sequence variations introduced by the inherent diversity, and mutational nature of *B. burgdorferi*. To date, protocols from the National Microbiology Laboratory (Manitoba), Mount Allison University (New Brunswick), and the Maine Medical Center Research Institute (Maine) have been acquired, and tested using purified B31 strain *B. burgdorferi* DNA. Preliminary results include optimized quantitative (q)PCR efficiencies, and amplicon detections via agarose gel electrophoresis for conventional (c)PCR and nested (n)PCR protocols. Future directions involve the semi-quantitation of cPCR and nPCR products via gel pixel density analysis, and test performances will be evaluated with negative tick DNA samples spiked with B31 DNA, followed by blinded tick samples confirmed through sequencing.

Research Grant: Sir James Dunn Animal Welfare Centre Research Grant, Canadian Institutes of Health Research

Student Support: Veterinary Summer Research Award, Boehringer Ingelheim Veterinary Scholar

Effect of benzoylphenylureas on the moulting process of copepod stage sea lice (*Lepeophtheirus salmonis*)

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The salmon louse (*Lepeophtheirus salmonis*) has become a global problem for the salmonid aquaculture industry due to the development of widespread drug resistance. Therefore the development of novel therapeutics is needed to maintain a sustainable industry. Benzoylphenylureas are known to disrupt chitin synthesis, an important biological process required for the moulting process between any of the sea lice's eight life stages. The exact mechanisms of this inhibition are not yet fully known. Sea lice were received at the copepod life stage and exposed for 3 hrs to one of four benzoylphenylureas treatments; 1500ppb hexaflumuron, 1500ppb teflubenzuron, 1500ppb lufenuron, and 50ppb lufenuron. Dimethyl sulfoxide was used as a vehicle for each treatment, and thus the control included a matching concentration. Samples were collected at 6, 12, and 24hrs post initial exposure for future qPCR and RNA analysis. Approximately 1500 copepods from each treatment were allowed to infect 6 Atlantic salmon (*Salmo salar*) housed at 15°C. Lice were counted, staged, collected, and stored in RNA later at 3, 7, and 14dpi. *P* < qPCR and RNA analysis not completed for this presentation.

Research Grant: Veterinary Student Research Award

Student Support: Unknown

Investigation of TSG-6-mediated hyaluronic acid modifications using SEC-MALS and a molecular crowding sensor

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Synovial fluid (SF) is a polymeric liquid critical to joint health and cartilage lubrication. The biophysical properties of SF are conferred by the hydrophilic polysaccharide polymer hyaluronic acid (HA). Changes in HA concentration, molecular weight (MW) distribution and conformation may significantly alter the properties of SF. In arthritis, a pathological modification by tumor necrosis factor-inducible gene 6 (TSG-6) can result in HA crosslinking, forming heavy chain-HA. It is unknown how this modification alters the properties of HA. We hypothesized that TSG-6-mediated crosslinking would alter the macromolecular structure of HA modifying its rheological and lubricating properties in SF. Size exclusion chromatography with multi-angle light scattering (SEC-MALS) was performed to determine how TSG-6 alters the quaternary structure of monodisperse HA. Recombinant TSG-6 induced the formation of higher molar mass HA, suggestive of intermolecular HA cross-linking or multimerization. A fluorescence resonance energy transfer (FRET)-based crowding sensor, comprised of two fluorophores separated by a flexible linker domain, was designed to analyze molecular crowding of HA. Increased osmotic pressure, due to increased polymer concentration and/or MW, brings the two proteins closer, resulting in an increased FRET signal. The sensor was validated using polyethylene glycol (PEG) and tested using dextran, an uncharged polysaccharide, and HA, a charged polysaccharide. Although most effective for lower MW polymers such as PEG, the sensor was effective at detecting increased osmotic pressure with increasing concentrations of 1.5 MDa HA. This methodology will be used to investigate the effect of TSG-6-mediated HA crosslinking *in vitro*.

Research Grant: NIH/NIAMS Award No: K08AR068469 (HR)

Student Support: NIH Training Grant: 5 T35 OD 10941-9 (JB)

Investigation of functional alleles of recurrent exertional rhabdomyolysis in Thoroughbreds and Standardbreds

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Recurrent exertional rhabdomyolysis (RER) is a painful exercise-triggered disorder characterized by rapid onset of skeletal muscle necrosis. RER affects 5-10% of Thoroughbred (TB) and Standardbred (STDB) horses. Expression of the clinical disease is affected by sex and environmental factors. Heritability ranges from 0.34-0.45% in both breeds. We hypothesized that multiple alleles disrupting genes and pathways involved in skeletal muscle function increase RER risk, and that some of these alleles may be shared by the two breeds. Whole genome sequencing (WGS) was used to identify potential functional alleles found in candidate protein-coding genes and to evaluate putative functional mutations and biologic pathways that may alter muscle physiology and RER. Alleles in 103 candidate genes from 9 regions of interest based on genome-wide association and random forest analyses were identified from WGS in 40 horses (10 cases and 10 controls of each breed). These alleles were genotyped in 211 STDB horses using a NuGEN Allegro genotyping-by-sequencing assay. Multiple bioinformatic programs were used to evaluate pathogenicity of the alleles, with particular focus on alleles in protein coding genes related to RER and genes with biological relevance to muscle diseases. At least four of the candidate genes contain one or more alleles that suggest deleterious functional effects. The comparison of the different functional alleles between the two breeds can lead to further knowledge of the genetics of altered muscle contractility, as well as improved prevention and treatment strategies.

Research Grant: University of Minnesota Wellstone Muscular Dystrophy Center, University of Minnesota Equine Center, and Morris Animal Foundation

Student Support: Minnesota Racing Commission and the College of Veterinary Medicine, University of Minnesota

Evaluation of the anabolic WNT pathway response to load in osteocytes using a fluorescence-based mouse model

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Osteocytes, the primary cells within the bone matrix, are postulated to be the sensors of mechanical load in bone and, consequently, direct the bone's response to the load by stimulating growth through the WNT pathway. Male and female TCF/LEF:H2B-GFP transgenic mice were used to access skeletal osteocytes' molecular response to load, specifically examining the canonical WNT pathway that ends in activation of the TCF-LEF promoter. The mice underwent tibial loading in one limb and after 24 hours were euthanized and the tibiae were removed from the loaded and contralateral, non-loaded control. Cryo-sections were produced from the bone samples. Microscopic evaluation of osteocytes was performed and the total number and percentage of fluorescent cells from the loaded and control limbs, as well as between sexes, were compared. Validating the use of these reporter mice for detecting mechanical activation of the WNT pathway is a key step in developing experimental approaches for examining the effects of targeted gene knockout, sex hormones, or pharmacologic agents, like parathyroid hormone, on mechanosensitive WNT pathway activation.

Research Grant: Purdue EVPRP New R01 Grant

Student Support: Boehringer Ingelheim

Death in racehorses at the Fair Grounds Race Course in New Orleans, LA (2009-2018)

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Horse racing is a popular sport around the world. Catastrophic injuries can lead to death of these Thoroughbred and Quarter horses housed at the racetrack. Injuries can occur while training and racing, while death due to other conditions might occur independent of exercise. The purpose of this study is to evaluate data from the last 10 years of horses that died or were euthanized at the New Orleans Fair Grounds Race Course (FGRC). We hypothesize that the incidence of racehorse deaths at the FGRC is low, there were fewer catastrophic injuries in racehorses at the FGRC during years 2014-2018 when compared to years 2009-2013, and younger horses were more likely to die at the racetrack than horses > 3 years-of-age. Information was obtained from the Louisiana Racing Commission on the number of fatalities, races, starters, and ages of the starters at the FGRC based on calendar year from 2009-2018. Focused necropsy reports from the Louisiana Animal Disease Diagnostic Laboratory at LSU were evaluated on all horses that died or were euthanized at the FGRC during these years. Racehorse deaths averaged 1.8 horses/1000 starters for all horses racing from 2009-2018. When compared to previous years, the last three years of racing have fewer deaths per year with an average of 1.4 horses/1000 starters. Fewer 2-year-old racehorses died while racing, but more 2-year-old racehorses died by other causes on the FGRC when compared to horses of other ages. It was also found that 68% of necropsied horses suffered from fractures. The number of catastrophic injuries in racehorses is low compared to data collected from other racetracks. From these data, it is concluded that horse racing is a relatively safe sport for horses in Louisiana.

Research Grant: Equine Health Studies Program

Student Support: Dr. Kenneth F. Burns Lectureship and Clinical Clerkship Foundation

Characterizing the inflammatory response in a mouse model of Mal de Meleda

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Palmoplantar Keratodermas (PPKs) are a group of rare diseases characterized by a thickening of the epidermis on the palms of the hands and soles of the feet. As part of the variation in clinical presentation among these disorders, some PPK patients experience intense pain in the affected area, while others do not typically experience pain. It is known that immune cells can release factors that influence the physiology of sensory neurons, specifically pro inflammatory cytokines IL6, IL22, and TNF α . Therefore, we hypothesize that these cytokines or other immune cell derived factors act on neighboring sensory neurons in order to drive the pain phenotype. The aim of this study is to better understand the underlying mechanisms of pain in PPKs and determine the role of the immune cells in that mechanism. Slurp2x $^{-/-}$ mice were used as a mouse genetic model of the hereditary and sometimes painful human PPK Mal de Meleda. Previous experiments in our lab revealed that these mice show increased pain-related behaviors, compared with wild-type controls. To mark the immune cells in the skin of the heel from Slurp2x $^{-/-}$ mice and wildtype controls, we immunostained the tissue for CD3, CD68, and Ly6. The results showed a five-fold increase in CD3 ($P < 0.001$) and CD68 ($P = 0.014$) positive cells and a 6-fold increase in Ly6 ($P = 0.017$) positive cells compared to the wildtype mice. These increases over all tested immune cell types show that there is a mixed lymphocytic and myeloid inflammatory response in the heel skin of SLURP2x $^{-/-}$ mice. This influx of immune cells could impact neighboring sensory neurons, perhaps through cytokine production, leading to hypersensitivity and potentially contributing to pain in these animals.

Research Grant: Neurosurgery Pain Research Institute at Johns Hopkins

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Genetic characterization of *Strongyloides stercoralis* isolates in dogs and cats for assessing zoonotic status

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Strongyloides stercoralis is a zoonotic parasitic nematode that infects humans, dogs and cats worldwide. Strongyloidosis is associated with poor sanitation and/or immunosuppression that range from subclinical to fatal. The role of dogs and cats as a reservoir for transmission to humans is unclear. Recent studies from Asia show that *S. stercoralis* isolates are genetically clustered in two clades: a zoonotic clade shared by humans and dogs; and a non-zoonotic clade found only in dogs. There is no genetic or epidemiologic information on *Strongyloides* infections in cats. Here, we provide preliminary genetic data on *Strongyloides* isolates from clinical samples to assess their zoonotic potential. Canine isolates were larvae isolated from dog feces from the Parasitology Diagnostic Laboratories at the University of Georgia and University of Pennsylvania. Feline isolates were obtained from intestinal tissues of cats necropsied at Ross University. Genomic DNA was extracted using a commercial kit. We performed PCR to target the cytochrome oxidase subunit I (cox1) region of the mitochondrial DNA. Phylogenetic analysis was performed using the Maximum Likelihood method in MEGA7, and included the sequences from isolates of 10 dogs, 2 cats, and available sequences of *S. stercoralis* isolates from other hosts around the world. All our isolates clustered with those of the zoonotic isolates in a well-supported clade. While our results confirm the presence of zoonotic *S. stercoralis* in dogs and cats, we cannot rule out the presence of the non-zoonotic clade. It is necessary to reiterate the public health importance of this by implementing appropriate preventative, diagnostic, and treatment protocols for strongyloidosis in dogs and cats.

Research Grant: none

Student Support: Boehringer Ingelheim, Veterinary Medical Experiment Station; UGA College of Veterinary Medicine

Prions are commonly present in muscle of CWD-infected animals

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Chronic wasting disease (CWD), a transmissible spongiform encephalopathy occurring naturally in wild cervids, represents a direct and growing threat to wildlife and an as-yet unknown threat to humans and domestic species. Muscle tissue is likely the most significant source of human and domestic animal exposure through the consumption of CWD-contaminated venison. Previous investigations have established the presence of PrP^{CWD}, the CWD infectious agent, in muscle harvested from CWD-positive animals, however, its prevalence and distribution in diseased cervids remains unknown. Here we have combined two powerful *in vitro* amyloid amplification techniques, protein misfolding cyclic amplification (PMCA) and real-time quaking-induced conversion (RT-QuIC), to detect PrP^{CWD} in muscle from experimentally-infected white-tailed deer (*Odocoileus virginianus*). We detected CWD muscle seeding activity in 19 of 27 deer - a prevalence of 70%. Further analysis indicated that 0.1mg of terminal muscle contains the amount of PrP^{CWD} equivalent to 10pg of CWD-positive brain - a concentration too low for reliable detection by conventional assays (e.g. immunohistochemistry or western blotting). Analysis of multiple muscle locations demonstrated that CWD prion seeding activity can be detected throughout skeletal muscle sites. These results are important in demonstrating that CWD prions are widely distributed in muscle at terminal. This ultra-sensitive amplification method can be used to track tissue pathways of prion spread during infection.

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Student Support: Colorado State University Veterinary Summer Scholars Program

Investigation of novel prognostic biomarkers in dogs affected with degenerative myelopathy

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Canine degenerative myelopathy (DM) is a progressive, fatal neurodegenerative disease of the spinal cord sharing many features with amyotrophic lateral sclerosis (ALS) in humans. It is an adult-onset disease that affects the hindlimbs causing a spastic paraparesis and general proprioceptive ataxia. Over a period of months lower motor neuron degeneration occurs leading to ascending flaccid paralysis and eventual tetraplegia. Similar to a genetic form of ALS, DM is associated with a mutation of superoxide dismutase 1 (SOD1) gene. Currently, there is no effective treatment available. There are a number of well described biomarkers of ALS that have not been investigated in DM. The aim of this longitudinal study is to identify non-invasive biomarkers that reflect stage of disease. Dogs diagnosed with DM will be evaluated on a monthly basis, will undergo physical and neurological examinations, blood and urine samples will be taken and retinal thickness will be measured utilizing optical coherence tomography (OCT). A comprehensive proteomic analysis (LC-MS/MS) will be performed using the plasma and urine samples. Four dogs have been recruited and undergone their initial evaluation and sampling. A preliminary proteomic analysis has been performed on 3 DM-affected dogs, 3 normal healthy controls and 3 dogs with chronic compressive myelopathy. There is inadequate data for conclusions at this time. We anticipate our findings may provide better understanding of disease progression and provide a means of monitoring therapeutic response in clinical trials.

Research Grant: Unknown

Student Support: NIH T35 Training Grant

Growing feline regulatory T cells for adoptive immunotherapy

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Regulatory T cells could be used in adoptive immunotherapy for autoimmune diseases. Felines have common autoimmune conditions that occur naturally and there are commercially available feline lymphocyte cell lines, making felines a great model for study. The aims of this study are to help characterize the phenotypic and functional characteristics of commercially available Mya 1 feline lymphocyte cells and establish a method for the growth of regulatory T cells *in vitro*. Lymphocytes are grown in media previously been shown to induce regulatory function in mouse and human regulatory T cells. Indicators of regulatory T cell subsets are positive stain for CD25 and CD4 surface markers as well as a FoxP3 intracellular marker, which are evaluated using imaging flow cytometry. Morphological differences are examined using the images captured to determine significant differences between effector and regulatory T cell populations. Our findings resemble those of previous experiments with mouse regulatory T cell culture. Growth media consisting of IL-2, TGF- β , and Rapamycin produce the highest percentage of CD4+, CD25+, and FoxP3+ (triple positive) cells. There is a significant morphological difference noticed between groups of triple positive cells and CD4+ Fox P3+ (double positive) cells. Feline Mya 1 cells have the potential capacity to be expanded and induced as effector regulatory T lymphocytes. Further research (such as cytokine profile analysis after stimulation), should be done to show maintenance of functionality *in vitro* for these regulatory lymphocytes. Continuation of this work will contribute to the knowledge of this elusive type of lymphocyte and their use in immunotherapy.

Research Grant: College of Veterinary Medicine Academic Research Enhancement Award

Student Support: Boehringer Ingelheim Veterinary Research Scholars Program

Characterization of the guinea pig placental histostructure

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Women harboring disease during pregnancy experience increased gestational complications that can lead to life-long health issues for their babies. It is not feasible to study the longitudinal effects of maternal infections on gestational development in humans. Therefore, the advancement of animal models to unravel these complexities is of utmost importance. We propose the use of the guinea pig as a model for these investigations because they possess: (i) a hemomonochorial placenta most closely representing the human placenta, (ii) susceptibility to a wide variety of insults (viruses, bacteria, diabetes, prions, CO2 emissions), and (iii) an intact immune response permitting investigation of immunological alterations at the maternal-fetal interface. This study was designed to characterize the placental histostructure of healthy guinea pigs throughout pregnancy. Placental tissue was collected at early (20-25 gestational days (gd)), mid (30-35 gd), and late (50 gd) gestation. These tissues were subsequently fixed, paraffin-embedded and probed for the presence of various cell types. Cells of mesenchymal origin were consistently found in the decidua and surrounding maternal blood lacunae in the labyrinth (anti-vimentin; Abcam ab92547). Epithelial cells, including trophoblasts, were probed using an antibody against cytokeratin (Dako Z0622). The data generated by this study will be a crucial foundational step in assessing the impact of maternal disease on immune cell profiles at the maternal-fetal interface and subsequent fetal outcomes. The overarching goal of this work is the development of intervention strategies to improve the health of mothers and their babies.

Research Grant: None

Student Support: Boehringer Ingelheim Veterinary Scholar Program

Viral RNA titers in kidneys collected from birds experimentally infected with infectious bronchitis virus

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Infectious bronchitis virus (IBV), a highly contagious viral disease of chickens with global distribution, is of primary concern in the poultry industry due to economic losses incurred from affected birds. The virus initially infects the upper respiratory tract, resulting in deciliation of the mucociliary apparatus. IBV can also replicate in and destroy other epithelial surfaces. In layers, lesions in the oviduct lead to declines in egg production and quality, while in broilers economic losses are primarily due to poor weight gain and feed efficiency. In addition, the IBV-QX strain is nephropathogenic, causing severe nephritis and high mortality. Current understanding of IBV-QX pathogenicity is limited. Thus, the objectives of this study were to determine if there are differences in kidney IBV viral titers in 1) layer versus broiler chickens, and 2) chickens inoculated with wild-type IBV-QX compared to the derivative QX vaccine. In total, 58 specific pathogen free (SPF) layers and 58 SPF broilers were included in this study. Of these, 24 of each were inoculated intranasally with the vaccine strain, 24 of each with the wild-type strain, and 8 of each were mock-inoculated as a control. Chickens were euthanized serially from day 1 through 8 post-inoculation, and kidney viral titers were determined using RT-qPCR. No significant differences were found between layers and broilers, however chickens inoculated with the wild-type virus had significantly higher titers than those inoculated with the vaccine strain. This information will be combined with histological and immunohistochemistry data from the same study to elucidate whether the presence of lesions and viral protein expression corresponds with viral RNA presence.

Research Grant: None

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Characterizing the biological and molecular consequence of PTEN loss in canine osteosarcoma

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Osteosarcoma (OS) is the most common bone malignancy in dogs and adolescent children. Early micrometastatic disease commonly occurs in both species and despite aggressive therapy, approximately 30% of children and 90% of dogs succumb to disease. The marked chromosomal heterogeneity of OS has hindered progress to identifying molecular drivers for targeted therapies. However, a growing number of studies have identified alterations in the PTEN/PI3K/Akt signaling pathway in canine and human OS suggesting this signaling pathway may play a critical role in mediating OS biology. Tumor suppressor phosphatase and tensin homolog deleted from chromosome 10 (PTEN) functions as a phosphatase and through its activity antagonizes the PI3K/Akt pathway. Loss of PTEN and subsequent activation of the PI3K/Akt signaling pathway is a common event in OS. The objective of this study was to characterize the biological consequence of PTEN loss in normal and malignant canine osteoblasts (Ob). PTEN transcript and protein expression was evaluated in 8 canine OS cell lines and normal canine Ob via Western blotting and Real time PCR. PTEN expression was significantly reduced or absent in 50% of canine OS cell lines compared to normal Ob. Concordantly, loss of PTEN correlated with an increase in Akt phosphorylation and activation. To define the role of PTEN in canine OS cell behavior, we are currently generating canine OS cell lines expressing lentiviral PTEN constructs or shRNAs for PTEN to evaluate the effect of PTEN loss on OS cell biology. Data generated from this study will aid in characterizing the role of PTEN in canine OS cell behavior with the ultimate goal of developing therapeutic strategies against the PI3K/AKT signaling pathway.

Research Grant: Steps for Sarcoma Foundation

Student Support: College of Veterinary Medicine Oncology Signature Program

A novel technique to quantify spasticity in dogs with spinal cord injury

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Spasticity is an important long-term complication of spinal cord injury (SCI) in people, resulting in increased muscle tone and painful spasms which negatively impact everyday life. There is little published information on the prevalence of spasticity in dogs with SCI and techniques to measure canine spasticity are limited. The aim of this study is to develop a novel technique to objectively quantify spasticity using a hand-held dynamometer, a device that measures resistance to passive muscle movement. The objective of this study is to evaluate reliability of a hand-held dynamometer and to assess its utility to quantify spasticity in dogs. We hypothesize that measures of muscle resistance obtained using the hand-held dynamometer will be reliable across testing sessions in normal dogs and will differ between normal and SCI-affected dogs. Ten clinically normal dogs will be initially enrolled. Dogs will be evaluated in both lateral recumbency and standing positions, and passive resistance to flexion and extension of several joints will be recorded in triplicate then averaged to produce a single value for each measure. Normal dogs will undergo two testing sessions separated by one hour and results compared between sessions. Once reliability of the technique has been established in normal dogs, SCI-affected dogs will be enrolled for comparison. The results of this study will provide partial-validation of a novel approach to measure one aspect of spasticity in dogs with SCI and will serve as preliminary data for future projects further evaluating the utility of SCI-affected dogs as a translational model of spasticity and for projects evaluating novel therapies to treat spasticity in SCI-affected individuals across species.

Research Grant: Gray Lady Foundation GRT # 60049797

Student Support: Gray Lady Foundation GRT # 60049797

Hepatic carcinogen metabolizing enzyme activity in dogs with transitional cell carcinoma

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Invasive transitional cell carcinoma (TCC) is the most common urinary bladder cancer in dogs. Canine TCC has strong cellular, molecular, and clinical similarities to human invasive TCC and can be used as a relevant disease model. Although smoking is a major risk factor for human TCC, 50% of patients have no known risk factors. Studies of canine TCC have identified prominent heritable and environmental risk factors that could apply across species. Scottish Terriers have a 20 fold increased risk for TCC, and exposure to lawn herbicides and pesticides or older generation flea control products further increases the risk. Alterations in carcinogen metabolism could explain both the genetic and the exposure risks associated with TCC in dogs. In this study, the activity of known carcinogen metabolizing enzymes was measured in liver samples from dogs with TCC and control dogs without TCC. The enzyme families chosen included cytosolic Glutathione-S-Transferases (GST) and Sulfotransferases (SULT), as well as membrane bound Cytochrome P450 (CYP), and UDP-glucuronosyltransferases (UGT). For GST activity, spectrophotometric analyses of the glutathione conjugate of 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene in the cytosolic fraction are being performed. LC/MS analyses are being used to assess CYP, UGT and SULT activities, and results are pending. GST activity has been highly variable between dogs, but in assays to date, the average GST activity in samples from dogs with TCC ($n=21$, 58.6 ± 51.2 nmol/min/mg) is not different from the GST activity in dogs without TCC ($n=6$, 58.5 ± 21.5 nmol/min/mg). Thus, preliminary results indicate that alterations in GST activity do not consistently contribute to TCC risk in dogs.

Research Grant: Private donations for bladder cancer research to the Purdue Comparative Oncology Program; Core research support from the Metabolite Profiling Facility – Bindley Bioscience Center, Purdue University

Student Support: Boehringer Ingelheim and Purdue University College of Veterinary Medicine

Evaluating telomere length as a biomarker of reproductive stress and radiation exposure in wild boar

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Following the Fukushima Daiichi Nuclear Power Plant disaster in March 2011, there has been a strong interest in researching animals in the exclusion zone to better understand the effects of chronic, low dose environmental radiation exposure. Wild boar regularly root through the soil, and so are exposed to Cesium-137, the radionuclide deposited into the topsoil and still contaminating the exclusion zones. Furthermore, the female's reproduce twice a year, making wild boar an ideal sentinel species for research in regard to chronic low dose radiation and potential effects on reproductive success. As both reproduction and environmental factors such as radiation exposure are stressors on these animals, telomere length will be analyzed. Telomere length shortens with cell division, as well as with a variety of stressors, so we hypothesize that it will serve as an informative biomarker of reproductive stress associated with chronic, low dose environmental radiation exposure. Wild boar were captured in control, low-exposure, and high-exposure radiation zones, then blood and tissue samples collected. DNA was isolated from blood samples for telomere length determination via qPCR. Telomere length will be analyzed in conjunction with reproductive parameters. Results will improve understanding of the effects of lifetime, chronic, low dose environmental radiation exposure.

Research Grant: Unknown

Student Support: Boehringer Ingelheim Veterinary Scholar Program

Seasonal effect on Equine Herpesvirus-1 shedding in Ontario broodmares

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Equine herpesvirus (EHV) 1 is ubiquitous in equine populations worldwide and can cause late gestation abortion, neonatal death, respiratory disease and myeloencephalopathy. As a member of the herpesviridae family, this virus can cause lifelong latent infections making it difficult to both identify asymptomatic carriers and predict the effects of reactivation. There is both a modified live and killed vaccine commercially available that have been proven to decrease the duration of viremia, level of viral shedding, and clinical signs, however, repeated outbreaks may still occur in vaccinated herds. A survey of breeders in Southern Ontario has been conducted, and over 30% choose not to vaccinate against EHV-1 for various reasons, and those that do not vaccinate did not report a higher incidence of abortion. Over 350 healthy broodmares from farms that completed the survey were sampled bimonthly to evaluate EHV shedding and humoral response status throughout a year's reproductive cycle. Samples collected included: nasal and vaginal swabs to gauge viral shedding, whole blood to identify viremia and latent infections, and serum for antibody levels. The overall project investigated the hypothesis that current vaccination protocols do not decrease the risk of viral shedding in healthy broodmares, and that infected broodmares will experience viremia, and shed virus intermittently. Additionally, antibody titer levels do not prevent or correlate with viremia or viral shedding. Here we present results of the viral shedding analysis at all time points to investigate seasonal trends in viral shedding, and a comparison of nasal and vaginal swabs as a method of viral detection.

Research Grant: Equine Guelph, Zoetis, OMAFRA

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Plasma lipopolysaccharide binding protein-1 as a healthspan biomarker in aging vervet monkeys

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Lipopolysaccharide binding protein 1 (LBP1) production is a response to microbial translocation and incites inflammation. LBP1 relates to healthspan in mammals, and predicts lifespan in invertebrates. Our aim was to determine if LBP1 predicts life- or healthspan in vervet monkeys. Plasma LBP1 concentrations were measured in 2 cohorts; 1) 57 animals eating healthy chow (CTL), unhealthy western diet (WD), or WD with time-restricted feeding (WD-TRF) and 2) 20 animals that died of natural causes, sampled at 17-18 years of age (≈ 65 human-year equivalent) and at a date closest to their death. The WD-TRF animals underwent intermittent fasting (IF) with food accessible between 06:00AM-2:00PM. Data from prior studies suggest IF reduces inflammation along with other benefits, independent of weight loss. We hypothesized that WD would increase LBP1 relative to CTL, and the WD-TRF would abrogate these changes. We then hypothesized that higher LBP1 values in CTL monkeys would indicate poor health trajectory and risk for shorter lifespan. WD caused 49% higher LBP-1 values ($P=0.10$) and IF resulted in lower LBP1 levels (33%; $P=0.08$). These results were similar to differences seen in glycemic control. The median age at death was 22.7 years (range 18.5-29.1), most commonly attributed to cancer, diabetes, cardiovascular and renal diseases. Lifespan did not relate to LBP1 levels but LBP-1 did increase closer to death. The increase in LBP1 was greater if LBP1 was lower at 17-18 years ($R=-0.59$, $P=0.02$). We conclude that LBP1 increases as health decreases and can be reduced by IF, but this may not increase lifespan. However, healthspan may not mirror lifespan and interventions such as IF may still be beneficial in adult populations.

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Student Support: T35OD010946

Optimization of the RT-PCR protocol for the detection of turkey arthritis reovirus

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Turkey arthritis reoviruses (TARVs) are non-enveloped, dsRNA viruses that are recognized as a significant cause of lameness in turkeys. They primarily affect male turkeys 12-17 weeks of age and present as recumbency with wing tip bruises, uni- or bilateral swelling of the hock, and other pathologic changes related to arthritis/tenosynovitis. In some instances, lameness can affect 35-70% of the flock. We have previously experimentally reproduced the disease in turkeys, fulfilling Koch's postulates. It was found that oral inoculation of TARVs can reproduce lameness and tenosynovitis in turkeys, but inoculation with chicken arthritis reovirus and turkey enteric reovirus did not cause lameness. Recently, we have observed that the RT-PCR used for the detection of TARVs is not very sensitive and this study was undertaken to address that problem. Currently, 3-6 legs are received at the MVDL (Minnesota Veterinary Diagnostic Laboratory). From these legs, 0.5 cm and 3.0 cm sections of gastrocnemius tendon and digital flexor tendon are collected, respectively, and these samples are pooled. The pooled samples are homogenized using a Genogrinder followed by centrifugation. The supernatant is used for virus isolation (egg and QT) and RT-PCR. In this study, we tested individual samples of tendons from individual legs rather than pooling them. We hypothesized that testing the samples individually will enhance the sensitivity of the RT-PCR. Preliminary results show that there are indeed cases where the pooled RT-PCR result was negative, but individual samples from that case were RT-PCR positive. This shows that there may be a dilution effect created by pooling the tendon samples.

Research Grant: Rapid Agricultural Response Fund

Student Support: Supported by the Office Of The Director, NIH, Award #T35OD011118

The effects of porcine reproductive and respiratory syndrome virus on T cell stimulation

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an economically significant disease in the swine industry. The pathogenesis is associated with its ability to cause immunosuppression, by infecting macrophages and diminishing the effector T cell response. The cellular and molecular mechanisms by which PRRSV dysregulates the host immune system is poorly understood. Better understanding of the mechanism of PRRSV associated immunosuppression will allow one to target alternative strategies to control and prevent PRRSV infection. The goal of the study was to evaluate expression of molecules associated with PD-1 axis (a receptor ligand complex that inhibits the function of T helper cells), in the lungs of PRRSV infected and control pigs. We hypothesized that porcine lungs infected with PRRSV would have a greater ratio of T cell inhibitory molecules than stimulatory molecules in infected individuals, signifying an over-stimulation of the PD-1 axis by PRRSV. This prediction was tested by evaluating the receptors and ligands associated with T cell activation and inhibition using quantitative PCR. We found that lungs from pigs infected with PRRSV had increased expression of both PD-1 ligands compared to controls. PD-L1 had average 14.3% expression increase, while PD-L2 had an average 18.0% expression increase. Our data further showed that mRNAs for PD-1 ligands were measurably increased in comparison to stimulatory molecules in infected pigs relative to control pigs. These findings are consistent with our previous results in vivo and suggest a role for the PD-1 axis in PRRSV-induced immune suppression. Developing means to inhibit the PD-1 axis in pigs may be one way to prevent viral and vaccine induced suppression.

Research Grant: Virginia Maryland College of Veterinary Medicine

Student Support: T35OD011887 NIH Office of Director

Neuroinflammation in the context of SIV/Rhesus Macaque Animal Model

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Human immunodeficiency virus (HIV) is a virus spread through certain body fluids that attacks the body's immune system, making it difficult to fight off infectious diseases. Recent studies suggest that HIV infection is followed by initiation of pathogenic processes including neuroinflammation. Simian immunodeficiency virus (SIV) is a retrovirus that causes a disease in monkeys similar to AIDS and that is closely related to HIV-2 of humans. HIV infection typically begins when a virion comes in contact with a cell surface containing a cluster of differentiation 4 (CD4) or C-C chemokine receptor type 5 (CCR5) for entry into target cell. Astrocytes are the predominant cell type in the brain that plays a key role in CNS physiology and can be found along with microglia, the residential macrophages in the brain. It has been a large topic of debate on if astrocytes can truly be infected with HIV because unlike microglia, they lack the receptors for CD4 and CCR5. Although astrocytes are phagocytic, it could be possible for there to be a non-receptor mediated mode of viral entry. Exosomes are small membrane-bound vesicles that can transport biological macromolecules from the site of production to neighboring or distant cells. The purpose of this study was to determine if activation of would be observed in astrocytes when exosomes from SIV positive and SIV negative rhesus macaques were added to them. The hypothesis is that when exosomes from an SIV positive animal model are added to an uninfected primary mixed glia culture there will be increased astrocyte activation.

Research Grant: Kenneth F. Burns

Student Support: Louisiana State University Summer Scholars Program

Neuronal migration and GSK3 β levels in the cortex of 129S6/SvEv mice, a genetic model of psychiatric disease

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Schizophrenia is a complex psychiatric disorder whose incidence is determined by many genetic and environmental factors. The 129S6/SvEv strain of mouse has a 25-base-pair deletion in *disrupted in schizophrenia 1 (DISC1)*-a gene known to confer genetic susceptibility to schizophrenia and other psychological disorders such as bipolar depression-and a DISC1 protein product that is thereby dramatically truncated. Moreover, 129S6/SvEv mice are known to exhibit behavioral phenotypes similar to those seen in human schizophrenia, bipolar depression, and major depressive disorder, i.e. despair, anhedonic demeanor and deficits in sociability, novel recognition, and working memory. Thus, this strain is considered to be a serendipitous genetic model of human psychiatric disorder. The goal of the present study is to evaluate this strain's merit as a model of some of the biomolecular characteristics of psychiatric disease observable in transgenic *DISC1*-knockout mice and bolster implications of such characteristics in the pathophysiology of such diseases. Based on data from *DISC1*-knockouts and the importance of DISC1 to neurodevelopment, we tested whether 129S/SvEv mice demonstrated aberrant neuronal migration, decreased perineuronal net formation, and decreased GSK3 β expression in the cerebral cortex relative to non-mutant controls, using immunofluorescent assays of upper-layer cortical neurons and perineuronal nets, as well as western blotting for GSK3 β . To elucidate a timeframe of manifestation for these pathophysiologicals, we compared these two strains at 5 different ages across postnatal neurodevelopment.

Research Grant: NIH R03 MH104851, NIH R03 AG052120

Student Support: NIH T35 Training Grant 5T35OD011151-15

Differentiation of early and late lytic gene expression using a Marek's disease alphaherpesvirus model

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The *Herpesviridae* family of viruses establishes a latent infection in hosts that can last for life, reactivating to produce infectious virions. Little is known about the genes involved in latency and reactivation. We are addressing these questions using Marek's disease virus (MDV), an alphaherpesvirus that affects poultry. Lymphocytes are targeted for latent infection and can be transformed to tumors. We constructed a recombinant MDV (v2001) that expresses monomeric red fluorescent protein (mRFP) inserted in frame with the early lytic RLORF4 gene (RLORF4mRFP) and enhanced green fluorescent protein (eGFP) inserted in frame with the late lytic UL47 gene (UL47eGFP). We hypothesize that viruses in the early lytic phase express only RLORF4mRFP and viruses in the late lytic phase express both RLORF4mRFP and UL47eGFP. Our goal is to develop transformed cell lines in which we can separate cells at early and late phases of lytic replication following reactivation. This approach will allow us to identify viral genes important for reactivation. Seven-day-old chickens (n=11) were infected with v2001. At 7, 14, and 21 days post-infection, the spleen, bursa of Fabricius, and thymus were collected from infected chickens and used to prepare cell suspensions of infected lymphocytes. Flow cytometry was used to detect viral antigens and host surface markers. B and T lymphocytes could be differentiated with this technique, but infection could not be verified. We are also developing *ex vivo* cell lines from tumors induced by v2001. The long-term goal of this project is to identify proteins important for reactivating herpesviruses from latently infected lymphocytes that could be used in the development of drug or antibody targets.

Research Grant: Agriculture and Food Research Initiative Competitive Grant 2013-67015-26787, USDA National Institute of Food and Agriculture

Student Support: Office of the Director, NIH, T35 OD011145

Optimization of methods to study the effect of cannabinoids on immune responses in stimulated canine PBMCs

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Current canine autoimmune disease treatments are not ideal due to limited efficacy or adverse side effects. Cannabinoids, such as cannabidiol (CBD) and tetrahydrocannabinol (THC), are compounds from the marijuana plant (*Cannabis spp.*) that have gained attention as potential treatments for autoimmune diseases. In mice it has been established that they are immunosuppressive and provide some benefit in autoimmune models, but in most other species there exists little data. The aim of this project was to examine the potential of cannabinoids as effective treatments for canine autoimmune diseases by determining whether they are immunosuppressive in canine peripheral blood mononuclear cells (PBMCs) in vitro. PBMCs were isolated from canine blood and treated with CBD or THC, followed by stimulation with ConA, LPS, or PMA/ionomycin (PI). Carboxyfluorescein succinimidyl ester (CFSE) staining was used in conjunction with flow cytometry to assess lymphocyte proliferation. RNA was also isolated to determine gene expression of cytokines interleukin-2 (IL-2) and interferon gamma (IFN γ) via qPCR. After assessing PBMC responsiveness under various conditions, the studies focused on PI as it was the most effective stimulant. Results from both flow cytometry and qPCR indicated that CBD and THC could affect proliferation and cytokine production, but that it was dog-specific; some dogs exhibited no effect, while other dogs exhibited modest suppression by CBD and THC. IFN γ consistently exhibited a higher magnitude of change in expression than IL-2. These findings provide important preliminary insight into the potential of these compounds for canine autoimmune diseases, and establish a knowledge base of effective methods for future studies.

Research Grant: NIH Training Grant T35 OD010432

Student Support: NIH Training Grant T35 OD010432

Increased expression of galectin-3 and CD25 as pre-neoplastic events in canine T zone lymphoma

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T zone lymphoma (TZL) is a common type of T cell lymphoma in dogs, with an indolent clinical course. The TZL phenotype is characterized by loss of the pan-leukocyte antigen CD45 on neoplastic T cells. Some older dogs have a small subset of CD45- T cells, which we call subclinical, but no other evidence of TZL. The goal of this study is to determine if these T cells exhibit phenotypic features also seen in neoplastic T zone T cells, thereby supporting the hypothesis that the CD45- T cells in subclinical dogs are pre-neoplastic. TZL cells express very high levels of intracellular galectin-3 and cell surface CD25. We hypothesize that CD45- T cells from subclinical dogs without TZL will exhibit upregulation of galectin-3 and CD25, demonstrating this is an early event in progression to TZL. We measured intracellular galectin-3 and cell surface CD25 expression by flow cytometry in CD45- T cells in subclinical dogs and TZL dogs. Results showed increased expression of galectin-3 in CD45- T cells, regardless of whether there was a diagnosis of TZL. Normal CD45+ T cells had lower expression of galectin-3. In subclinical dogs, CD45- T cells had increased CD25 expression compared to normal T cells. These results suggest CD45- T cells in subclinical dogs are beginning to exhibit phenotypic characteristics of TZL cells.

Research Grant: Clinical Immunology, Colorado State University

Student Support: Canine Lymphoma Foundation

Hepatocyte growth factor mimetics as a potential therapeutic for muscle wasting diseases

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Muscle wasting diseases including the multiple forms of muscular dystrophy (MD) represent a family of untreatable diseases that are rapidly debilitating and universally fatal. Current treatment strategies, which are purely palliative in nature, can neither alter disease progression nor restore lost motor function. The aim of this study is to repurpose a Hepatocyte Growth Factor mimetic, MD-123, which was developed at Washington State University and is currently in clinical trials as a treatment for Alzheimer's disease, as a disease modifying treatment for MD capable of stopping disease progression and restoring lost motor function. The hypothesis of the study is that the administration of MD-123 to healthy rats will alter skeletal muscle and thus motor function in one or more of the following ways: increased activation and proliferation of resident muscle stem cells, increased angiogenesis, and increased muscle innervation. Twenty male Sprague Dawley rats are currently receiving subcutaneous injections of either MD-123 at 1mg/kg (N=10) or vehicle (N=10) every other day. Motor function will be tested twice weekly until clear performance differences exist between treated and control groups. Once differences in motor function are apparent, several muscles will be isolated from rats in both groups and relevant cellular markers will be quantified using fluorescent microscopy and cell morphometric software. These markers will include: BrDU (stem cell activation and proliferation), Endothelial Factor VIII (angiogenesis), and α -bungarotoxin (muscle innervation). Final analyses are expected to be finished in late August.

Research Grant: M3 Biotechnology

Student Support: WSU College of Veterinary Medicine Summer Research Fellowship Program

Evaluating the effect of UVB exposure on protein electrophoresis and biochemistry findings in Leopard Geckos

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Vitamin D plays many important roles in vertebrates, the most notable being calcium homeostasis and bone growth. However, other important roles that are not as well studied include its role in metabolism, neuromuscular function, reproduction, cardiovascular health, and immune function. Many captive reptiles are deficient in vitamin D as a result of low concentrations of the hormone in their diet or due to a lack of exposure to UVB light. It is imperative for us to better understand the specific roles of vitamin D in reptilian species, in order to make recommendations for best practices in these animals. Leopard geckos are one of the most commonly bred lizard species, and although crepuscular, are known to increase their circulating concentrations of 25-hydroxyvitamin D₃ after exposure to UVB light. The purpose of this study was to identify whether geckos exposed to short term UVB radiation resulted in any significant changes in systemic health or immune function. In this study, 17 adult male leopard geckos were split into two groups, a treatment group receiving UVB radiation and a control group receiving no UVB. Baseline blood chemistries and protein electrophoresis values were compared to samples collected at the end of the 30-day study. We hypothesize that leopard geckos exposed to UVB will have higher circulating 25-hydroxyvitamin D₃ concentrations, significantly higher circulating alpha, beta, and gamma globulin concentrations, and potentially altered calcium to phosphorus ratios as compared to leopard geckos that were not exposed to UVB.

Research Grant: Kenneth F. Burns Lectureship and Clinical Clerkship Foundation

Student Support: Louisiana State University School of Veterinary Medicine & Kenneth F. Burns Foundation

Evaluation of transdermal flunixin and meloxicam in piglets at processing age

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Transdermal non-steroidal anti-inflammatory drugs in food animals is a potential application for pain control. In Europe, the use of analgesia when processing piglets, which includes castration and tail docking, is required and due to increasing emphasis on animal welfare, the US will likely follow suit. This study aims to assess the absorption and distribution of meloxicam and flunixin following transdermal application. Piglets (n=3) were dosed with either 3.3mg/kg of transdermal Banamine (FLU) or the same dose of Banamine mixed with 0.66mg/kg meloxicam (MIX). The dose was applied lateral to dorsal midline. Blood was collected via a jugular catheter and interstitial fluid (ISF) via a subcutaneously placed probe for 72 hours post-dose. Flunixin plasma concentrations of MIX piglets reached C_{max} sooner and were lower (66.09 ± 16.12 ng/ml; T_{max} 16.0 ± 6.9 h) compared to FLU piglets (C_{max} 72.60 ± 40.61 ng/ml; T_{max} 20.0 ± 13.9 h), although concentrations were not significantly different between treatment groups at any time point. Meloxicam plasma concentrations in MIX piglets reached C_{max} 124.46 ± 57.35 ng/ml and T_{max} 20.0 ± 6.9 h. Flunixin ISF concentrations for FLU (C_{max} 1733 ± 789 ng/ml; T_{max} 22.1 ± 0 h) and MIX (C_{max} 7668 ± 865 ng/ml; 16.1 ± 8.5 h) were significantly different ($P < 0.05$) at 12, 24, and 36 hours despite the low number of replicates. Meloxicam ISF concentrations were much lower and peaked later with C_{max} 241.6 ± 104.7 ng/ml and T_{max} 28.1 ± 8.5 h. This suggests that flunixin could be more extensively and rapidly distributed to tissues. Future studies will also include clinical measurement of pain alleviation and residue determination since findings indicate that both drugs are still present in the body at 72 hours.

Research Grant: North Carolina Pork Council (NCPC) Grant 560553

Student Support: Boehringer Ingelheim Veterinary Scholars Program (VSP)

The effects of hydrocortisone, vitamin C, and thiamine on cytokines in an ex vivo bacterial sepsis model

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Bacterial sepsis is a leading cause of death in foals and infants. Recent studies have demonstrated that administration of hydrocortisone, vitamin C, and thiamine in combination reduces organ dysfunction and mortality in people with severe sepsis, and can alter leukocyte function and inflammatory responses. Thus, this therapy might enhance the foal's immune system's ability to combat bacterial sepsis. We hypothesized that the cytokine responses to bacterial antigens would differ between adult horse and foal leukocytes, and that cytokine production would be altered when leukocytes were exposed to bacterial antigens in the presence of hydrocortisone, vitamin C and thiamine in both foal and horse cells. Peripheral blood mononuclear cells (PBMCs) were isolated from 6 healthy adult horses and 7 one-day old foals via density gradient centrifugation. PBMCs were exposed to lipopolysaccharide (LPS) or killed whole-cell *Staphylococcus aureus* in the presence or absence of hydrocortisone, vitamin c, and thiamine, alone and in combinations. Cells in media only served as a negative control to assess basal cytokine responses. PBMCs were incubated in the above conditions for 6hrs, and supernatants were collected and stored at -80C for batch analysis of cytokine concentrations with validated immunoassays. Preliminary analysis revealed that the quantity of pro- and anti-inflammatory cytokines produced differed significantly between horse and foal PBMCs. Further, exposure to LPS in the presence of hydrocortisone and thiamine in combination significantly decreased TNF-alpha production from both horse and foal PBMCs. Further evaluation of these compounds as potential immune-modulating therapies in equine neonatal sepsis is warranted.

Research Grant: Steve Lee Summer Research & Grants on the Edge Funding, UGA College of Veterinary Medicine

Student Support: Steve Lee Summer Research Stipend, UGA College of Veterinary Medicine

Incidence of non-toxigenic and toxigenic *Clostridium difficile* isolated from animals

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C. difficile causes almost half a million infections and an estimated 29,000 deaths per year in the U.S. alone. Instances of both non-toxigenic and toxigenic strains of *C. difficile* have been documented in both humans and animals. However, little is known about the prevalence of this bacterium in domestic animals, and the role it plays in human transmission and disease. This study characterizes the toxin gene profile and prevalence of *C. difficile* strains isolated from animals. Fecal samples (199 total) from six host species including dogs, cats, horses, donkeys, sheep, alpaca, and chickens were obtained from the Veterinary Hospital Microbiology Diagnostic Laboratory. *C. difficile* was discovered in 21% of samples (42/199), which included 33 dogs, 2 cats, 5 horses, and 2 donkeys. These isolates were confirmed *C. difficile* positive by 16S rRNA sequencing. The primary mediators of inflammation in *C. difficile* infection (CDI) are large clostridial toxins toxin A (tcdA, gene) and toxin B (tcdB), and, in some strains, the binary toxins (cdtAB). We ran a five plex PCR method to screen for toxin genes listed above using the 16S rRNA as an internal control. PCR on *C. difficile* positive samples revealed five different profiles: tcdA+, tcdB+, cdtAB- (22); tcdA-, tcdB-, cdtAB- (12); tcdA-, tcdB+, cdtAB+ (3); tcdA+, tcdB-, cdtAB- (4); and tcdA+, tcdB+, cdtAB+ (1). In our study, 21% of animals tested were carrying either a non-toxigenic or toxigenic strain of *C. difficile*, suggesting that this could represent a reservoir for zoonotic transmission.

Research Grant: None

Student Support: NC State College of Veterinary Medicine Boehringer Ingelheim Veterinary Scholars Program

Annexin V: A possible role in the development of endometrial degenerative fibrosis in the mare?

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Mares with endometritis and subsequent endometrial degenerative fibrosis (EDF), also known as endometrosis, resulting in infertility represent enormous economic losses to the equine industry. Despite its sizeable impact, the etiology and pathogenesis of EDF is currently unknown. Previous studies suggest that periglandular accumulation of inflammatory cells with consequent destruction of the basal lamina and expression of vimentin play a role. Recent studies in our laboratory have demonstrated that high levels of annexin V are associated with endometritis/EDF and that mares with persistent breeding-induced endometritis have significantly higher levels compared to normal mares ($P=0.02$). Annexin V, a marker of apoptosis and chemotactic factor, has been implicated in the pathogenesis of idiopathic pulmonary fibrosis in a rat model. We hypothesize that pattern of expression of annexin V will differ in biopsies from mares with endometritis/EDF compared to normal mares. The aim of this preliminary study was to optimize the immunofluorescence protocol and localize annexin V in endometrial biopsies from normal mares ($n=5$), mares with endometritis ($n=5$) and EDF ($n=5$) using immunofluorescence. Immunofluorescence analysis was performed on samples using mouse monoclonal anti-annexin V antibody (Abcam ab54775) and Invitrogen Gt anti-Ms IgG (H+L) Alexa Fluor 488 conjugate, then visualized via confocal microscopy. Data collection and protocol optimization for this study are ongoing. Results could lead to better understanding of annexin's role in the development of endometrial scarring and aid in the development of methods to prevent or minimize these conditions, thus improving fertility and economic return to the breeding industry.

Research Grant: OMAFRA and Ontario Veterinary College CORE program

Student Support: Andrea Leger Dunbar Studentship

Bone marrow supernatant is an alternative to fetal bovine serum in MSC isolation and expansion

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Equine bone marrow derived mesenchymal stem cells (MSCs) are routinely expanded in a culture media supplemented with fetal bovine serum (FBS). For clinical use, the presence of xenogen should be avoided because of the potential for immune recognition and disease transmission. As an alternative, we investigated autologous bone marrow supernatant (BMS) as an alternative to FBS for MSC isolation and expansion. We hypothesized there would be no significant difference in isolation or expansion, immunomodulatory function, or characterization of MSCs grown in BMS compared to FBS. Bone marrow was drawn from the sternum of six horses and plated for culture in a media containing either 10% FBS or autologous BMS. Colony forming unit-fibroblast assay (CFU-f), total cell number after three passages, and population doubling times were compared. We found a significant increase in CFU-f in the BMS group compared to the FBS group. There were no significant differences in the overall population doubling time or in the total cell number in the BMS group as compared to the FBS group. Our initial results indicate that BMS supplemented media is an adequate replacement for FBS supplemented media in the expansion of MSCs.

Research Grant: Link Endowment for Equine Research at Texas A&M University and the Dennis and Linda Clark Endowment for Equine Orthopedic Research from the Department of Large Animal Sciences at Texas A&M University

Student Support: Boehringer Ingelheim VSP, Texas A&M College of Veterinary Medicine & Biomedical Sciences

Hypertension in vervet monkeys and sodium-lithium countertransport (SLC)

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Hypertension is a chronic, complex disease that affects one out of every three American adults. It is influenced by sex, genetics, environmental factors, and dietary salt. Sodium lithium countertransport (SLC) is one of a few mechanisms in which sodium is transported across the red blood cell (RBC) membrane. Studies have shown that SLC activity is heritable in primates, but not rodents, and that there is an increase in the maximum rate of RBC SLC activity in some patients with essential hypertension. The gene variants and molecular mechanisms responsible for this occurrence, however, are unclear. To identify the gene variants and mechanisms by which these variants influence blood pressure, we proposed to use a pedigree-genomically-sequenced population of Caribbean-origin vervet/African green monkeys (*Chlorocebus aethiops sabaes*) located at the Clarkson Campus at Wake Forest University School of Medicine due to their genetic and anatomical similarities to humans. Furthermore, a portion of this vervet colony develops spontaneous hypertension, but the genes and mechanisms contributing to this phenotype are unknown. Therefore, we hypothesized that hypertensive vervet monkeys from this colony will have increased SLC activity and will serve as an appropriate animal model to study SLC activity in relation to hypertension. To test our hypothesis, we measured blood pressure values and collected blood samples from a group of normotensive and hypertensive animals to assess RBC SLC activity and intracellular (ICNa) concentration. Through this experiment, we found that vervets may be useful animal models in studies pertaining to essential hypertension in humans.

Research Grant: Wake Forest University School of Medicine Summer Research Fellowship

Student Support: NIH T35 Training Grant

Endometrial hyperplasia and pyometra in African painted dogs: An overview of the female reproductive pathology

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African painted dogs (APD), *Lycaon pictus*, are endangered with less than 7,000 dogs living in the wild. The APD is considered to be more susceptible to developing endometrial hyperplasia (EH) and pyometra than other canids. These conditions are often fatal if untreated or lead to permanent infertility. We hypothesize that the longer a female remains barren or is given hormonal contraceptives, the more likely it will develop EH. Histological sections from uteri and ovaries and the demographics/medical history of APDs submitted to the lab through the AZA Reproductive Health Surveillance Program contraceptive database were examined, lesions categorized, and data analyzed. We expect most APD females will have reproductive lesions, including endometrial hyperplasia (cystic and adenomatous), adenomyosis, metritis, pyometra, and neoplasia and predict that there will be a correlation between the number of reproductive cycles without pregnancy or parturition. With a better understanding of the healthy and pathologic reproductive system of the African painted dog, we can better mold reproductive management strategies. This will improve both long term reproductive health in the zoological setting and help with conservation efforts.

Research Grant: Association of Zoos and Aquariums Reproductive Management Center

Student Support: Boehringer Ingelheim Veterinary Scholars Grant to Michigan State University

Molecular dissection of mosquito resistance to heartworm infection

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Heartworm disease is caused by the parasitic nematode *Dirofilaria immitis*, which requires mosquitoes for both larval development and transmission. Due to its high prevalence in North America and in evidence of parasite resistance to drugs of the macrocyclic lactone class, there is a need to develop novel transmission-blocking strategies, such as targeting *D. immitis* within its vector. Our experiments aim to disrupt parasite development by over-activating components of mosquito immune signaling. Using a *D. immitis* infection-susceptible strain of the mosquito species *Aedes aegypti*, we found that activating the Toll pathway through RNAi-mediated silencing of the pathway inhibitor Cactus (I κ B) resulted in a strong decrease in the number of transmission stage heartworm larvae (L3), which were developmentally arrested in mosquito Malpighian tubules. Co-silencing the Toll pathway transcription factor REL1 (NF- κ B) alongside Cactus rescued parasite development. These data suggest that Toll signaling can block *D. immitis* development through transcription of Toll pathway target genes in Malpighian tubules. We have begun to identify and screen Toll signaling target genes to determine their role in restricting parasite development. Our preliminary data show that co-silencing Cactus with a component of the mosquito complement pathway, LRIM1, also leads to reversal of the effects of the Cactus silencing phenotype. Thus, REL1 and LRIM1 may represent potential targets for over-activation in transgenic mosquitoes as a measure to control heartworm transmission.

Research Grant: NIH T35OD010919 and Boehringer Ingelheim

Student Support: Meriel and Morris Animal Foundation Veterinary Student Scholars Program

Molecular taxonomy of *Neorickettsia* spp. from *Nanophyetus salmincola* and dogs with salmon poisoning

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Salmon poisoning is a lethal infection of dogs caused by *Neorickettsia helminthoeca*. This disease most commonly affects dogs in the Pacific Northwest and Canada. The bacterium is vertically transmitted in the trematode *Nanophyetus salmincola* through a complex life cycle that involves snails (*Juga plicifera*) as the first intermediate host, salmonid and other freshwater fishes, fishes and the Pacific giant salamander as second intermediate hosts, and fish-eating mammals as definitive hosts. Metacercariae of *N. salmincola* from a salmon in Oregon was identified as a vector of a second species of *Neorickettsia* known as *Stellanchamus falcatus* (SF) agent. This bacterium has been found to cause clinical signs in dogs that are similar, yet milder, to salmon poisoning disease caused by *N. helminthoeca*. The purpose of this study is to determine the prevalence of each species of *Neorickettsia*, and perhaps others, in cercariae shed by *Juga* snails, metacercariae of salmon, and blood from afflicted dogs in an attempt to expand our knowledge of their roles in salmon poisoning disease. To date, we have collected several samples from these categories, which will soon be screened using general neorickettsia primers (a real-time PCR protocol). Samples positive for neorickettsial DNA will be verified using a nested PCR protocol amplifying a 1370bp fragment of the 16S rRNA gene as described by Greiman et al. (2014) with subsequent sequencing of PCR products for molecular taxonomy.

Research Grant: Boehringer Ingelheim Veterinary Scholars Program

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Role of microRNAs in the lupus-gut microbiome axis

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Systemic lupus erythematosus is a chronic inflammatory autoimmune disease. Lupus can affect many organs throughout the body and inflict a large variety of symptoms with no known cure. microRNAs (miRNA) are known regulators of genes including those from the immune system. Understanding dysregulation of vital regulatory factors such as miRNAs in the immune system is integral to creating diagnostic tests and treatments. Preliminary studies have looked at the link between the host gut microbiome in relation to lupus symptoms and how treatment with antibiotics (such as vancomycin) influences the progression of the disease by decreasing intestinal permeability and the level of lipopolysaccharide in the plasma. It is unknown whether vancomycin exerts its protective effects against lupus through regulating lupus-associated miRNAs; however, as these lupus-associated miRNAs are upregulated as lupus progresses, we anticipate that vancomycin treatment, or the removal of certain gut bacteria, will downregulate these miRNAs. The aims of this study were to identify lupus associated miRNA targets through next-generation sequencing and validate the miRNA targets through RT-qPCR. Sequencing data showed 34 upregulated and 16 downregulated miRNAs from which 5 miRNAs from the spleen: miR-26b-5p, miR-409-3p, miR-342-3p, miR-451a, 211-5p, and 4 miRNAs from the kidney: let-7j, miR-145a-5p, miR-1843b-5p, miR-146b-5p were chosen for validation based on known autoimmune and lupus association. Validating these target miRNAs with RT-qPCR would further support the involvement of these miRNA sequences in the pathogenesis of lupus.

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Dwarf Deerhounds: genetic investigation of the *COMP* gene in dogs with pseudoachondroplasia

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Pseudoachondroplasia (PSACH) is a skeletal dysplasia that causes dwarfism. All human cases of PSACH have been traced exclusively to the *COMP* gene, encoding cartilage oligomeric matrix protein. To date, 111 PSACH-causing mutations have been described in human *COMP*. PSACH is clinically unrecognizable at birth, but becomes evident by 2 years, and is characterized by gait changes and decelerated growth ultimately resulting in disproportionate short stature. PSACH has been described in Scottish Deerhounds (SD) with clinical, radiologic, and pathologic findings in striking parallel to human PSACH. The mode of inheritance in humans is autosomal dominant, with occasional reports of autosomal recessive; the latter appears to be the SD pattern of inheritance based on pedigree analysis. However, human families with phenotypically normal parents and PSACH offspring, initially suspected to have autosomal recessive inheritance, have instead revealed germline/somatic mosaicism in one of the parents. This scenario should also be considered in dogs. In the present study, we used a candidate gene approach to investigate the cause of PSACH in a family of SDs. DNA was extracted from archival bone samples of 3 dwarf and 2 normal SD littermates. We Sanger sequenced all *COMP* exons, as well as flanking intronic sequence, in one dwarf and one normal SD to screen for mutations. Variants observed in the dwarf SD, for which the normal SD was identical to the canine reference genome, were sequenced on all other SDs to confirm or exclude the variant as a potential cause of PSACH.

Research Grant: Funding provided by start-up funds of Dr. Kari Ekenstedt

Student Support: Boehringer Ingelheim, Purdue University College of Veterinary Medicine

Microglial density and morphology following CTB-saporin induced respiratory motor neuron death

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Motor neuron death and increased microglia (resident immune cells in the CNS) are observed in many neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). Loss of motor neurons (*e.g.*, phrenic and intercostal) innervating inspiratory muscles (*e.g.*, diaphragm and intercostal muscles) leads to impaired respiratory function and eventually death in ALS. In addition, increased microglial density corresponds with motor neuron death, but whether this increase is beneficial or harmful remains unknown. Microglia can exist in multiple states including resting (ramified morphology) and activated (amoeboid morphology). Increased microglial density is also observed in the phrenic motor nucleus in a novel rat model of respiratory motor neuron death induced by intrapleural injections of cholera toxin B conjugated to saporin (CTB-SAP); however, less is known about microglia in the intercostal motor nucleus. In this study, microglial density and morphology in the intercostal motor nucleus as well as microglial morphology in the phrenic motor nucleus will be analyzed. Cervical and thoracic spinal cord sections containing the phrenic and intercostal motor nuclei from CTB-SAP treated rats will be stained for microglia using immunohistochemistry techniques, visualized using confocal microscopy, and analyzed using ImageJ and IMARIS software. We hypothesize that there will be an increase in microglial density in the intercostal motor nucleus, as well as amoeboid microglial morphology in both the phrenic and intercostal motor nuclei. If the data support our hypotheses, this would indicate increased microglial activation in areas controlling inspiration and suggest that microglia may play a role in respiratory function.

Research Grant: Committee on Research (COR) grant

Student Support: Committee on Research (COR) grant

The *In vitro* effects of zoledronic acid and radiation therapy in canine osteosarcoma

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Osteosarcoma accounts for roughly 85% of all canine bone tumors. In addition, spontaneously occurring canine OS shares many characteristics with human OS, making dogs an ideal comparative model. In both species, surgery is typical for local therapy; however, targeted radiation therapy is used in patients with non-surgical disease. In other cancers affecting bone, combination radiation therapy and bisphosphonates are synergistic. The objective of our study is to evaluate the combined effect of zoledronic acid and radiation therapy on canine osteosarcoma cell growth, proliferation, and death. We hypothesize that there will be a significant increase in apoptosis in cells treated with combination radiation therapy and zoledronic acid, compared to either therapy alone and cause synergistic apoptosis. Abrams and D17 canine osteosarcoma cell lines were grown in 96 well plates and incubated for 24 hours at 37°C. Subsequently, the cells were treated with zoledronic acid at biologically achievable concentrations: 0.1, 1, 10, and 100 µM. The cells were irradiated (200, 400, 600, and 800 cGy) at 48 hours. A control group of untreated cells were processed in parallel. Cell viability, cytotoxicity, and apoptosis were determined using the Triplex ApoTox assay. We have previously shown a statistically significant increase in apoptosis in Abrams and D17 canine osteosarcoma cell lines treated with radiation and zoledronic acid (400 cGy and 10 µM), compared to either treatment alone. Future studies include using a comet analysis to determine quantity and quality of double strand DNA breaks in these cell lines.

Research Grant: Comparative Oncology Laboratory Start-up Funds

Student Support: The Mizzou Advantage Initiative in One Health / One Medicine

Evaluation of a commercial *Haemonchus contortus* vaccine in non-domestic hoofstock

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Similar to small ruminants, non-domestic hoofstock in human care face significant challenges with parasite management. *Haemonchus contortus* is of particular concern, causing significant morbidity and mortality in zoological collections within the United States. Management of this nematode is often complicated by the high stress nature, unique social structures, behavior, and particular habitat requirements of these non-domestic hoofstock species. This study utilized Barbervax, a vaccine that has shown efficacy in sheep, goats, and alpacas at producing antibodies against the gastrointestinal intraluminal membrane protease complex, H11/H-gal-GP responsible for nutrient metabolism within *H. contortus*. This vaccine is further effective at lowering mortality associated with infection. Specific aims of this study were to determine the safety of Barbervax administered to Chinese gorals (n=4) and pronghorns (n=3) and determine if vaccination would result in antibody production directed to the protease complex that, under normal conditions, is not detected by the host immune system. All animals involved were vaccinated at regular intervals and kept on parasite contaminated pastures during the summer months. Serum was collected to evaluate antibody production. The animals showed no detectable local or systemic indications of adverse vaccine reaction. We also investigated appropriate secondary antibodies for optimization of the non-domestic hoofstock ELISA assay to detect the H11/H-gal-GP specific antibodies. The gorals and pronghorns produced antibodies following vaccination. These results demonstrate potential for Barbervax to be a beneficial adjunct for *H. contortus* management in non-domestic hoofstock in human care.

Research Grant: OSU and Columbus Zoo and Aquarium Cooperative Grant

Student Support: Boehringer Ingelheim

Identification of phages binding to epithelial and to mesenchymal (EMT) phenotypic breast cancer cells

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Therapeutic drugs that have been made available that have not efficiently treated tumors in individuals; the absence of specificity has harmful effects on patients. During phage display, the peptide and protein libraries are expressed on the surface of the specific phage with diversity of 109 peptides of different permutations. This will then lead to a selection of peptides and proteins with high affinity and specificity. Initially, a depletion assay was performed to isolate phages that express an affinity to human cells. We isolated a phage diversity of 1020 from 109. In addition, subsets of population were used to determine the phage and breast cancer cell interaction in vitro using ELISA and Plaque Binding assay. Exposure of the phages to epithelial and mesenchymal breast cancer cells and fibroblast cells become prominent. Due to immunofluorescence and tissue microarray, findings were determined. A phage profile that targeted tumor cells based on their transition and change from epithelial to mesenchymal. In addition, L-24, a phage subset, indicated a greater affinity for more mesenchymal-like tumor cells. Adding to the information, an increase in the binding affinity for MDA-MB-231 in comparison to the control fibroblast cells was identified. The identified phage profile exhibits a preferential affinity for more mesenchymal like tumor cells, which are known to be aggressive. The capability of chemotherapy for this specific phage is further suggested. Experimentation in the future will include a further validation of selected phages and development of peptides to be employed as imaging agents to study EMT in vivo and as diagnostics to detect circulating tumor cells.

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Student Support: Boehringer Ingelheim Research Scholarship

Behavioral correlates of canine tail position in a leash-associated aggression model

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A dog's tail is used as an important inter- and intra-specific communicative signal. However, there is little scientific literature to help pet owners or veterinarians interpret the dog's tail activity and predict their behavior. Most prior research on tail position has been subjective, but with the advent of new technologies, we aim to quantify tail use with depth cameras. We hypothesize that tail-wag frequency and tail position patterns in response to dog- and non-dog related stimuli will be different for aggressive and non-aggressive dogs. We recruited 56 dogs, 40 with unfamiliar dog-directed aggression and 16 with no aggression, and recorded their interactions with their owner, dog related (model dogs, unfamiliar real dog, and/or familiar real dog) and non-dog related stimuli. In addition to quantifying the tail using depth cameras, behavioral coding (length of barking, whining, growling, number of lunges and owner-directed social referencing) was performed to correlate the tail with other behavioral signaling. Preliminary results suggest that aggressive dogs show higher rates of barking and lunging at the dog related stimuli than the non-dog related stimuli. The aggressive dogs also showed higher rates of barking and lunging at the dog related stimuli than the non-aggressive dogs. Importantly, dogs wag their tails while displaying these behaviors. Analysis of the depth camera data is ongoing; use of these cameras appears feasible. In conclusion, our data supports the tail as an important communicative tool, and we aim to provide objective parameters to best interpret this aspect of dogs' body language.

Research Grant: Margaret Gruen's practice plan

Student Support: North Carolina State College of Veterinary Medicine Summer Research Internship Program

Influence of *Trypanosoma cruzi* strain and vector species on selected canine Chagas cardiac disease outcomes

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Chagas disease is an infectious cardiac disease of humans and animals caused by the zoonotic protozoan parasite *Trypanosoma cruzi* and transmitted by triatomine vectors. The clinical manifestations of canine Chagas disease range from asymptomatic infections to chronic heart disease or sudden death, with little understanding of what contributes to variation in outcome. This study aims to determine how parasite genetic strain type, triatomine vector species, and geographic location contribute to variation in clinical outcomes of infection. We hypothesized that there would be variation in the frequency and severity of selected cardiac abnormalities across dogs of various infection status. We enrolled 120 dogs from 10 kennels across different ecological regions of Texas, including 56 pairs of seropositive and seronegative dogs in which pairs were matched based on breed, age, and location. While at the kennels, we applied 24-hour ECG Holter monitors and collected blood for measuring anti-*T. cruzi* antibodies, parasite DNA load, strain type, and cardiac troponin (cTnI), a biomarker of cardiac injury; we also surveyed the kennels for triatomines. Preliminary analysis of ambulatory ECGs showed atrioventricular block and ventricular arrhythmias in seropositive dogs. The average cTnI levels for seropositive dogs (0.09 ng/mL $\pm$ SD 0.11) trended higher than that of seronegative dogs (0.04 ng/mL $\pm$ SD 0.04; $P=0.14$). We collected 199 triatomines representing 4 species and found 31% (9/29) infection prevalence including strain types TcI and TcIV. The information acquired from this study will help define prognostic indicators and clinical outcomes of dogs infected with *T. cruzi*.

Research Grant: American Kennel Club Canine Health Foundation No. 02448

Student Support: National Institutes of Health #5T35OD010991-14

Effect of printing material and parameters on the mechanical properties of 3-dimensionally printed bone models

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Three-dimensionally printed bone models are used for clinical, educational, and research purposes in the medical and veterinary fields. Many printers are able to achieve anatomical fidelity of specimens, however, it can be challenging to create a model with similar tissue characteristics. The objective of this study was to evaluate the effects of printing material and printing parameters on the mechanical properties of 3D printed bone models. We hypothesized that by selecting parameters for internal fill, contour width, and contour number 3D models of a canine radius and equine proximal phalanx could be printed with mechanical properties comparable with normal orthopaedic bone. Threading torque, insertional torque, and screw pull out force were measured and compared to the values of the original bone.

Research Grant: Morris Animal Foundation

Student Support: Morris Animal Foundation

Optimization of protocols for RNA, DNA, and chromatin isolation for epigenetic annotation

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Since the Encyclopedia of DNA elements (ENCODE) project in 2003, our understanding of mammalian epigenetic function has vastly improved. These factors have wide ranging effects on health, growth, and reproduction. While ENCODE focused on profiling cell lines, succeeding projects expanded to include murine models to reference epigenomes in primary cells. Such work has just recently begun in agriculturally important species. The regulatory elements of interest are relatively conserved across species, but every cell and tissue require optimized methods. We are working with investigators of the Functional Annotation of Animal Genomes (FAANG) Consortium to standardize the techniques used to reference epigenomes of target reproductive tissues and peripheral blood mononuclear cells (PBMCs) of the chicken. Blood was collected and PBMCs isolated from production chickens using a Ficoll-Paque density gradient. Monocytes, B-cells, and CD4+ T-cells were positively isolated using antibody-bound magnetic beads. Target reproductive tissues, including ovary, oviduct, and shell gland, were collected from euthanized birds and subsequently flash frozen, ground, and homogenized using a frozen tissue grinding protocol. DNA and RNA were isolated for reference libraries using the AllPrep DNA/RNA Mini kit (Qiagen). Cells and tissue were prepared for chromatin immunoprecipitation sequencing (ChIP-seq) by cross-linking in 1% formaldehyde followed by cell lysis and subsequent washes. Nuclei were collected, and chromatin sheared using a Covaris focused-ultrasonicator. Optimized protocols will be used to annotate regulatory elements and elucidate their functions in the chicken related to enhanced immunity, disease resistance, and reproductive health.

Research Grant: USDA

Student Support: Boehringer Ingelheim Veterinary Research Scholars Program. WesternU CVM Fellowship

Environmental persistence of MAP as a barrier to Johne's disease elimination

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Johne's disease is pervasive on dairy farms in the United States and is notoriously difficult to control. One suspected reason for the difficulty in managing Johne's disease is the ability for *Mycobacterium avium* ssp. *paratuberculosis* (MAP), the causative agent, to persist in the environment. Animals become infected with MAP by ingesting contaminated material in their environment, and once infected can shed large quantities of MAP in their feces. With fecal shedding coupled to the ability of MAP to persist in the environment, dairy farmers may be unable to rid their herd of Johne's disease without intense focus on environmental management. Although it is well documented that environmental contamination is an important factor in MAP transmission, few simulation models include an environmental component, and no agent-based model with explicit environmental transmission exists. We developed an agent-based model of Johne's disease in a US dairy farm with explicit environmental MAP transmission. Individual cows, grouped by age and production characteristics, could become infected through exposure to MAP in their simulated environment. Infected animals could contaminate their environment by shedding MAP quantities proportional to the amount of manure produced and their infection stage. Using this model, determined if herd level MAP elimination was possible through intensive environmental management. We also determined if a threshold of environmental MAP contamination exists that prevents elimination. We found that MAP elimination is possible with vigorous cleaning alone, but the level of hygiene necessary for elimination is likely impossible to achieve in a true herd.

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Autism-relevant behavioral outcomes in an antigen-driven rat model of maternal autoantibody-related autism

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Autism spectrum disorder (ASD) affects over 1% of children in the United States, yet there remains little understanding of the underlying causes and mechanisms of ASD. We present a preclinical model to investigate one potential cause of ASD - maternal autoantibodies targeting fetal brain tissue found in a subset of mothers of children with ASD. We hypothesize that these maternal antibodies cross the placenta during pregnancy and interact with fetal brain proteins important for neural development, resulting in long lasting changes in offspring brain and behavior. In this model, rat pups are continuously exposed to maternal antibodies throughout gestation, mimicking the exposure experienced by human fetuses. We predict that rat pups exposed to these antibodies prenatally will show deficits in social behavior as paralleled in children with ASD. In addition, we are piloting a study to evaluate expression of Parvalbumin (PV) cells most found in the basolateral nucleus in the amygdala using a Nissl and PV stain. The amygdala is an area of the brain important in species typical social behavior populated with interneurons (PV cells) that play a role in the balance of excitation and inhibition in perception of social interactions and threats. An emerging theory is that individuals with ASD perceive social interactions as threatening, and therefore avoid socialization as a means to alleviate the fear that it triggers. Future directions include detailed analysis of reciprocal social interactions, quantitative analysis of PV+ cell numbers in the basolateral amygdala and integrating *in vivo* neuroimaging to provide insight into underlying neuropathology associated with prenatal exposure to autism-associated maternal antibodies.

Research Grant: Nancy Lurie Marks Foundation

Student Support: National Institutes of Health (NIH), UC Davis School of Veterinary Medicine STAR Fellowship

Assessment of nutritional, clinical and hematological status in dogs fed a plant-based versus meat-based diet

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For both humans and their companion dogs, plant based diets have been gaining popularity over the years for economic, environmental, and health reasons. Small animal clinicians that have recommended nutritionally adequate plant-based diets to their dog patients have observed clinical improvements in some medical conditions such as food and skin allergies, chronic diarrhea and vomiting. However, there is a lack of scientific data documenting the potential medical benefits of nutritionally adequate plant-based diets in the dog. The aim of this ongoing study is to assess the nutritional, clinical and hematological status in adult clinically healthy dogs fed a commercial plant-based versus meat-based diet. A total of 10 dogs were included in our study. All dogs have been fed either a commercial meat based-diet, or a plant-based diet for at least six months. Complete Blood Count, Chemistry panel, physical examination as well as body scores were performed to confirm the dogs' health statuses. We will also include the fecal microbiome analysis as part of the health status assessment for all 10 dogs in our study. Blood work was performed at the Pet Health Center at Western University using IDEXX analyzers. Fecal samples are being collected from all dogs to analyze their fecal microbiomes. Hydrosoluble vitamins as well as lipid soluble vitamins will also be analyzed. Analysis of the samples is ongoing and we expect to have results that will objectively assess the health and nutritional statuses of dogs fed a commercially available plant-based diet for at least six months, as compared to dogs fed a commercial meat-based diet. We will then discuss the data relevance in the context of nutrition and health.

Research Grant: Western University of Health Sciences, College of Veterinary Medicine (intramural funds)

Student Support: Petsmart Charities Sponsored Student Project

Cell mediated immunogenicity of TGF- β 2-treated MHC-mismatched equine mesenchymal stem cells

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Autologous bone marrow-derived mesenchymal stem cells (BM-MSCs) require a lengthy culture period but offer promising results for treating horses with musculoskeletal injuries. Use of allogeneic BM-MSCs would allow for immediate treatment of patients, but is not practical due to recipient recognition of mismatched-major histocompatibility complex (MHC) molecules leading to in-vivo rejection. Studies have shown that 7-14 days post-injection of MHC-mismatched BM-MSCs there can be production of antibodies that are cytotoxic to MSCs. Transforming growth factor- β 2 (TGF- β 2) treatment has been shown to downregulate constitutive MHC I and MHC II surface expression and partially block IFN- γ -induced upregulation of MHC expression on equine BM-MSCs. This study aims to compare the cytotoxicity of TGF- β 2-treated and untreated MHC-mismatched equine BM-MSCs via an in vitro cytotoxicity assay. Our hypothesis was that TGF- β 2-treated BM-MSCs would have reduced cell-mediated cytotoxicity compared to untreated BM-MSCs. A one-way mixed leukocyte reaction was performed using peripheral blood leukocytes to generate MHC-specific cytotoxic effector cells. A standard chromium-51 release assay was performed to measure the cell-mediated cytotoxicity of untreated and TGF- β 2-treated MSCs. Results for 6-hour cytotoxicity assays showed that TGF- β 2-treated MHC-mismatched BM-MSCs showed a strong trend in reduced cytotoxicity compared to untreated MHC-mismatched BM-MSCs. Intracellular cytokine staining is being completed to look at interferon production in an effort to examine the immune response variation in cytotoxic effector cell activity of the different horses used in this study.

Research Grant: Morris Animal Foundation D18EQ-055 and D16EQ-405

Student Support: Veterinary Research Scholars Program

Blood-brain barrier pathology in an experimental model of blast-induced neurotrauma

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The signature injury of modern warfare is blast-induced neurotrauma (BINT) due to the use of explosive devices. The clinical symptoms of BINT have been correlated with neuropathology including edema, hemorrhage, neuronal necrosis, and increased paracellular permeability of the blood-brain barrier (BBB). Herein, we aim to investigate the pathological alterations of the BBB in rats following single or repeated blast exposure. The BBB is composed of endothelial cells with tight junctions, a basement membrane, pericytes, and astrocyte endfeet. BINT was induced by using an open-ended blast apparatus to deliver single or triple 150 kPa shockwaves to 3-month-old male Sprague Dawley rats. After 24 hours, the brains were excised, flash frozen, and cryosectioned into 5 μ m thick sections. Qualitative and quantitative evaluation of the BBB was accomplished using immunofluorescence microscopy and Zen blue analysis software. We observed alterations in expression with endothelial cells, tight junction proteins, tight junction adaptor proteins, basement membrane, and pericytes. Our preliminary findings demonstrated a 1.82-fold ($P=0.037$) increase in the expression of claudin-5, a tight junction protein, in the single blast model compared to the control. There was a 2.01-fold ($P=0.018$) increase in the expression of PDGFR- β , a pan-pericyte protein, in the single blast model compared to the control. The triple blast model demonstrated a 1.87-fold decrease ($P=0.028$) in PDGFR- β expression compared to that of the single blast. This is the first comprehensive pathologic analysis of the BBB in BINT in an experimental model. These data will support the development of a robust and reproducible experimental model of blast-induced neurotrauma.

Research Grant: None

Student Support: Boehringer Ingelheim and Purdue University College of Veterinary Medicine

Genome editing at the AF-associated *Pitx2* Locus: a role for the lncRNA *Playrr* in cardiac arrhythmias

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Atrial Fibrillation (AF) is the most common arrhythmia worldwide in humans, with serious implications such as stroke, cardiac arrest, and death. The most significantly associated AF risk loci map to variants in a noncoding, putative regulatory gene desert upstream of the *Pitx2* locus. Indeed, loss of *Pitx2* in mice and humans is associated with congenital cardiac defects, increased susceptibility to atrial fibrillation (AF), development of bilateral sinoatrial nodes (SAN), and sinus node dysfunction (SND). However, the genetic mechanisms by which *Pitx2* and its associated cis-regulatory elements at the locus contribute to the development of AF remain unclear. Previously we discovered *Playrr*, a conserved enhancer-associated long noncoding RNA (lncRNA), arising from the *Pitx2* locus gene desert. To investigate the role of *Playrr* in regulating *Pitx2*, we used CRISPR/Cas9 genome editing in mice to target the *Playrr* RNA transcript while leaving its associated enhancer element intact (*Playrr*^{Ex1sj}). By adapting an awake surface ECG device (AliveCor) for use in mice, we detected cardiac arrhythmias in *Playrr*^{Ex1sj} mutants and validated these results with telemetry ECG. We demonstrate that adult *Playrr*^{Ex1sj} mutant mice exhibit bradycardia and irregular R-R intervals that are indicative of sinus node dysfunction (SND), a bradyarrhythmia and risk factor for the development of AF. Finally, programmed stimulation reveals that *Playrr*^{Ex1sj} mutants are predisposed to pacing-induced AF, strikingly similar to *Pitx2* loss-of-function mutant heterozygous mice. Our data suggests that *Playrr* modulates *Pitx2* gene dosage in the heart leading to conduction abnormalities and predisposition to important cardiac arrhythmias.

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Student Support: NIH F30 OD021454-03

Optimization of embryo electroporation to enhance genetic modification in rats using CRISPR/Cas9 technology

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CRISPR/Cas9 genome editing is a widely used technique for genetic modification that has enabled researchers to effectively alter the genome of rats without the need for embryonic stem cell targeting. Traditionally, reagents for the CRISPR/Cas9 system are delivered to rat embryos via a laborious and technically challenging microinjection technique. The purpose of this study was to optimize a simple and high-throughput electroporation method to deliver CRISPR/Cas9 reagents into rat embryos. Mature female Sprague Dawley (SD) rats were superovulated and mated with SD stud males. Embryos were then collected and subjected to electroporation to deliver genome editing reagents. The embryos were cultured to the blastocyst stage and analyzed genetically. We compared embryo survival rates and gene knock-out efficiency when electroporation was performed in different electroporation mix buffers (water, Opti-MEM, and TE buffer). Additionally, we compared the gene knock-in efficiency after electroporating embryos at either the one-cell or the two-cell stage. We hypothesized that the longer G2 phase and open chromatin structure during the two-cell stage would allow for an increased knock-in efficiency. Our results show that the electroporation mix buffers tested do not significantly impact *in vitro* embryo survival or gene knockout efficiency. On-going studies involve manipulating embryos using these same conditions and transferring them into pseudopregnant surrogate dams to evaluate live pup rates and genetic knock-out/knock-in efficiencies. Overall, our findings will guide the optimization of rat embryo electroporation and effective delivery of CRISPR/Cas9 reagents in order to mediate efficient genome editing in rats.

Research Grant: NIH 2P40 OD011062 (E.C.B.)

Student Support: American Society of Laboratory Animal Practitioners Foundation

Determining the *in vitro* effects of extracellular vesicles on peripheral nerve growth

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Extracellular vesicles (EVs) mediate a recently discovered form of intercellular communication that is crucial for many developmental and physiological processes. EVs, which contain mRNAs, miRNAs, and various proteins, are released into the cytoplasm by parent cells. Shortly after, the EVs fuse to the plasma membrane of distant recipient cells, resulting in altered cellular phenotypes due to changes in their transcriptional profiles. In the peripheral nervous system (PNS), neurons exchange EVs with one another, as well as with supporting glial cells. These inter-neuronal and neuro-glia interactions have been shown to play an important role in neuroregeneration. Here, we study olfactory sensory neurons (OSNs) because they undergo a high rate of neuronal regeneration due to their constant exposure to damaging molecules from the external environment. We used primary neuronal tissue culture models derived from FVB and B6 mice, grown between 24h to 48h, as well as immunofluorescence, to study the effects on neuronal growth when applying EVs containing cell-adhesion proteins (two protocadherins and one neural cadherin) to OSNs. To determine what kind of effect has occurred, we compared total neurite length and number of branches in experimental conditions to those in controls. Determining the role of EVs in neuroregeneration may shed light on therapeutic potentials for neurodegenerative conditions such as Alzheimer's and Parkinson's diseases. Once we can elucidate the effects of neuronal and glial EVs on neuronal growth in the PNS, we can hopefully harness these components to promote axonal regeneration in an efficient way that does not require sacrificing a functioning nerve.

Research Grant: NIH Training Grant

Student Support: CUCVM's Veterinary Investigators Program

Characterizing *in vivo* and *in vitro* TLR4 expression and response to LPS in immature and mature mouse intestine

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Necrotizing enterocolitis (NEC) is a devastating gastrointestinal (GI) disease in premature neonates. The Hackam Laboratory has demonstrated the activation of TLR4, a cell surface receptor for LPS, on the intestinal epithelium is essential for the disease to manifest. Interestingly, human outcomes data has shown NEC lesions to afflict different GI segments depending on gestational ages at birth, with ileum being affected in severely preterm babies shifting to colon as gestation progresses toward term. This suggests some mechanism of developmental regulation is occurring in the intestine that allows for regional sensitivities to inflammatory stimuli. We hypothesize that 1) TLR4 expression differs between anatomic intestinal segments, and 2) TLR4 expression and activation within the developing intestine is different than that in a mature intestine. To test our hypotheses, we established an *in vivo* model to evaluate TLR4 activity in intact intestine and an *in vitro* model to evaluate TLR4 activity in the intestinal epithelia alone. For the *in vivo* model, C57BL/6 and TLR4^{-/-} mouse pups and adult mice were treated with LPS or saline control. Duodenum, jejunum, ileum, cecum, and colon were harvested 8 hours later. Our *in vitro* model used organoids propagated from intestinal stem cells which were treated with LPS or regular media. qRT-PCR was performed to investigate TLR4 expression and markers of cellular differentiation, proliferation, and inflammation. While data from this project is currently being analyzed, results should aid in the understanding of how and why NEC lesions affect different regions of the intestine depending on age at birth, and may generate hypotheses on how this disease can be mitigated in the clinic.

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Student Support: Boehringer Ingelheim Veterinary Scholars Program

The effect of cryopoor plasma addition to adsorbed plasma to correct prothrombin time: an in vitro study

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Anticoagulant rodenticides are pesticides that function by inactivating vitamin K epoxide reductase, which plays a critical role in the coagulation cascade. This leads to the failure of the activation of vitamin K dependent coagulation factors II, VII, IX, and X and thus increases prothrombin time (PT) and then partial thromboplastin time (PTT). The standard treatment is transfusion with fresh frozen plasma but is very expensive. Cryopoor plasma, a cheaper alternative, is regarded as a byproduct but has been shown to have concentrations of the coagulation factors II, VII, IX, and X similar to those in fresh frozen plasma. The aim of this in vitro study is to mimic the conditions caused by anticoagulant rodenticide toxicity using plasma adsorbed by oral barium sulfate suspension and to compare the efficacy of cryopoor plasma to fresh frozen plasma at clinically relevant doses. We hypothesize that similar amounts of cryopoor plasma added to adsorbed canine plasma is as effective as fresh frozen plasma to correct the prothrombin time in adsorbed canine plasma. Plasma depleted of the vitamin K dependent coagulation factors was created by mixing fresh frozen plasma with oral barium sulfate suspension. A mixing study was conducted by the addition cryopoor plasma or fresh frozen plasma to the barium sulfate-depleted plasma to determine if and to what level PT or PTT was corrected. Preliminary data indicates that cryopoor plasma may be utilized to correct prothrombin time. Additional data collection is on-going and may indicate cryopoor plasma can be a suitable cost-effective alternative to fresh frozen plasma transfusions for anticoagulant rodenticide toxicity.

Research Grant: Unknown

Student Support: Boehringer-Ingelheim Animal Health

Assessing the development of the postnatal canine gastrointestinal microbiome utilizing the dysbiosis index

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The gastrointestinal (GI) microbiome is critical to the development of canines as it assists in the maturation of the immune system, protection against enteropathogens, and utilization of nutrients. Few papers have been published describing the development of the canine microbiome. Those studies have shown that initially aerobic bacterial groups including *Escherichia coli*, predominate. As puppies age, their microbiomes increase in anaerobic bacterial abundance leading to a decrease in *Proteobacteria*. The Dysbiosis Index (DI) is a recently developed, rapid quantitative PCR-based assay that measures eight key bacterial taxa in the GI tract to quantify the degree of microbial dysbiosis in adult dogs. Because puppies have high *Proteobacteria* at the time of birth, we hypothesized that DI is initially increased and decreases with increasing age at which point it reaches a value that is normal for adult dogs. The aim of this study was to utilize the DI to approximate the age at which development of the postnatal canine's GI microbiome stabilizes. Fecal samples from healthy puppies aged 1 week to 1 year old (n=94) were analyzed to measure changes in bacterial abundance. Our findings demonstrated that during the first few weeks of life, DI was significantly increased as *Proteobacteria* predominated. DI decreased significantly in the 9 week old samples compared to the 2 week old samples ($P < 0.001$) as *E. coli* decreased, *C. hiranonis* increased, and *Faecalibacterium* increased. In conclusion, the postnatal microbiome changes rapidly from birth to 9 weeks of age at which point it stayed within the reference interval of healthy adult microbiota.

Research Grant: None

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Genotype assay development for alleles associated with relevant phenotypes in pet and working German Shepherds

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Canine breeding through artificial selection has been performed to the best of the abilities of their human counterparts for millennia. Selection is based on traits observed in familial groups that are deemed favorable to pass on. These traits include personality, conformation, and aptitude for various tasks. Because of a lack of understanding of genetic inheritance, favorable and unfavorable traits were passed together. For instance, the slanted back of the German Shepherd Dog (GSD), a contemporarily favorable conformation, led to an increase in incidence of hip dysplasia. Personality and aptitude traits have been linked to various alleles of genes in many species. Selection for these traits may also lead to propagation of unfavorable disease characteristics. Advancement in genomic sequencing and molecular techniques for elucidating genotypes will lead to more informed breeding of pets and captive wildlife by direct genetic analysis. This project aims to develop genotype assays to assess and compare local populations of pet and working GSDs for polymorphisms that have been associated with personality, aptitude, and disease traits. Using buccal swabs, we collected DNA and will perform various molecular techniques including PCR-RFLP, allele specific PCR, and sequencing on 20 pet and 20 working GSDs. We hypothesize that we can demonstrate successful PCR genotyping of two selected genes, SOD1 for Canine Degenerative Myelopathy and OPRM1 associated with greeting behavior, using RFLP and allele specific amplification respectively. Additionally, successful amplification of two regions of the Oxytocin Receptor (OXTR) containing SNPs associated with friendliness will be demonstrated with intent to genotype by sequencing.

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Student Support: College of Veterinary Medicine Research Student Summer Research Stipend

Covariation between personality and immunity in the presence versus absence of parasitism

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Personality has been described in many animal species as consistent behavioral responses to different conditions. There is growing evidence suggesting a link between personality and immune responses, but this relationship is rarely examined in the context of parasitism. Our preliminary work in wild cotton rats (*Sigmodon hispidus*) showed that while some personality traits were closely associated with relative abundance of phagocytic cells, these relationships disappeared following gastrointestinal helminth clearance. The purpose of this study was to continue exploring links between personality traits and immunity in parasitized versus non-parasitized animals. Rats were trapped at sites in Athens, Georgia, and their personality traits (boldness, docility, exploration, activity) were characterized using trappability, handling bag, and novel environment tests. Individuals were trapped four times. Starting at the second trapping event, and at each subsequent event, individuals received a combined dose of the anthelmintic drugs ivermectin and pyrantel or distilled water. Blood samples were collected at each trapping session for white blood cell differentials and bacteria killing assays. Initial results show that before drug treatment, activity level in a novel environment is negatively correlated with neutrophils, but this pattern disappears after treatment suggesting that parasite clearance affects the relationship between personality and immunity. Ongoing analyses will investigate these relationships in more detail.

Research Grant: None

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Biofilm formation in commensal and pathogenic *Enterococcus cecorum* strains isolated from broiler chickens

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Enterococcus cecorum (EC) is the dominant enteric commensal of adult chickens and sporadically causes serious infection in humans. In contrast to other enterococci, which cause opportunistic infections, pathogenic EC are frank pathogens of chickens causing morbidity and mortality comparable to other important infectious diseases of poultry. Comparative genomic analysis of pathogenic and commensal EC identified potential mediators of virulence, including genetic loci homologous to mediators of biofilm formation in other enterococci. As these biofilm encoding genes were conserved in pathogenic EC, we hypothesized that pathogenic EC strains would form biofilms while commensal strains would not. To test this hypothesis, a biofilm assay was optimized for EC and used to compare *in vitro* biofilm formation of commensal and pathogenic EC. Contrary to our hypothesis, the commensal strains were strong biofilm formers, while the pathogenic strains formed biofilms comparable to a biofilm forming *E. faecalis* V583. Overall, the pathogenic strains were weaker biofilm formers with all tested media. The commensals formed robust biofilms in high dextrose concentrations at 42°C (normal chicken body temperature); however, growth media significantly impacted biofilm formation. Some commensal strains demonstrated significantly increased biofilm formation in Todd Hewitt broth compared to Brain Heart Infusion broth while some strains showed the opposite. Our results demonstrate, for the first time, the ability of EC isolates to form biofilms. As the biofilm forming phenotype was conserved in all isolates, but more strongly expressed in the commensals, additional work is needed to elucidate the role biofilm formation plays in EC virulence.

Research Grant: None

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Understanding the effects of oxidative stress on the pyroptotic pathway of programmed cell death

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The development of disease is often due to programmed cell death, where constituent cells die by specific pathway-driven mechanisms. A recently discovered form of cell death is pyroptosis, which is characterized by activation of the inflammasome. This in turn activates the protease caspase-1, which cleaves Gasdermin-D. Cleaved Gasdermin-D then perforates the plasma membrane causing cell death. Pyroptosis is canonically activated by bacterial infection. However, how pyroptosis contributes to pathology in the absence of infection is unknown. A major driving component of many diseases is oxidative stress. Thus, deeper mechanistic insight of how oxidative stress causes pyroptosis is needed to better understand how this form of cell death contributes to pathogenesis. Consequently, our research will investigate the ability of oxidative stress to stimulate pyroptosis. We hypothesize that oxidative stress activates the inflammasome and caspase-1 leading to Gasdermin D cleavage and cell death. Cultured fibroblasts will be treated with oxidants and analyzed by Western Blotting to determine activation/cleavage of the inflammasome, caspase-1, and Gasdermin D. Gasdermin D will be overexpressed in the fibroblasts, and activation of the pyroptotic pathway and cell death measured following oxidative stress. Antithetically, fibroblasts will be depleted of Gasdermin D by siRNA and oxidant-induced activation of pyroptotic proteins and cell death determined. We anticipate that oxidative stress will induce pyroptosis. Elevated Gasdermin-D will sensitize cells to oxidative stress, whereas Gasdermin-D depletion will protect cells. Together these studies will provide new insight into how pyroptosis may contribute to the development of disease.

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Evaluation of impacts by probiotics expressing *Listeria* adhesion protein on porcine intestinal epithelium

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Commercially grown piglets are exposed to a variety of stressors, including weaning, food deprivation, and heat stress, as they are transitioned onto grower farms. These stressors cause long-lasting changes to the secretory and barrier functions of the piglet intestine, leaving them highly susceptible to bacterial and viral diseases. Furthermore, as concerns over antibiotic resistance grow, probiotics are being increasingly used to reduce the risk of infection and promote protective immunomodulatory effects on the intestine. One set of recombinant probiotics being investigated include those that possess *Listeria* adhesion protein (LAP), an adhesion protein of *Listeria monocytogenes* that may be used to prevent infection and provide general gut-health benefits. IPEC-J2 cells, a porcine jejunal enterocyte cell line, were exposed to several strains of *Lactobacillus casei* to test efficacy of adhesion and subsequently infected with *L. monocytogenes* to elucidate the protection provided by the probiotics under both ideal (37°C) and heat stress (41°C) conditions. These include a wild-type strain, a recombinant strain expressing LAP from pathogenic *L. monocytogenes*, and a recombinant strain expressing LAP from nonpathogenic *L. innocua*. Bacterial adherence was enumerated by plating the bacteria and the level of cytotoxicity to the enterocytes was measured via an LDH assay. Development of effective probiotic interaction with host cells is a critical initial step in preventing the significant economic losses intestinal disease causes through the increase of piglet mortality and the reduction of piglet growth. The swine intestine also bears the most similarity to the human intestine, setting the future framework for human studies.

Research Grant: BioMatrix

Student Support: Boehringer Ingelheim and Purdue Veterinary Medicine

Effects of S-equol on obesity-associated depression and anxiety in male and female mice

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S-equol is a non-steroidal estrogen metabolized from daidzein by intestinal microbiota. Previous studies attribute beneficial neurological and metabolic effects of this metabolite, including anti-depressive and anti-obesity effects. A correlation exists between obesity and mood disorders; therefore, the question remains whether S-equol can potentially mitigate the effects of obesity-induced depressive-like or anxiety-like behaviors. With both obesity and mood disorders on the rise, we sought to examine the potential ability of S-equol to improve depressive- or anxiety-like behaviors in a diet-induced mouse model of obesity (DiO). We hypothesized that mice dosed with S-equol would have reduced depressive- and anxiety-like behaviors compared to those not receiving this supplementation. Twenty-eight C57 mice were placed on a HFD and randomly assigned to 4 groups: 7 females on S-equol, 7 females on control, 7 males on S-equol, and 7 males on control. The mice were orally dosed (10mg S-equol daily/kg body weight) for the total duration of the studies (8 weeks). Beginning at week 7, the following tests were performed: elevated plus maze (EPM), forced swim test (FST), and tail suspension test (TST), which collectively assess depressive- and anxiety-like behaviors. When compared to the control mice, we hypothesize the S-equol mice will have higher mobility times in the FST, increased time spent on the open arms of the elevated plus maze, and higher mobility times during the TST. Such finding will support the hypothesis that S-equol alleviates depressive- and anxiety-like behaviors in mice fed an obesogenic diet. Such results could have important ramifications for treating depression and anxiety disorders in humans.

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Student Support: University of Missouri College of Veterinary Medicine Office of Research

Using ImageJ to differentiate histiocytic sarcoma on cytology through automated and manual measures

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Definitive diagnosis of disseminated histiocytic sarcoma currently requires histology and immunohistochemistry. Automated cytology could provide a quicker, less expensive, and minimally invasive alternative. The primary aim of this study was to use ImageJ to determine if there is a difference between nuclear area, perimeter, or circularity on cytology when comparing dogs diagnosed with histiocytic sarcoma and macrophagic inflammation or other neoplasms. In addition, this study compares manual and automated analysis on ImageJ. The results showed that area and perimeter were significantly different between histiocytic neoplasia (n=12) and macrophagic inflammation (n=10). No parameter was significantly different between histiocytic and other neoplasms (n=10). Agreement between manual and automated measurement was best for area, with the automated measurements having a small negative constant bias (-242 pixels/inch, 95% confidence interval -398, -91) and minimal proportional bias (slope 0.98, 95% confidence interval 0.97, 0.99). The results showed that while there were statistical differences between histiocytic sarcoma and inflammation, these differences were not sufficient to diagnose histiocytic sarcoma by nuclear measurement alone. However, nuclear area and perimeter may be useful components of a future multifactor model. This study also revealed that automated analysis of area agrees well with manual analysis, suggesting automated analysis alone could potentially be used in future cytology studies using ImageJ.

Research Grant: None

Student Support: Boehringer Ingelheim VSP, Texas A&M College of Veterinary Medicine & Biomedical Sciences

Exosome deficiency leads to an increased T cell-mediated inflammatory response in Rab27dKO mice

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Exosomes are a nano-sized subset of extracellular vesicles that play a key role in cell communication by cell to cell transportation of various molecules such as DNA/RNA transcripts and proteins. This area has mainly been explored in cancer and stem cell biology, but less so in the context of immune repertoire development or T cell phenotype and function. Recent literature demonstrated that mice lacking both genes responsible for exosome production, Rab27a and Rab27b (Rab27dKO), displayed heightened cytokine secretion and bone-marrow cell proliferation, enabling an acute inflammatory response. We therefore hypothesized that T cells from Rab27dKO mice would exhibit a stronger inflammatory phenotype compared to wild-type (WT) T cells. We stimulated T cells with various combinations of proinflammatory cytokines. Compared to WT, Rab27dKO CD8+T cells demonstrated increased effector cytokine production. By 72 hours, Rab27dKO CD8+T cells proliferated and differentiated more rapidly than WT. To examine behavior in vivo, we infected Rab27dKO and WT mice with *L. monocytogenes* and tracked antigen-specific CD8+T cell with tetramer. The magnitude of the CD8+T cell response was larger in Rab27dKO mice and antigen-specific cells expressed increased PD-1, marker of exhaustion. To examine how the maternal phenotype affects the neonatal immune system, we developed a way to examine immune development in Rab27^{+/-} mice born from Rab27dKO or WT mothers. Neonatal T cells born from a Rab27dKO mother showed higher frequencies of inflammatory immune cell types, but lower numbers of regulatory immune subsets across organs. Overall, these results demonstrate the key role that exosomes play in dampening the CD8+T cell-mediated inflammatory response.

Research Grant: Boehringer Ingelheim Veterinary Scholars Program

Student Support: Cornell University Veterinary Investigators Program

Mesenteric lymph node cytology in cats for diagnosing small cell granular lymphocyte intestinal lymphoma

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Intestinal lymphoma is the most common lymphoma in cats, and granular lymphocyte (GL) type is a common variant. When there is concern for intestinal lymphoma, clinicians often sample mesenteric lymph nodes (mLNs) as a surrogate tissue for the intestine. It is currently not known how many GLs are present in normal mesenteric lymph nodes, and how many are indicative of underlying intestinal lymphoma of GL type. The hypothesis of this study was that mLN aspirates of normal cats will have < 5% GLs. More than 5% GLs will be associated with the presence of a clonal T-cell granular lymphocyte population and underlying intestinal granular T-cell lymphoma. mLN aspirates were evaluated using three methods in this study: differentially counting GLs using routine Wright Giemsa staining; granzyme B immunostaining to more sensitively identify GLs and confirm the accuracy of GL counts using routine morphologic stains; and T cell molecular clonality PCR to confirm either lymphoma or reactivity in each node. When available, accompanying intestinal biopsies were also interrogated, using PCR and granzyme B immunohistochemical staining, to confirm the pathologic diagnosis. Results from each cytology and biopsy were correlated, and each case was sorted into one of four diagnostic categories: normal, inflammation/IBD, small cell lymphoma (SCL) non-granular, and SCL granular. Preliminary results indicate that mLNs of cats with normal or inflamed intestine have < 1% small granular lymphocytes while those of cats with intestinal GL small cell lymphoma have 2.78-57.5% small granular lymphocytes. These findings support our hypothesis and provide useful guidance for the diagnostic workup of cats with intestinal disease.

Research Grant: None

Student Support: School of Veterinary Medicine STAR Student endowment funds

Expression and localization of Ezrin in embryonic wild-type kidney and a model of cystic kidney disease

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Polycystic kidney disease (PKD) affects 13 million people worldwide. PKD is characterized by massively enlarged kidneys with fluid-filled cysts and fibrosis that replace renal parenchyma and contribute to renal failure. Epithelial dedifferentiation and aberrations in cell cytoskeletal and migration behavior contribute to cystogenesis however, underlying molecular mechanisms remain unclear. Ezrin is a cell membrane to actin cytoskeleton linker protein critical for cytoskeleton remodeling, cell migration, and brush-border formation. Ezrin expression and localization is unknown in wild-type (WT) embryonic kidney and a widely used embryonic explant model in which cysts are induced by 8-Br-cAMP treatment. We hypothesized that ezrin will be highly expressed in renal tubular cells and its localization will be altered in cystic tubules. We performed co-immuno-fluorescence assay to study ezrin expression in embryonic WT kidney and 8-Br-cAMP-induced cystic model. In WT kidney, ezrin was detectable in Six2+ve mesenchymal nephron progenitors and their early epithelial differentiating structures. Ezrin was highly expressed in proximal tubules and ureteric bud marked with LTL- and DBA-lectin, respectively. In vehicle-treated renal explants, ezrin was highly expressed at cell-cell junctions and at brush-border of proximal tubules. In contrast, in cystic tubules of 8-Br-cAMP-induced renal explants, ezrin was mis-localized from cell-cell junctions; and was dramatically reduced in cystic proximal tubule cells that lost their brush border (indicated by the loss of LTL staining) and have undergone dedifferentiation. Together, these results indicate that ezrin may have a role in nephrogenesis and in the pathogenesis of cystic kidney disease.

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Student Support: National Institutes of Health 2T35OD010432-17

Defining the early stages of skin immunity against a soil-transmitted helminth

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Soil-transmitted parasitic helminths cause significant morbidity in both humans and livestock across the globe and current treatment and vaccine options are inadequate. Penetration of host skin is a critical initial step in disease pathogenesis for several species of parasitic nematodes, but the early immune response surrounding larval migration to their predilection site in the small intestine is poorly defined. This project uses *Strongyloides ratti*, a murine nematode related to the human pathogen *Strongyloides stercoralis*, as an experimental model to interrogate this early immune response, focusing on the interaction between migratory infectious larvae and myeloid antigen presenting cells. We employed a novel transgenic strain of *S. ratti* that stably expresses GFP in its body wall to enable *in vivo* visualization using 2 photon microscopy. Given that dendritic cells (DCs) initiate immune responses through antigen presentation and production of polarizing cytokines, we chose to use CD11c-YFP reporter mice to track the CD11c-expressing DC response to infection within the skin. Experiments are underway that will visualize the DC response to early infection and characterize local cytokine milieu by real time PCR and immunofluorescence staining. Additionally, we will compare these parameters of DC infiltration between primary and secondary infection in the skin in order to address the hypothesis that CD11c expressing cells are recruited more quickly and produce increased levels of type 2 promoting cytokines during secondary infection. Taken together, these studies could lead to novel insights into the early events that control helminth specific immunity and could bolster efforts at rational vaccine design.

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Student Support: NIH Grant T35OD010919, Boehringer Ingelheim grant

Mast cells exhibit bone morphogenetic protein signaling in response to inflammation

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Mast cells are innate immune cells that are most infamous for their roles in anaphylaxis and allergy. Recently, they have been recognized as key orchestrators of anti-inflammatory responses. For example, they are required for prevention of graft-versus-host disease. Our group has demonstrated that mast cells produce bone morphogenetic proteins (BMPs), cytokines in the TGF- β superfamily that have anti-inflammatory and anti-fibrotic effects. BMPs are attractive therapeutics for inflammatory bowel disease (IBD), a condition in which excess fibrosis leads to intestinal strictures. The objective of this study was to determine stimuli that trigger upregulation of BMPs, demonstrate the presence of autocrine signaling, and elucidate downstream signaling pathways in mast cells. We hypothesized that inflammatory stimuli upregulate BMPs and that signaling occurs via the SMAD1/5/8 pathway. Murine bone marrow derived mast cells (BMMCs) were used to characterize expression of BMPs and their signaling molecules in response to pro-inflammatory stimuli by immunofluorescence and Western blotting. We demonstrated that BMP7 was 2.45-fold increased in BMMCs stimulated with lipopolysaccharide (LPS) and IgE versus control cells ($P=0.008$). Furthermore, we demonstrated elevated expression of phosphorylated SMAD1/5/8 in the nucleus of LPS/IgE-treated BMMCs following subcellular fractionation. These findings indicate that inflammatory stimulation of mast cells causes BMP upregulation and signal transduction through the SMAD pathway under conditions of acute inflammation, likely as an anti-inflammatory counter-regulatory mechanism. Future studies will refine the signals and time course that result in BMP upregulation.

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Student Support: University of Tennessee Center of Excellence

Effects of temporary dry ice storage on cryopreserved C57BL/6 mouse sperm

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Sperm cryopreservation and transportation are essential aspects of distributing mutant mice genetics to research facilities worldwide. While liquid nitrogen (LN₂; -196 °C) is a reliable media to store and transport cryopreserved sperm, it is a hazardous material and incurs additional shipping expense as a result. Dry ice theoretically provides low enough temperatures (~ -79 °C) to maintain cryopreserved sperm and does not carry the same hazards and shipping expense as liquid nitrogen. The goal of our study was to determine the effects of transient dry ice storage for a week on sperm progressive motility, in vitro fertilization ability, and embryonic development. Sperm was collected from the cauda epididymis of five inbred C57BL/6 mice. Motility and morphology of each sample were assessed immediately after collection, after cryopreservation in LN₂, and after being transferred and maintained in a Styrofoam shipping container filled with dry ice for 7 days. Some of the samples temporarily stored on dry ice were used to perform in vitro fertilization and embryo culture to confirm fertilization and embryo development ability. Percent progressive motility for sperm that were freshly collected, stored in LN₂ only, or stored in LN₂ and subsequently stored on dry ice for a week were 71.8%, 28.0%, and 14.4% respectively. Percent embryonic development to the two-cell stage after in vitro fertilization using fresh, LN₂ only, or dry ice stored sperm were 84.7%, 49.6% and 46.5%, respectively. These results suggest that despite significant decline in progressive motility ($P < 0.01$), comparable fertilization and embryo development ($P > 0.01$) can be achieved with C57BL/6 mouse sperm that are transiently stored on dry ice for a week.

Research Grant: None

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Contributing effect of organochlorine exposure on Atlantic bottlenose dolphins during a morbillivirus outbreak

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Dolphin morbillivirus (DMV) is a threat to cetacean populations as it causes high morbidity and mortality. Exposure to chemical pollution is suspected to increase susceptibility to DMV infection in cetaceans. A recent outbreak along the US East Coast resulted in the death of over 1600 Atlantic common bottlenose dolphins. Prior research has found a correlation between elevated blood polychlorinated biphenyls (PCBs) and dichloro-diphenyl-trichloroethane (DDT)-related compounds and suppressed immune responses in bottlenose dolphins from this region. Therefore, we hypothesize that dolphins that died from morbillivirus infection had significantly higher mean Σ PCB and mean Σ DDT blubber concentrations than live bottlenose dolphins that inhabited the same areas and were sampled before the DMV outbreak. Blubber samples from 15 bottlenose dolphins that died from DMV infection are being analyzed by GC/MS for 51 PCB congeners and six DDT-related compounds. Additionally, brain samples from each individual will be analyzed because these contaminants have not been measured before in brain tissue from bottlenose dolphins, including those that died with morbilliviral infection, but may be important due to the presence of pathological lesions in brain as a result of infection. Lipid content and lipid classes are also being measured to compare individual congeners in blubber and brain tissue. Overall, this study will examine if there is an association with contaminant exposure and increased susceptibility to DMV infection that may have contributed to the large number of deaths during the outbreak.

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Student Support: UCDavis School of Veterinary Medicine endowment funds, Dr. Tracey Goldstein discretionary funds

TET2's DNA demethylation in CD8 memory

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Strong immunological memory is vital to public health in humans and animals. When CD8 T cells lack expression of TET2, a key enzyme in DNA demethylation, they exhibit markers of increased memory during acute lymphochoriomeningitis virus (LCMV) infection compared to wild type (WT) mice. This increased memory phenotype is characterized by a dominant presence of memory precursor effector cells (MPECs), identified by cell surface markers CD127^{hi} KLRG1^{lo}, over short-lived effector cells (SLECs), which are CD127^{lo} KLRG1^{hi} and do not contribute to long-term immunological memory. We propose that demethylation regulated by TET2's catalytic domain (CD) controls this cell fate choice such that reconstitution of TET2 knock-out (KO) CD8 cells with the TET2 CD should revert them to a wild-type phenotype. To test this hypothesis, we generated retrovirus expressing WT and catalytically inactive mutant HxD (H1382Y/D1384V) TET2 CD. We transduced both WT and TET2 KO murine CD8 cells with these viruses and their empty vector controls. Our preliminary experiment showed that TET2 KO CD8 cells expressing WT TET2 CD virus had reduced MPECs compared to TET2 KO with empty vector, though not to wild-type levels, and overexpressing the WT TET2 CD in WT mice resulted in enhanced SLEC differentiation. Next steps are to repeat this experiment to confirm the importance of TET2's catalytic activity in regulating CD8 T cell memory and to evaluate other TET2 domains to see if they play a role in this process.

Research Grant: None

Student Support: NIH grant T35OD010919 and a grant from Boehringer Ingelheim

Comparison of uropathogenic *Escherichia coli* isolates from human and companion animal patients

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Urinary tract infections are often caused by extraintestinal uropathogenic *E. coli* (UPEC) strains. Past studies have found similarities between companion animal and human UPEC strains, but the significance of this overlap and the potential for cross-species transmission are not understood. An overlap may indicate that the patients were infected from a common reservoir. In this study, we ran minimum inhibitory concentration (MIC) assays using the NARMS gram negative panel to determine the lowest concentration of antimicrobial necessary to inhibit growth. There were 34 isolates collected and analyzed with a panel of 11 antimicrobial agents of differing concentrations. Isolates with resistant and intermediate MICs are as follows (human isolates in parenthesis): Amoxicillin/Clavulanic Acid: 4(2)=R, 1=I; Ampicillin: 9(3)=R; Ceftiofur: 3(1)=R, 2=I; Chloramphenicol: 1=R; Ciprofloxacin: (1)=R; Naladixic Acid: 3(1)=R; Streptomycin: 4(1)=R; Sulfisoxazole: 26(10)=R; Tetracycline: 3(2)=R; Trimethoprim/Sulfamethoxazole: 5(1)=R. While 9 isolates were resistant to the penicillin-type antibiotic Ampicillin, only 3 were resistant to Amoxicillin/Clavulanic Acid. Further, while there was resistance to the second-generation cephalosporin, Ceftiofur, there was no bacterial growth in the third-generation cephalosporins, Ceftriaxone and Cefepime. The greatest resistance was to Sulfisoxazole, with 76% of the isolates showing resistance. It is important to note that only human isolates expressed resistance to Ciprofloxacin. This study shows that understanding the resistance mechanisms of each strain will help select appropriate treatments. Moreover, comparing the UPECs will help predict the zoonotic potential and identify a common reservoir.

Research Grant: Unknown

Student Support: NIH T35 Training Grant T35OD010977

Optimization of canine CAR T cell production and expansion for re-directed t cell therapy

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Adoptive T cell therapy is a novel approach to treating primary, metastatic, and relapsing neoplastic diseases. T cells are transduced with a viral vector that expresses a tumor-specific chimeric antigen receptor (CAR), enabling them to specifically target and kill tumor cells. Previous studies developing CAR T cell therapy for dogs with Diffuse Large B Cell Lymphoma used the same VSV-pseudotyped lentiviral vector that is used for CAR T cell generation in humans, but it resulted in low transduction efficiencies (< 10%) of primary canine T cells. High transduction efficiency is a critical step in generating sufficient CAR T cells to rapidly eliminate malignant B cells and prevent disease progression and death. Thus, the goal of this study was to optimize viral transduction of primary canine T cells. To do so, we focused on key factors that influence the transduction process including T cell activation, viral vector, viral envelope, MOI, spinoculation, and viral entry enhancers. We used viral vectors expressing GFP to assess transduction. Preliminary results indicate that activation with artificial antigen presenting cells expressing CD80, CD83, CD86, and 4-1BBL led to higher transduction efficiency than activation with CD3/CD28 coated magnetic beads. We also found increased transduction using RD114-pseudotyped gammaretrovirus, and a Moloney Murine Leukemia Virus promoter resulted in greater GFP expression than a MSCV promoter. Further exploration of spinoculation and viral entry enhancers is underway. This work aims to improve our current canine T cell transduction protocol, and we believe this will contribute to improved clinical responses to CAR T cell therapy in the canine clinic.

Research Grant: Animal Cancer Foundation and the Parker Institute for Cancer Immunotherapy
Student Support: Boehringer Ingelheim and NIH Grant T35OD010919

Apoptosis in neutrophils and lymphocytes in septic and critically ill dogs

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Sepsis in people is the leading cause of morbidity and mortality. Apoptosis is an important mechanism for regulating immune cell clearance during sepsis. Failure of neutrophil apoptosis results in inappropriate neutrophilic inflammation and tissue damage during sepsis in people. Concurrently, in people with sepsis, lymphocyte apoptosis results in immunosuppression and secondary infections. Similar pathology is suspected in dogs, but this has not been studied previously. The purpose of this study is to look at spontaneous peripheral blood neutrophil and lymphocyte apoptosis in dogs with sepsis, critical illness and healthy controls. We hypothesize that dogs with sepsis will have reduced spontaneous neutrophil apoptosis and increased lymphocyte apoptosis compared to dogs with other forms of critical illness or healthy dogs. Dogs will be selected from the intensive care unit and assigned to the sepsis or critically ill groups based on diagnosis. Blood will be collected and apoptosis measured using flow cytometry. To investigate mechanisms of neutrophil immortality and lymphocyte apoptosis, we will artificially creating critical illness, *in vitro*, by incubating blood from healthy dogs with pathogen associated molecular pattern motifs (PAMPs), stress hormones and cytokines. It is expected that dogs with sepsis will have reduced neutrophil apoptosis and increased lymphocyte apoptosis compared to critically ill dogs and healthy dogs. It is also expected that PAMPs, stress hormones and cytokines will be involved in apoptosis signaling. Better understanding of the pathophysiology may aid in the development of treatments, diagnosing and predicting the outcome of sepsis.

Research Grant: None

Student Support: Mizzou Advantage Initiative in One Health One Medicine

Examining the genetic role in *Cytauxzoon felis* survival and mortality

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Cytauxzoon felis is a protozoan parasite that causes an infectious disease with a 97% mortality, 40% mortality in treated cats. The lonestar tick (*Amblyomma americanum*) transmits *C. felis* from the natural reservoirs of bobcats (*Lynx rufus*) and pumas (*Puma concolor*). Why most bobcats, pumas, and some domestic cats survive a *C. felis* infection without treatment, while other cats do not, is unknown. *C. felis* resistance in wild felids and susceptibility in domestic cats is likely due to genetic differences within these species genomes. The hypothesis is genetic variants influence the susceptibility or resistance of *C. felis* in bobcats, pumas, and domestic cats. DNA from both a survivor and non-survivor domestic cat has been submitted for genome sequencing. Candidate genes involved in parasitic infections caused by *Theileria* and *Plasmodium* species, such as *SIRPBI* and *PIEZO1*, will be specifically examined due to their related disease etiologies. DNA variants will be identified between bobcats, pumas, and domestic cats to determine if these candidate gene variants influence the survival or fatality of domestic cats infected with *C. felis*. In the future, the candidate gene variants will be examined in a cohort of cats infected with *C. felis* to determine their frequencies in survivors and non-survivors. Target treatments to the functional pathway of the genes might allow veterinarians to increase the survival of *C. felis* infected cats.

Research Grant: MU CVM Gilbreath McLorn Endowment and 99 Lives Genome Sequencing Project

Student Support: Department of Veterinary Pathobiology, University of Missouri, College of Veterinary Medicine

Assessment of Zika virus tissue tropism and persistence after subcutaneous and sexual infection

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Zika Virus (ZIKV) has recently surged in human populations throughout the Americas. Although mainly transmitted through the bite of *Aedes spp.* mosquitoes, ZIKV can also be transmitted through sexual activities and from mother to fetus. The goal of this study was to determine if route of infection produces different outcomes of disease. Six guinea pigs were infected subcutaneously (nape of neck) and six vaginally with ZIKV MR766 to mimic mosquito-borne and sexual transmission, respectively. Guinea pigs from each group were euthanized 7, 21 and 30 days post infection (dpi) to assess tropism and persistence in tissues, including the genitourinary tract, spleen, lymph nodes, and sensory ganglia innervating the genitals and neck. Immunofluorescent staining showed presence of ZIKV antigen in the lumbosacral dorsal root ganglia (DRG), specifically in the satellite glial cells surrounding the sensory neurons, at acute (7 dpi) and convalescent (30 dpi) time points after both vaginal and subcutaneous infection. However, ZIKV was only detected in cervical DRG in one animal during the acute time point after vaginal infection. ZIKV antigen was also detected in spleen and lymph node, but not in trigeminal ganglia, kidney, or external vaginal tissue. Detection of ZIKV antigen in satellite glial cells, but not neurons, of the lumbosacral DRG following both subcutaneous and vaginal infection suggests that the virus gains access to the sensory ganglia innervating the genitals through hematogenous dissemination. Detection of antigen at both acute and convalescent time points shows that ZIKV persists in the sensory ganglia for at least 30 days after infection, providing a potential source for viral shedding in genital secretions.

Research Grant: Virginia-Maryland College of Veterinary Medicine (VMCVM)

Student Support: Virginia-Maryland College of Veterinary Medicine (VMCVM)

Identification of genetic markers for familial subvalvular aortic stenosis in Rottweilers

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Subvalvular aortic stenosis (SAS) is one of the most common congenital heart diseases of dogs, and Rottweilers have a proportionally high incidence of disease. A fibrous ridge below the aortic valve narrows the outflow tract and increases blood flow velocity, leading to devastating side effects in diseased dogs. We hypothesize that SAS is a heritable condition in Rottweilers with a single significant region of genome-wide association. This study aims to identify a chromosomal region with candidate genes containing SAS-associated variant(s). Results from this study can also inform future investigations toward the development of a genetic test for SAS in Rottweilers. A total of 71 Rottweilers were phenotyped based on aortic flow velocity (44 control, 11 mild, 2 moderate, and 14 severe) via a 2D, m-mode, color and spectral Doppler echocardiogram. DNA was extracted from 2-3 mL of venous EDTA blood, and DNA samples were submitted for genotyping on the Illumina 230k Canine HD SNP BeadChip. Analysis of 3- to 5-generation pedigrees provided for each dog supports an autosomal recessive mode of inheritance. A genome-wide association study (GWAS) is underway using Golden Helix SVS software. Preliminary results did not identify a significant region of association. After utilizing EMMAX to correct for population stratification and removing 4 outliers (3 control and 1 case) based on principal component analysis results, the single SNP with the highest disease association was on chromosome 17 ($P = 1.12 \times 10^{-5}$). Due to persisting population stratification within this study ($\lambda = 1.22$), alternative association methodologies are being tested.

Research Grant: American Kennel Club Canine Health Foundation;
University of California at Davis School of Veterinary Medicine – Center for Companion Animal Health
Student Support: National Institutes of Health

Development of a data-driven algorithm from swine to improve management of human cardiovascular disease

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According to the Centers for Disease Control and Prevention, hypertension is the primary cause or a contributing cause of death in more than 1,000 people each day in the US. Hypertension is a complicated expression of various factors, which include heart function as well as blood flow and pressures. Current methods to diagnose hypertension involve evaluating heart rate and blood pressure readings. As a result, it is often difficult to identify the root cause for hypertension in a specific patient, as these methods do not provide a complete picture of cardiovascular function. We propose that properly assessing cardiovascular health and function requires at least seven hemodynamic properties: stroke volume, ejection period, heart rate, systolic pressure, diastolic pressure, peripheral resistance and aortic capacitance. To estimate properties not routinely measured in clinic, we developed a hemodynamic computational method using experimental data from healthy and hypertensive swine, applying the physical principles of mass conservation and the mechanical elasticity of blood vessels. Currently, clinical human data is being compiled into a large, longitudinal data repository, with the goal to refine and validate this computational method for humans. Once validated, this model will be used to describe the complete cardiovascular function state for humans, to enhance clinical monitoring and care, ultimately improving cardiovascular health.

Research Grant: NIH R01 HL 112998 (Emter, PI)
Student Support: Stipend for Sierra Crotty is supported by an endowment established by IDEXX-BioResearch

Determining the role of environmental chemicals in Western Pacific Parkinsonism-Dementia Complex (PDC)

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The Western Pacific Amyotrophic Lateral Sclerosis and Parkinsonism- Dementia Complex (ALS/PDC) is a slowly progressive neurodegenerative disease virtually endemic to the Western Pacific islands such as Guam and the Kii peninsula of Japan. PDC can manifest with parkinsonian features such as tremor, rigidity, slow movement and cognitive decline as well as progressive mental deterioration, memory deficits, and disorientation seen in Alzheimer's disease. Exposure to food and medicine from Cycas, a plant indigenous to the tropics, is the main hypothesis as the cause for the disease. Cycads are palm-like gymnosperm plants whose seeds contain toxic compounds yet are traditionally eaten as a source of dietary starch. Major cycad toxins include methylazoxymethanol (MAM) and β -N-methylamino-L-alanine (BMAA) which are neurotoxic and damage motor neurons in the spinal cord and brain. The aim of this study is to derive neurons and astrocytes from induced pluripotent stem cells (iPSCs) from lymphocyte cells of age and gender-matched PDC affected and healthy subjects to determine the role of these toxins in PDC. After exposure to toxins, immunocytochemistry was used to compare phenotypic changes that occur between affected and healthy neurons, before and after treatment. The results demonstrate that PDC patients have more reactive astrocytes, evident through co-localization with neurotoxin reactive marker, C3 and Galectin-3. In addition, as reactive astrocytes induce cell death, there is a decrease in neuron density in PDC-affected subjects. Our results support the hypothesis that cycad toxins have a significant etiological role in PDC.

Research Grant: Western University of Health Sciences, College of Veterinary Medicine (intramural funds)

Student Support: Boehringer Ingelheim Veterinary Research Scholars Program

Detecting the sry gene in bone-marrow derived mesenchymal stem cells from *Equus caballus*

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Mesenchymal stem cells (MSCs) have been investigated in animals and humans as an intra-articular therapy to treat osteoarthritis. A recently completed study in our laboratory showed that allogeneic bone marrow derived-MSCs (BM-MSCs) mitigated the development of post traumatic osteoarthritis (PTOA). A concern in any stem cell therapy is tumor formation from the implanted MSCs. The motivation for this study was to develop a PCR based method to determine if male MSCs, transplanted into female horses, survive after intra-articular injection in horses with surgically induced PTOA. Two sets of primers for reverse transcriptase-PCR (RT-PCR) were generated against *Equus caballus* SRY mRNA and tested in RNA isolated from BM-MSCs from a castrated male horse, female horse, and equine testis. RNA was chosen over DNA as the test material because the prospective in vivo study was designed to isolate RNA from synovial sample nanostring analysis. Several optimization steps were performed for the RT-PCR reactions, but only the testis showed bands indicating SRY gene expression. These results suggest that the SRY gene is not expressed at a detectable level in BM-MSCs of a gelded horse. Primers were then generated against the *E. caballus* SRY gene for conventional PCR. DNA was isolated from equine testis, and BM-MSCs from a stallion, gelding, and female horse. Conventional PCR will be performed using these DNA samples. We expect to detect the SRY gene in both the testis, stallion, and gelding BM-MSCs but not the female BM-MSCs. If our expectations are proved to be true, conventional PCR using DNA primers for the *E. caballus* SRY gene would be the preferable method to detect the residual presence of male BM-MSCs injected into female horses.

Research Grant: Fortier Faculty activity award and Xintela Inc., Lund Sweden

Student Support: Cornell University VIP and NIH Training Grant

Effects of ovarian hormones on blood pressure regulation in middle aged female rats lacking central serotonin

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Central 5-hydroxytryptamine (5-HT) neurons project to pre-sympathetic and parasympathetic neurons that regulate autonomic drive to the heart and vasculature. However, the ultimate effects of central 5-HT on blood pressure in adult animals, and whether any effects of 5-HT depend on sex, are unclear. Also, the role of 5-HT on arterial blood pressure (ABP) regulation may depend on state of vigilance, as 5-HT neurons have maximal activity during wakefulness (QW), with progressively reduced activity following the transition to non-rapid eye movement (NREM) and REM sleep. Recently, we showed that 1-year-old rats lacking central 5-HT had increased ABP; this phenotype occurred only in males, and was most evident during REM. It may be that in females, ovarian hormones compensate for the loss of 5-HT, preventing a rise in ABP. We hypothesize that ovariectomy will lead to a greater increase in the ABP of female rats deficient in central 5-HT compared to female wild-type (WT) controls. To test this, we will use 1-year-old female rats deficient in tryptophan hydroxylase 2 (TPH2^{-/-}), an enzyme necessary for 5-HT synthesis, as well as WT controls. Using femoral catheters, ABP will be measured as the rats cycle between vigilance states (determined by behavioral observation). TPH2^{-/-} and WT females will then be ovariectomized to deplete systemic ovarian hormones. Following a two-week recovery period, ABP will be measured again and compared to baseline values. It is expected that after ovariectomy, the ABP of female TPH2^{-/-} rats will increase to a greater degree than female WT. Our interpretation would be that ovarian hormones protect adult females from the hypertensive effects of central 5-HT deficiency.

Research Grant: American Heart Association Scientist Development Grant (14SDG18560022; PI: KJC), National Heart, Lung and Blood Institute Grant (1R01HL136710-01A1; PI: KJC)

Student Support: Department of Biomedical Sciences, University of Missouri College of Veterinary Medicine

HDAC inhibitors interfere with meiotic chromosome synapsis in the domestic dog

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There is a need to develop safe and reliable methods for feline and canine contraception. The objective of this study is to undertake a systematic and superresolution analysis of spermatogenesis in the dog and investigate whether this process can be arrested by in vitro treatment with histone deacetylase inhibitors (HDACi), such as trichostatin A (TSA). We previously characterized the sister chromatid cohesion protein, structural maintenance of chromosome 3 (SMC3), as a marker of chromosome synapsis during mouse and equine spermatogenesis. Here, we hypothesize that SMC3 is enriched at the XY chromosome axes in canine pachytene cells where it is associated with a unique process of X chromosome self-synapsis. Spermatocytes were quantified by immunochemical localization of SMC3 during the pachytene stage from canine testis samples (n=3). Our analysis demonstrates a strong correlation between SMC3 enrichment and X chromosome self-synapsis. Additionally, canine seminiferous tubules from each of the samples were treated with TSA in tissue culture and analyzed for abnormal homologous chromosome synapsis at 48 and 96 hours using SMC3 and the DNA double strand break marker γ H2AX. Notably, TSA treatment resulted in an increase (32%) in the number of abnormal spermatocytes following 96 hours of TSA treatment compared to the control (4%). Our results demonstrate for the first time the applicability of tissue culture methods established in the mouse, for canine testicular tissue and, thus, will enable analyses of the spermatogenic process to other inhibitors. Future studies will focus on using fluorescent markers of canine germ cells in live cell imaging experiments and the application of these techniques for feline models.

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Student Support: NIH Office of Research Infrastructure Programs, Grant Number 5T35OD010433-12

Identifying a variant associated with cerebellar cortical degeneration in Scottish Terriers

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Hereditary ataxias are neurodegenerative diseases that cause progressive cerebellar degeneration. The causative mutation has been described in 10 dog breeds but remains elusive in others, including Scottish Terriers. Preliminary research mapped the locus to the X chromosome and produced whole genome sequencing (WGS) data of affected and normal dogs. This project aims to identify pathologic variants that segregate with phenotype. Previously, sequence data from seven affected and five normal dogs was aligned to the reference sequence and compared with our WGS database to generate a list of variants. This data was filtered and analyzed for X-linked recessive disease segregation with our phenotypes. Variants were inspected for gene locations maximizing the likelihood of protein alterations, such as exonic regions. To test if mutations could cause neurodegeneration, genes with potential variants were analyzed for function and expression levels. One variant meets these standards thus far, a single nucleotide polymorphism located at X:105,410,917 in an intron of the FAM122B gene. The function of FAM122B has not been fully described, although it is estimated to have moderate cerebellar expression levels. This variant was further analyzed in 49 additional affected and 49 normal dogs with Sanger sequencing. We had proper disease segregation in 81.6% of affected and 85.7% of normal dogs. Further work entails confirming sex and diagnosis of sequenced dogs, then analyzing gene function. Ultimately, we hope to identify the mutation leading to cerebellar degeneration in Scottish Terriers and design a genetic screening test to wean the mutation and disease out of the population.

Research Grant: Scottish Terrier Club of America, AKC Canine Health Fund Foundation

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Three-dimensional motion of equine hoof components during axial loading and unloading

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Equine laminitis is a common and often devastating condition with no definitive treatment. Corrective shoeing is often used to promote hoof tissue healing by minimizing third phalanx (P3) motion within the hoof. There is little information on P3 within the hoof wall in normal and inflamed hooves. The purpose of this study was to quantify the motion of P3 and surrounding hoof wall during forces consistent with walking. The tested hypothesis was that three-dimensional P3 motion within hooves affected by laminitis is significantly great than in hooves without inflammation. Three unaffected and three laminitic hoof specimens were prepared by immobilizing the proximal pastern joint with an internal fixation system and methyl methacrylate potting. To measure P3 and hoof motion, a window was created in each hoof wall. Infrared markers detectable with an active motion detection system were placed on P3 and proximodorsal, proximolateral, and distolateral hoof wall. A materials testing system with custom fixtures was used to apply 6,000 N of pressure to each hoof over 5 loading cycles (1 mm/sec). Three dimensional motion of P3 and the distance of P3 from each marker on the hoof wall compared to baseline (no load) was determined at 1500 N, 3000 N, 4500 N and 6000 N during loading and unloading. Results will be compared between unaffected and laminitic hooves at each load and between loads for each disease cohort.

Research Grant: United States Department of Agriculture (USDA)

Student Support: Louisiana State University School of Veterinary Medicine and Boehringer Ingelheim

Can IS711 be used as a surrogate for MLST genotyping of *Brucella ceti* in wild cetaceans?

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Brucella ceti, identified in dolphins, whales, and porpoises beginning in the 1990s, has 3 main sequence types (ST): ST23, ST26, and ST27, which vary by species and geographic region. *B. ceti* produces a range of pathologic lesions and can induce abortions, negatively affecting already-declining wild cetacean populations. Additionally, as the human-cetacean interface increases, so does the need for a rapid genotyping assay to determine the zoonotic risk to exposed individuals, as at least ST27 has been identified as zoonotic. Although the ST of *B. ceti* isolates can be differentiated using the nine-gene multilocus sequence typing (9-MLST), the method is cumbersome for diagnostic testing. An insertion sequence called IS711 can be used to differentiate between STs of many *Brucella* species, but it is unknown if it can differentiate among *B. ceti* STs. Here, we investigate the use of IS711 genotyping for differentiation of ST23, ST26, and ST27 in *B. ceti* isolates. We hypothesize that IS711 genotype will be predictive of 9-MLST genotype, allowing for the identification of *B. ceti* type by a single genetic element. Both the IS711 and 9-MLST methods were used to genotype *B. ceti* isolates (n=56) that were collected from cetacean tissues on necropsy. 9-MLST genotyping identified the isolates as ST23 (n=3), ST26 (n=25) and ST27 (n=28). Preliminary comparisons indicate that the IS711 genotyping may provide a more-rapid method for distinguishing among *B. ceti* STs of pathogenic importance.

Research Grant: University of Illinois College of Veterinary Medicine

Student Support: University of Illinois College of Veterinary Medicine

Effects of Gulf War-related chemical exposures on mouse motor behaviors: therapeutic modulation by LNFPIII

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Over 25% of the 1991 Gulf War veterans suffer from a chronic, multi-symptom illness termed Gulf War Illness (GWI), which includes a variety of neurological and immunological dysfunctions. The etiology of GWI continues to be elusive, but exposures to deployment-related chemicals like the nerve agent prophylactic pyridostigmine bromide (PB) and the pesticide permethrin (PM) are strongly linked to GWI. Currently, the long-term behavioral deficits associated with GWI are not well characterized and effective and safe treatment options are not available. However, because of its immunomodulatory effects, the glycan conjugate LNFPIII merits evaluation as a potential therapeutic component. Here, we used a mouse GWI model (PB-PM exposure for 10 consecutive days) and evaluated (i) the effects of this exposure on multiple behaviors months after exposure termination and (ii) the ability of LNFPIII to modulate effects of PB-PM when LNFPIII treatment is initiated months after PB-PM exposure. From the behavioral data analyzed up to date, muscle strength (measured by Grip Strength Test; GST) was not affected by the GWI chemicals or LNFPIII. Interestingly, outcomes from a multi-parameter Gait Analysis indicated that mice with prior exposure to PB-PM took more steps during testing, an effect prevented by LNFPIII, and that LNFPIII, independent of the PB-PM/control treatment, increased stride length and decreased stride variability. Thus, as it relates to motor function, it appears that LNFPIII treatment not only improves the deficit caused by PB-PM, but it also improves gait in general. This is a promising outcome and LNFPIII as a GWI treatment option warrants further exploration.

Research Grant: Department of Defense, Grant Number W81XWH-16-1-0586

Student Support: NIH Office of Research Infrastructure Programs, Grant Number 5T35OD010433-12

Effect of antimicrobial administration on respiratory and gastrointestinal microbiota of healthy foals

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Microbiota-host interactions have been shown to direct immune responses in many species. Our long-term goal is to characterize how altered microbiota in foals affects immunity, and therefore susceptibility, to *Rhodococcus equi* infection. In adult horses, administration of antimicrobials causes reduced diversity of bacterial microbiota. However, these effects have not been confirmed in foals. In this study, our goal was to determine the effects of antimicrobials on bacterial microbiota at various gastrointestinal and respiratory sites in foals. Twenty-four (24) healthy foals were randomly assigned to one of three treatment groups: untreated, Excede-treated (6.6 mg/kg IM q 96h twice), and Azithromycin-treated (10 mg/kg once daily for 7 days). Pharyngeal swabs, transtracheal washes, and bronchoalveolar lavages, as well as gastric and fecal samples, were collected from foals on days 0 (before treatment), 3, 7, and 35 (4 weeks after treatment). DNA extraction and 16S rRNA bacterial analysis will be performed on all samples. Descriptive analysis of alpha diversity will be performed. Measures of beta diversity will be used to determine differences between treatment groups. Preliminary data in adults revealed differences in phylum-level relative abundances between gastric and fecal samples, and reduced species richness in fecal samples after treatment with Excede. Based on this data, we expect that foals will have distinct communities of bacteria at each site, and decreased diversity after antimicrobial administration, at all sites. Results of this study will be used to direct future studies investigating the effects of altered microbiota on immunological parameters of foals susceptible to *R. equi*.

Research Grant: University of Georgia, Faculty Research Grant

Student Support: Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Stem-cell transplantation into stallion testes: Optimization of a method

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Stem cell transplantation into the testes has been successfully performed in numerous lab and farm animal species but not in horses. The main obstacle in applying this procedure to stallions is technical difficulty in delivering stem cells into the seminiferous tubules (STs). In our lab, an ultrasound-guided approach was recently used to infuse dye into the equine STs, using gravity force. After dissection, fluid was found in STs, but also in the blood vessels and connective tissue within the testes, which is undesirable. Therefore, the aim of this study was to optimize a method of delivering dyed fluid into the STs of stallion testes, using a syringe pump with controlled infusion rates. Fifteen testes were obtained from stallions ranging from 1-4 years old and were assigned to an infusion rate (5 mL/hour, 10 mL/hour, or 20 mL/hour) so there were 5 testes infused per rate. Trypan blue (TB) dye mixed with ultrasonic contrast agent was infused under ultrasonographic guidance into the rete of each testis. After each infusion, testes were dissected and evaluated for the patterns of dye dispersion and for the presence of TB dye in the lumens of STs. Similar patterns of dye dispersion were observed in all infusion rate groups. TB dye was found in STs of 3/5 testes from 5 mL/hour group, 5/5 testes from 10 mL/hour, and 4/5 testes from 20 mL/hour group. There was a trend for the infusion rate 10 mL/hour to yield the highest percentage of successfully infused STs ($P < 0.1$; Kruskal-Wallis AOV). Our data suggest that 10 mL/hour is the most optimal rate for infusing stallion STs, but further studies with a larger sample size are necessary to confirm these findings.

Research Grant: University of Florida; 2018 CVM Spring Consolidated Faculty Research Development Award Grant Competition

Student Support: Florida Veterinary Scholars Program and Boehringer Ingelheim Veterinary Scholars Program

Comparison of an internal versus novel external teat sealant for the prevention of mastitis in dairy cattle

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Mastitis is a prevalent disease endemic to dairy herds within the U.S. Blanket dry cow therapy (BDCT) is commonly used on commercial dairies to prevent the development of new mastitis infections and to treat current infections during the dry period. Treatment options include internal teat sealant (ITS) or external teat sealant (ETS) with or without antibiotics (+/-Ab). The aim of this study is to investigate if a novel ETS is a viable alternative to a traditionally used ITS product. Cows that are unlikely to benefit from antibiotic administration prior to dry off were defined as “low risk cows” according to a previously established algorithm. 81 low risk cows were randomized to one of four treatments: ETS or ITS application +/-Ab into/onto all 4 quarters at dry off. Quarter level milk samples were retrieved prior to dry off and again at 1-7 days in milk. Aerobic cultures at these time points were used to assess new infections of ETS compared to ITS treated cows. Out of 139 quarters enrolled, 11 quarters treated with ETS and 9 quarters treated with ITS had a new infection. Of 148 quarters enrolled, 14 quarters treated with ETS and 12 quarters treated with ITS had a new infection. The median linear score of the first test date of the new lactation of ETS (n=4) and ITS (n=3) treated cows was 1.95 and 3.9 respectively; the median linear score of ETS +Ab (n=4) treated cows was 1.85 whereas the median linear score was 4.1 for ITS +Ab (n=3) treated cows. Similar pathogens infected both ETS and ITS treated cows. Preliminary analysis indicates that there are no important differences between new infection frequencies or linear score between ITS and ETS treated cows suggesting that ETS may be an effective ITS alternative.

Research Grant: DeLaval Corporation

Student Support: Cornell University Veterinary Investigators Program

Psychotropic drug use to combat stress-related disease in animal shelters

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Stress is linked to multiple health conditions including cardiac, metabolic and infectious diseases in humans and is suspected to impact various diseases in cats and dogs. It is hypothesized that additional stressors prevalent in animal shelters contribute to the outbreak of infectious diseases, specifically upper respiratory tract disease (URTD) in cats and stress colitis in both dogs and cats. Although veterinarians utilize psychotropic medications to decrease stress and anxiety in shelter animals, no studies have evaluated the efficacy of these medications in shelter environments. This study aims to determine the impact of gabapentin and trazodone, two commonly prescribed psychotropic medications, on stress-related clinical signs of disease in cats and dogs, respectively. Owner surrendered cats and dogs relinquished to a Colorado humane society are being entered into the ongoing study. Within 24 hours of surrender, each animal is randomly assigned to a treatment or control group. In the treatment group, dogs are administered 3-7 mg/kg trazodone orally and cats are administered 100 mg gabapentin orally on Days 1 - 3. Control animals are administered a placebo orally. On Days 1-7, masked observers apply a standardized fecal score to feces from both cats and dogs, and an additional standardized respiratory and ocular score to cats. Differences have been noted between animals on the study to date, but the study is still masked and case accrual and clinical scoring is ongoing.

Research Grant: Young Investigator's Grant, Center for Companion Animal Studies

Student Support: PetSmart Charities Summer Scholar Program

Developing optimal conditions for 3D *in-vitro* co-culture of human mesenchymal stem cells and macrophages

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Periprosthetic osteolysis and subsequent orthopaedic implant failure are detrimental potential complications of orthopaedic surgery. They result from an inflammatory response to wear particles from the implant, driven primarily by activated tissue macrophages. Extensive research and murine cell models have identified a seemingly contradictory role of macrophages in mediating both osteolysis and osteogenesis, making them an interesting target for both implant failure studies, as well as potential cell therapies in the future. Studies have shown that polarized M1 macrophages play a vital role in the inflammatory response to orthopaedic implants, while M2 macrophages encourage bone matrix deposition by mesenchymal stem cells (MSC). However, the intricacies of this dynamic macrophage-MSC interaction remain poorly understood. The ultimate goal is to co-culture human macrophages (hM) and human mesenchymal stem cells (hMSC) in a 3D gelatin scaffold to develop a better *in-vitro* model for studying hM associated osteolysis and osteogenesis. Previous experiments in the laboratory attempting to co-culture murine macrophages and MSCs indicated that an essential element in hM media, macrophage colony stimulating factor (MCSF) can have an inhibitory effect on bone growth by MSCs. The present study intended to see if there is a concentration of MCSF that is both sufficient for hM survival, while not having deleterious effects on hMSC bone matrix deposition. We hypothesized that such a growth medium could be developed, and through the use of microscopy, histology and ELISA to assess cell vitality and evidence of bone deposition, concluded that a concentration of 50ng/ml was optimal for supporting both cell types.

Research Grant: NIH NCATS 1UG3TR002136-01

Student Support: NIH/ORIP T35 OD010989

Genetic testing for progressive rod-cone degeneration and centronuclear myopathy in Labrador Retrievers

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The overall objective of this study was to develop Atlantic Veterinary College into a centre of excellence for canine genetic testing, making genetic testing more accessible to Atlantic and Eastern Canadian dog owners. Progressive rod-cone degeneration (PRCD) is a form of progressive retinal atrophy (PRA) caused by a point mutation on exon 1 of the *Prcd* gene, resulting in vision loss. Centronuclear Myopathy (CNM) is the result of a short interspersed nucleic element (SINE) insertion on exon 2 of the *Ptpla* gene. CNM causes a decrease in skeletal muscle function, leading to muscle atrophy, weakened reflexes and limited locomotion. The focused objective of this study was to replicate current standard canine genetic testing protocols for PRCD and CNM in Labrador Retrievers. Breeders from Atlantic and Eastern Canada submitted buccal swabs from their dogs for blinded study; when known, breeders submitted information separately regarding their dog's PRCD and CNM genetic predispositions. DNA was then extracted and amplified via polymerase chain reaction (PCR). Restriction fragment length polymorphism analysis (with *RsaI*) was used to determine the status of an individual's PRCD predisposition. For detection of an individual's predisposition to CNM, electrophoresis was used to detect the SINE insertion on the PTPLA gene. DNA from individuals with two wild copies of the PRCD gene, when amplified using known primers, was unaffected by *RsaI* resulting in a single 512BP band upon electrophoresis. An affected allele resulted in two fragments of the PRCD gene, one of 369BP and one of 116BP. Expected results once affected CNM DNA is analyzed is a 610BP fragment from wild type alleles and an 848BP fragment from affected alleles.

Research Grant: KRESCENT, CIHR & AVC Research Fund

Student Support: Veterinary Student Research Award

Subunit vaccine-induced CD8 T-cell immunity to mucosal and systemic viral infection

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The most effective vaccines are based on antibody induction, but it has become clear that vaccines against many intracellular pathogens need to elicit memory T cells. Live vaccines are best at generating T cell immunity but are contraindicated in the pregnant or immune compromised. There is a need to develop safe subunit protein vaccines that elicit potent T cell memory. Adjuvax, a commercial adjuvant, has shown promise in this area. The aim of this study is to determine whether Adjuvax-adjuvanted subunit (Chicken Ovalbumin; OVA) vaccine administration induces protective T cell memory when given via different routes (SQ vs IN), and if this immunity protects against pathogens introduced via different routes. Mice are grouped based on vaccination and infection routes. Three weeks after booster, five mice per group will be euthanized without viral challenge, and samples will be analyzed using flow cytometry to assess the type and numbers of memory T cells in the respiratory tract and spleen of vaccinated mice. The remaining twenty mice will be challenged with recombinant vaccinia virus expressing OVA antigen by IN or IP route. We will quantify antigen-specific T cells and viral titers in tissues of virus-challenged mice. We hypothesize that: (a) IN vaccination induces tissue resident memory T cells in the respiratory tract and protects against IN viral challenge; (b) SQ vaccination induces systemic memory T cells in spleen and protects against IP challenge. We expect these studies to improve understanding of the tenets of protective immunity to mucosal and systemic infections. This has implications in the development of safe and effective T-cell-based subunit vaccines against intracellular pathogens of animals and humans.

Research Grant: 1U01AI124299-01 (MARULASIDDAPPA,SURESH)

DHHS, PHS, NATIONAL INSTITUTES OF HEALTH

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3.00 Cal.

Title: Novel Adjuvant Combination for Systemic and Mucosal CTL Memory \$250,000

Student Support: National Institute of Health T35 Training Grant T35OD011078

Enhancing wild animal semen quality through nanotechnology tools

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Assisted reproductive techniques (ARTs) have been widely used in wildlife species for conservation of threatened species. However, approaches to obtain high quality semen through purification are not reliable in all wildlife species. Thus, there is a need to develop a multi-species compatible technique for efficient preservation of viable spermatozoa. This study aims to identify the compatibility of a sperm nanopurification technique in wildlife species through applications with domestic feline and threatened cattle species. Domestic feline epididymal sperm and ejaculated Ankole and Sahiwal bull semen samples were mixed with magnetic nanoparticles designed to interact with non-viable acrosome-reacted spermatozoa. Mixed semen was placed under an electromagnetic field trapping moribund sperm, enriching the dose with viable cells. Motility characteristics were analyzed, and felid samples cryopreserved and utilized for in vitro fertilization and microscopic analysis. Comparable motility characteristics were found in all species with microscopic analysis of felid nanopurified sperm showing an increased proportion of acrosome intact cells. In vitro fertilization of nanopurified felid sperm showed improved 8-cell embryonic growth 3 days post-fertilization. Findings indicate sperm nanopurification is compatible with feline and bovine species. Additionally, sperm nanopurification does not interfere with sperm viability, cryopreservation, or in vitro fertilization potential. Additional research is being conducted to confirm current findings for future implementation in conservational reproductive breeding programs.

Research Grant: USDA ARS 58-6402-3-018

Student Support: Morris Animal Foundation; Office of Research and Graduate Studies Mississippi State University

Effect of fecal microbiota transplantation on the fecal microbiome of healthy dogs treated with antibiotics

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The intestinal microbiome consists of a variety of microorganisms present in large amounts, and provides essential benefits to the host health. Gastrointestinal diseases but also antibiotic treatment have been shown to severely alter the intestinal microbiota. Fecal microbiota transplantation (FMT) involves the transfer of feces taken from a healthy donor to a recipient with the purpose of restoring the recipient's intestinal microbiome. This procedure has been used successfully in people to treat gastrointestinal diseases such as recurrent *Clostridium difficile* infections. Only a few case reports are available in dogs. We hypothesize that FMT will result in accelerated recovery of canine fecal microbiota after healthy dogs have received tylosin, an oral antibiotic shown to significantly impact the fecal microbiome. A single fecal donor was selected for the whole study. FMT solution was prepared with fresh donor feces after 1:5 dilution with saline, filtering, and addition of glycerol, and immediately frozen at -80C. Sixteen dogs received tylosin orally for 7 days, and were subsequently divided in 3 groups. Six dogs were given FMT once via enema, 6 other dogs received gelatin capsules containing fecal solution for 2 weeks, and 4 dogs received placebo for 2 weeks. Feces were collected from all dogs at the beginning of the study, after giving the antibiotic, weekly for three weeks after initiation of FMT, once more 5 weeks after beginning FMT, and frozen until analysis. High throughput sequencing as well as qPCR will be used to evaluate the fecal microbiome and determine the dysbiosis index. Select fecal metabolites and bile acids will also be analyzed.

Research Grant: Hunting Retriever Club Foundation

Student Support: CGS/Idexx Veterinary Student Summer Scholar Award, LSU School of Veterinary Medicine

Molecular characterization of helminth parasites from North Atlantic wildlife

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Biomedical Sciences (Egers, Robbins, Greenwood), Pathology and Microbiology (Mahjoub, Bourque, Jones, Conboy), Canadian Wildlife Health Cooperative (Bourque, Jones), Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI; Provincial Biologist - Retired (Curley), PEI

GenBank is a worldwide database used to store and share genetic sequences. Unfortunately, there is a poor representation of helminth parasite sequences from North Atlantic wildlife. This lack of information can be problematic in determining the species of parasite for diagnosis, especially if the researcher does not have an extensive background in parasite morphology. The goal of this research is to utilize morphological and molecular diagnosis and phylogenetic analysis to confirm genus and species of collected helminth parasites from opportunistic cases submitted to the Atlantic Veterinary College Diagnostic Services, Morphologic Pathology. One discovery was the presence of nematodes in the nasal sinuses of mink (*Mustela vison*), which were morphologically identified as *Skrjabinigylus nasicola*; the first reported case in Prince Edward Island. A GenBank search retrieved only two submissions for *Skrjabinigylus nasicola*, and both were partial sequences of the COX 1 gene. To aid in confirmation of the morphological diagnosis, polymerase chain reaction (PCR) was done using the NC18SF1/NC5BR primers for the small ribosomal subunit (SSU) and 391F/501R primers for the large ribosomal subunit (LSU). A preliminary consensus sequence was constructed and sequence similarity calculations were performed. The nematode identified as *Skrjabinigylus nasicola* shares the highest DNA sequence similarity of 96.7% and 94.8% with the *Skrjabinigylus chitwoodorum* SSU and LSU sequences in GenBank. A phylogenetic analysis of the SSU sequence places the collected *Skrjabinigylus nasicola* firmly within the Metastrongyloidea, a superfamily in the phylum Nematoda. These SSU and LSU sequences, and sequences from future discoveries, will be added to GenBank.

Research Grant: Atlantic Veterinary College research funds (Greenwood and Conboy)

Student Support: NSERC-USRA, VetSRA-Boehringer Ingelheim Veterinary Scholars Award

Canine gastrointestinal histamine receptor-1 expression in normal and inflammatory bowel disease biopsies

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Histamine is released upon the degranulation of several different cell types, particularly mast cells, which are abundant in the gastrointestinal (GI) tract. After its release, histamine binds to histamine receptors (H1-H4) located throughout the body. Upregulation of histamine and its' receptors is well documented in various GI disease in human medicine. In dogs, based on full thickness biopsies collected via surgery, H1-H4 receptors were identified throughout the entire canine GI tract. In this study, our aim was to identify and score the expression of H1 receptors in pinch biopsies of the GI tract of dogs collected via endoscopy. Our study utilized immunohistochemistry (IHC) to investigate the expression of H1 receptors in the GI tract of healthy dogs and dogs diagnosed with inflammatory bowel disease (IBD). Our hypothesis was that expression of the H1 receptor would be more abundant in the GI tract of dogs with IBD compared to healthy dogs. Biopsies were collected from four dogs with no clinical signs of IBD (control) and archived samples from 17 dogs diagnosed with mild or moderate/severe IBD. IHC staining was performed on endoscopic samples from the stomach, small intestine and colon. IHC staining was scored on abundance and intensity on a scale of 0 to 3. H1 receptor expression varied by anatomic site and tissue location (superficial vs deep) in the control and IBD dogs, with the most abundant expression in the stomach. There were no significant differences between control and IBD (mild vs moderate/severe) H1 receptor expression. The results of our study suggest that the H1 receptor can be identified using endoscopically collected biopsies, and that dogs with IBD do not have increased H1 receptor expression.

Research Grant: Dr. Sullivant Indirect

Student Support: NIH T35 Training Grant T35OD010432

Investigating CpG ODN-mediated attenuation of lung metastasis in a preclinical model of osteosarcoma

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Osteosarcoma (OS) is an aggressive bone neoplasm that is estimated to impact over 10,000 dogs per year. New therapies are desperately needed, as the majority of dogs with OS develop pulmonary metastasis, and survival times have plateaued. Innate immunotherapeutics are promising for the treatment of OS and also have the added ability to be used across species. Our lab has determined that the toll-like receptor agonist CpG ODN attenuates lung metastasis and increases survival time in a preclinical model of murine OS. However, the mechanism of metastatic attenuation and minimal effective dose of CpG ODN are still unknown. We sought to elucidate whether specific immune cells, metastasis-associated proteins, or pro-inflammatory cytokines are critical to the efficacy of CpG ODN. In addition, we attempted to determine the minimum effective therapeutic dose. Using a well-described preclinical model of murine OS (K7M2 cell line), we used immunodeficient mice to determine if specific immune cells are functional in CpG ODN's mechanism of action (MOA), as well as immunocompetent mice to determine the minimum dose requirements of CpG ODN. TNF α depletion experiments were also performed to investigate the potential involvement of TNF α in CpG ODN's MOA. Western blot analysis was used to investigate whether metastasis-associated proteins are decreased post-CpG ODN exposure in vitro. Our results suggest that CpG ODN has a distinct minimal effective dose and exhibits direct effects on OS cells. These findings serve to increase our understanding of CpG ODN's MOA and efficacy levels,providing increased knowledge for the application of CpG ODN as an immunotherapy in dogs with spontaneous OS.

Research Grant: Midwestern University College of Veterinary Medicine Intramural funds

Student Support: Boehringer Ingelheim Veterinary Scholars Program Fellowship

Changes in gonadotropin-like receptor expression in the spawning anemone *Aiptasia pallida*

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Although the health of coral reefs globally depends on continued successful reproduction, endocrine reproductive mechanisms of cnidarians as a phylum are not understood. To begin to characterize these, we built on our previous genomic work in a model cnidarian, the anemone *Aiptasia pallida*, in which we cloned several members of the LGR superfamily, encoding for G-protein coupled receptors including gonadotropin receptors required for mammalian fertility. *A. pallida* express LGR-A, -B, and -C receptors, with LGRA similar to receptors for FSH and LH. Exposure of *A. pallida* to human gonadotropins resulted in rapid and robust increases in cAMP, suggesting functional signal coupling. The current study explores changes in LGR receptor expression in anemones that are reproductively cycling, via incubation in light and feeding conditions mimicking normal lunar cycles, such that spawning occurs routinely 5 days following “new moon” conditions. *Aiptasia* samples were harvested at multiple timepoints within these induced reproductive cycles, and RNA was isolated and converted to cDNA, which we are probing for expression of LGRA2 and housekeeping gene L11, using real-time qPCR. Preliminary results point to robust changes in gonadotropin-like receptor expression during the monthly cycle, further implicating this gonadotropin-like pathway in cnidarian reproduction. This research demonstrates for the first time hormonal changes in reproductively patent cnidarians, and can be used to further model coral spawning, as they remain in decline and are key habitats to an array of crucial species.

Research Grant: NSF: OISE 1042509. EAGER: Building networks and study systems to advance research on the biology of Pacific corals

Student Support: Boehringer Ingelheim Veterinary Scholar Program

Identification of genetic variants associated with two Mendelian diseases in the horse

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Equine myotonia and hemochromatosis are debilitating, likely Mendelian diseases. Horses with myotonia have delayed muscle relaxation due to abnormal muscle membrane conduction, often necessitating euthanasia. Hemochromatosis causes weight loss, lethargy, and permanent liver damage. Historically, identifying genetic variants associated with Mendelian diseases has been difficult. However, recent work in humans and cattle demonstrated that whole genome sequencing (WGS) of individuals with Mendelian diseases accompanied by a catalog of genetic variation from a healthy population enables identification of causative mutations. We hypothesized that causative mutations for equine myotonia and hemochromatosis can be identified using this approach. WGS (12X) was performed on horses with myotonia (3) and hemochromatosis (1). Reads were mapped to EquCab3 and single nucleotide polymorphisms (SNPs) were identified and annotated using publicly available software. SNPs in candidate genes were investigated using programs that predicted functional effect and conservation scores. 170 variants predicted to be moderate or high impact were identified in 38 candidate genes for myotonia. 16 variants predicted to be moderate or high impact were identified in 31 candidate genes for hemochromatosis. The allele frequency (AF) of these variants was determined in a population of 230 horses. These variants were considered unlikely to be disease causing, due to either poor conservation across species and/or AFs $\geq 5\%$ in the 230 horse population. While definitive pathogenic variants are yet to be identified for these diseases, this project demonstrates the utility of WGS methods for ruling out multiple candidate genes for Mendelian diseases in the horse.

Research Grant: Minnesota Agricultural Experiment Station, USDA-NIFA grant no. 2017-67015-26296, and the American Quarter Horse Foundation.

Student Support: Boehringer-Ingelheim (Merial)

Activation of canine mesenchymal stem cells to enhance control of inflammation, arthritis, and wound healing

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Mesenchymal stem cells (MSC) have been demonstrated to track to sites of inflammation and downregulate inflammation through immunologic mechanisms. MSC have toll-like receptors (TLRs) and are able to alter their phenotype following exposure to various TLR ligands and cytokines. Our hypothesis is that pre-activation of MSC with TLR ligands and pro-inflammatory cytokines will enhance the anti-inflammatory action of canine MSC and promote wound healing. To investigate this hypothesis we utilized canine adipose derived MSC from three different canine donors and pre-activated the cells with TLR agonists poly-IC and LPS as well as TNF α and IFN γ to simulate an inflammatory environment. MSC were exposed to these compounds for 24 hours and conditioned media was collected. Production of cytokines by MSC following pre-activation was assessed by ELISA. In vitro assays were utilized to assess wound healing, and macrophage polarization in response to MSC conditioned media. Pre-activation of MSC improved fibroblast survival, affected the cytokine production profile and stimulated macrophage polarization to promote a wound-healing phenotype. Pre-activation of MSC may be a valuable tool to individualize stem cell therapy and to maximize wound healing and anti-inflammatory effects of stem cells prior to delivery.

Research Grant: State of Colorado Oedit Grant (APP-103508)

Student Support: NIH Grant T35OD01530-06

Corticosterone monitoring to assess the effect of transport distance on stress levels in mice

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Animals used in experimental studies are often subjected to a wide variety of transportation, from short-distance movement between rooms or buildings to long-distance transportation from vendors that may be thousands of miles away. While stress associated with transportation between vendors and research institutions is well-documented, the effect of transportation duration or modality is not well-understood. Glucocorticoids induced by stressful events can alter metabolism, immune function, and animal behavior. Therefore, characterizing transport stress matters from a welfare perspective and from one of scientific rigor. In this study, we aimed to characterize transportation stress in mice. Fecal corticosterone, complete blood counts, and blood serum biochemistry were used to analyze stress in mice shipped from the vendor, hand-carried between buildings, or transported between buildings on a cart. We hypothesize that corticosterone levels and glucocorticoid-induced enzymes will be significantly increased for all methods of transportation, with the lowest increases when animals are carried by hand. We also hypothesize that regardless of transport distance, mice will still require at least 7 days to acclimate after transportation stress. This information will be used to provide a stronger scientific justification for acclimation periods for research animals transported from a vendor or from nearby facilities and also inform researchers of least-stressful ways to transport animals. This will help reduce animal stress and confounding factors resulting from glucocorticoid release.

Research Grant: Colorado State University Office of the Vice President for Research

Student Support: American Society of Laboratory Animal Practitioners

Laser plume generation and potential associated risks

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Over the years, the use of lasers (YAG, CO₂ and diode) in equine upper airway surgeries continue to increase when compared to traditional scalpel surgery due to an associated reduction in pain and bleeding while resulting in improved recovery rates. Plumes are generated as a by-product of laser use and studies in human medicine show that they not only contain toxic gases (e.g. carbon monoxide), but also have the potential to possess infectious viruses and bacteria. Though exposure to laser plumes can be decreased by use of local exhaust ventilation (LEV), these systems are not required or commonly applied, leaving personnel at risk. To our knowledge, no studies have yet characterized plumes generated from lasers in the veterinary field. We proposed to evaluate potential health risks of plume exposure to veterinarians and staff. A diode 980 nm laser was applied to laryngeal and pharyngeal tissues of 5 horses and resultant plumes were collected for characterization of PM_{2.5} particle size distribution, organic carbon and elemental carbon (OC/EC) concentrations, and volatile organic compounds composition. Preliminary results indicate particle size concentrations > 10 times higher than permissible total dust exposure limits and > 20 times higher than the respirable fraction recommended by the Occupational Safety and Health Administration. Additionally, OC/EC measurements resemble results obtained from wildfire and prescribed fire studies. Therefore, we identified risks to operating personnel that indicate mitigation and protection from laser plumes is appropriate. Our future aim is to further categorize surgical plumes with the use of mass spectrometry and Next-Gen Sequencing ex vivo and in vivo.

Research Grant: None

Student Support: Boehringer Ingelheim Veterinary Scholar Program

Tumor lysate vaccines with immune blockade inhibitors and adverse effects in the treatment of canine glioma

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Glioma is the most common form of primary brain tumor in humans and the second most common in canines. It has a poor long-term prognosis in both species regardless of treatment including surgery, radiation and chemotherapy. Treatment is a challenge due to the location, presence of the blood brain barrier, and the ability to evade the immune system by exploiting checkpoint blockades. Autogenous tumor lysate (ATL) vaccines made from the patient's tumor cells can stimulate an immune response to tumor antigens. However, gliomas are poorly immunogenic and express high levels of CD200, an immunosuppressive checkpoint protein. Administration of a CD200 inhibitor can help overcome tumor immune tolerance. The purpose of this study was to assess the safety and efficacy of treating dogs with high-grade glioma (HGG) with ATL vaccines and P3, a CD200 peptide that overcomes the immunosuppressive effects of CD200. Solitary intra-axial masses were debulked and extent of resection checked by immediate postoperative MRI at the University of Minnesota. Dogs then received ATL vaccines combined with P3 and were assessed by MRI at 4, 8, and 12 months and post mortem histological examination of the brain. Data from medical records, email correspondence from owners and other clinicians, MRI, biopsy reports, and post mortem reports were analyzed and compared to data from dogs with HGG treated with surgery and ATL vaccines alone. Median overall survival is 249 days and is 386 days when non-tumor associated deaths are censored, compared to 194 days in dogs treated with vaccines without P3. Two owners reported lethargy after vaccine administration. 4 of the 20 dogs in the CD200 trial exhibited seizures within 24 hours after vaccine administration.

Research Grant: Funding from Humor To Fight the Tumor and NIH U01 CA224160-01

Student Support: NIH T35 Training Grant

Detection of IBV using Sanger vs MinION nanopore sequencing

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Avian infectious bronchitis virus (IBV) is a major source of disease affecting poultry and results in substantial financial losses. Due to IBV's high mutation rate, recombination, and more than fifty variants, effective diagnosis and control of outbreaks is a challenge. Reverse transcription PCR along with Sanger sequencing has been a standard method for IBV differentiation; however, this method requires access to fully equipped laboratory resources, may take several days if using external sequencing services, is limited in its ability to detect samples with more than one genotype and, due to the relatively low sensitivity of IBV S1 primers, requires culturing of the virus to enhance detection. A rapid, in-house, real-time technique for detection and differentiation of IBV lineages would be useful in the field and cost effective. MinION nanopore sequencing has been proposed to be an accurate and cost-effective application to replace conventional and current diagnostic practices. The goal of this study was to determine the ability of MinION sequencing to detect and differentiate IBV variants using clinical samples. Ten tracheal swab samples were sequenced by reverse transcription PCR coupled with Sanger sequencing, utilizing various strain specific primers. Additionally, samples were amplified using the S1 primers and then sequenced with MinION, classified with Centrifuge, and visualized with the Pavian web application. Sanger sequencing results were compared to MinION nanopore sequencing. This study shows that MinION sequencing can detect and differentiate IBV variants, including in samples containing multiple variants, supporting MinION's potential as a diagnostic technique for the poultry industry.

Research Grant: Agriculture and Food Research Initiative Competitive Grant no. 2018-67015-28306 from the USDA National Institute of Food and Agriculture.

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Evaluating the use of deuterium oxide to determine body composition in the big brown bat, *Eptesicus fuscus*

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Evaluating body composition of wild and captive big brown bats (*Eptesicus fuscus*) as a proxy for fitness is an important component of research and conservation. However, current methods of evaluation including morphological indices and chemical extraction can be inaccurate, are time consuming, or require destruction of the animal. Dual-energy X-ray absorptiometry (DXA) is used to determine body composition in live, anesthetized bats, but this method requires access to an expensive DXA machine and transport of the animal to the DXA machine increasing cost, time requirements, and stress for the animal. Deuterium oxide dilution has been used for many years as a safe and rapid technique to evaluate body composition in humans. In wild bats, this technique could allow evaluation of body composition in the field without removal from the site of capture or destruction of the animal. The purpose of this study is to compare the use of DXA with deuterium oxide dilution in bats. We hypothesize that use of deuterium oxide dilution would be a valid substitution to DXA to determine lean body mass and fat mass in big brown bats. Ten captive and ten wild big brown bats will be used in this study. Under anesthesia, bats will undergo DXA scans followed by deuterium oxide administration. Blood samples drawn before and two hours after deuterium oxide administration will be analyzed and used to calculate body composition. We expect that results of the DXA scan and deuterium oxide dilution for each bat will be similar. Such results would indicate that deuterium oxide dilution would be a cost-effective and low stress substitute for DXA evaluation of body composition in big brown bats.

Research Grant: USDA Forest Service; Missouri Department of Conservation

Student Support: IDEXX-BioResearch

The cytotoxic effects of iodinated contrast media on renal proximal tubular epithelial cells

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Iodinated contrast media (ICM) are used in millions of cardiovascular procedures every year to analyze vessel morphology and determine the severity of atherosclerotic disease. Many of these procedures are performed on individuals who have preexisting renal insufficiency. Following the administration of ICM, these patients are at increased risk for the development of worsening renal function, a condition known as contrast-induced acute kidney injury (CI-AKI). CI-AKI is clinically defined as a ≥ 0.5 mg/dL or 25% increase in serum creatinine concentration. This loss of kidney function is reversible, typically lasting a maximum of 5 days, however it has been shown to cause long term adverse effects. Compared to those who have not developed CI-AKI post administration patients, who do, have a higher probability of morbidity and mortality. The exact mechanism(s) for this disparity are unknown, however direct cytotoxic effects to the renal epithelium are thought to be involved. Recent studies suggest that proximal tubular epithelial cells are more sensitive to the toxic effects of ICM than those in the distal tubule. One key difference, and what we believe may have causative role, is the different apical transporters that they express. To test this hypothesis, we applied chemical inhibitors of various apical transport mechanisms to RPTEC/TERT1 cells to determine if they reduce the cytotoxic effects of ICM. In future studies we plan to use this model to evaluate targeted therapy to reduce incidence of CI-AKI.

Research Grant: National Institutes of Health

Student Support: Biomedical Research for University Students in Health Sciences Program

Is pain associated with nerve inflammation in Guillain-Barre Syndrome?

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Guillain Barre Syndrome (GBS) is the leading cause of acute paralysis in the U.S. This illness is characterized by ascending limb paralysis. NOD CD-86^{-/-} mice have been found to develop Spontaneous Autoimmune Peripheral Polyneuropathy (SAPP) after 20 weeks of age. Previous work determined that SAPP closely mirrors the Acute Inflammatory Demyelinating Polyneuropathy (AIDP) form of GBS and that these inbred mice with SAPP can serve as a good model for the study of GBS. Mice that developed SAPP had increased inflammation in their sciatic nerves and dorsal root ganglia. We hypothesize that NOD CD-86^{-/-} mice that displayed signs of pain will have increased inflammation in their peripheral nerves compared to the control groups. To test our hypothesis, we had two groups of mice: NOD WT (control) and NOD CD-86^{-/-} (experimental). The NOD WT and NOD CD-86^{-/-} mice were put through the open field test and their performance recorded on a camcorder for two to three minutes. These videos were analyzed using the Mouse Grimace Scale to determine pain levels. After the mice were humanely euthanized, they were necropsied and their brachial plexus, dorsal root ganglion, and sciatic nerve were sectioned and stained with anti-CD3 and anti-F4/80 antibodies to detect t-cells and macrophages, respectively. Thereafter, nerve sections were analyzed using morphometry to determine levels of inflammation. We found increase infiltration of t-lymphocytes and macrophages in the peripheral nerves of NOD CD-86^{-/-} mice when compared to NOD WT mice. Preliminary results of the study indicates a correlation between pain phenotypes and increased inflammation in NOD CD-86^{-/-} mice.

Research Grant: These studies were funded with funds from National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Grant No. U19AI090872

Student Support: NIH grant R25HL103156

Cloning canine CD16 for directed NK cell-immunotherapy

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Osteosarcoma (OS) is the most common bone cancer in people, affecting predominantly children. Overall survival has not improved significantly in the last few decades; OS is invariably fatal and its rarity makes it challenging to study. Recent studies demonstrated biological and molecular features that are common to both canine and human OS. Canine OS is similarly immunogenic to its human counterpart, but occurs more commonly; therefore, the dog could provide a model to study novel immunotherapy strategies. NKs are powerful cytotoxic effectors with a great immunotherapeutic potential, but their effectiveness is limited by their poor persistence in vivo and by the lack of antigenic specificity. In human medicine it is well known that CD16 is the most important NKs' activator and the only means of NK cells interaction with anti-neoplastic antibodies, in order to trigger Antibody Dependent Cell-mediated Cytotoxicity (ADCC). However, the role of CD16 in dog NKs is unclear. The aims of this project were: 1) to confirm and quantify expression CD16 expression in dogs' White Blood Cells (WBCs), and 2) to clone canine CD16 to transfect a cell line, in order to make further in-depth studies possible. WBCs from healthy dogs were used for mRNA extraction and cDNA retrotranscription. qPCR was used for the quantification of CD16 expression. For cloning, primers were designed for the entire length of the gene and the PCR technique was used for the expansion of the product of interest. By means of restriction enzymes (EcoRI and XbaI), the gene was cut and cloned into the "pLVX-IRES-ZsGreen1" vector. After checking that the cloning had taken place, the cell line was transfected.

Research Grant: NIH K01OD017242, U of M ACCR Program, Skippy Frank Fund for Translational Medicine

Student Support: College of Veterinary Medicine, University of Minnesota

Variation in cytosine arabinoside protocols based on dosage and route of administration in canine patients

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Cytosine arabinoside (CA) is one of many chemotherapeutics used as an immunosuppressant or anti-neoplastic agent to treat a variety of diseases in veterinary medicine. It is particularly useful in the treatment of diseases of the central nervous system due to its ability to cross the blood brain barrier. However, given its side effects (myelosuppression, GI disturbance, hepatotoxicity), it is critical to monitor patients that receive CA. The goal of this study is to collect and compare data about CA treatment protocols in veterinary medicine. Delegates of the American College of Veterinary Internal Medicine were offered a 26-question survey via email about the administration, monitoring, and side effects in dogs being treated with CA. The University of Illinois Web Services Toolbox was used to collect and collate responses. Survey data were tabulated for each response and for selected cross-tabulations. Compiled survey data were compared statistically to similar data extracted from records of dogs treated with CA at the University of Illinois Veterinary Teaching Hospital. Results of this study will provide insight into best practices for use of CA in veterinary medicine.

Research Grant: None

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Effects of pharmacological inhibition of RIPK1 in *Mycobacterium tuberculosis*-infected mice

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Background. *Mycobacterium tuberculosis* (MTB) manipulates host cell death pathways in infected cells to promote bacterial survival and propagation. Necroptosis is a pro-inflammatory form of programmed cell death mediated by Receptor Interacting Protein Kinases (RIPK). RIPK1 is the critical protein determining cell fate downstream of necroptosis stimulation by TNF α . As necroptosis is a form of regulatable necrosis that may be exploited by MTB to favor pathogen survival and dissemination, necroptosis inhibitors represent a potential host-directed therapy for treating MTB-infection.

Methods. C3HeB/FeJ mice were infected with MTB and treated with a novel RIPK1 inhibitor (GSK'547) for 8 weeks beginning at week 3 post infection. Bacterial burden was assessed by plating homogenized lungs and spleens from infected mice. To determine the effects of necroptosis inhibition on systemic pro-inflammatory signaling we compared levels of TNF α , IFN γ , IFN β , IL-1 β , and IL-10 in serum and broncho-alveolar lavage fluid (BALF) between treated and untreated animals. Colocalization of necroptosis mediators with either vimentin or CD68+ was assessed using immunofluorescent antibodies against MLKL, Caspase 3 and 8 and IKK- β in lung lesions.

Results. A minimum inhibitory concentration assay confirmed that GSK'547 does not have direct antimycobacterial activity. Additional data are expected in the coming month.

Conclusion. While several studies have looked at the role of necroptosis in MTB-infected macrophages *in vitro*, our study is the first to examine the effects of RIPK1 inhibition *in vivo*. Inhibition of necroptosis using RIPK1- or related pathway inhibitors may have therapeutic potential in TB.

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Student Support: Boehringer Ingelheim Veterinary Scholars Program

Does induction of HIF-1 α in alveolar type II epithelial cells play a role in influenza pathogenesis?

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Influenza A virus (IAV) is a public health concern as it is highly contagious and may progress to potentially fatal acute respiratory distress syndrome (ARDS). Alveolar type II (ATII) epithelial cells synthesize pulmonary surfactant and regulate the depth of the alveolar lining fluid. In severe influenza, IAV replication within ATII cells has been shown to alter cell function and reduce mitochondrial respiration. We hypothesized that following IAV infection, ATII cells transition from oxidative phosphorylation to aerobic glycolysis (the Warburg effect). Further, we proposed that this metabolic shift occurs through hypoxia-inducible factor-1 α (HIF-1 α), which has been shown to upregulate glucose transporters and glycolytic enzymes. To test this hypothesis, we inoculated C57BL/6 mice with influenza A/WSN/33 (H1N1), inducing severe ARDS by day 6 post-inoculation. Immunohistochemistry was used to visualize HIF-1 α and glycolytic enzyme expression in ATII cells. In other mice, ATII cells were isolated via lung digestion and negative selection. Expression of genes encoding glycolytic proteins will be quantified by qRT-PCR using ATII cell RNA. Expression of glycolytic enzymes, HIF-1 α , and prolyl hydroxylases (which regulate HIF-1 α) will be measured by Western blot. Finally, the effects of treatment with HIF-1 α inducers and inhibitors on the severity of IAV-induced ARDS will be determined using pulse oximetry and lung function assays. We expect that IAV triggers HIF-1 α stabilization and increases glycolysis for cell survival. Findings may elucidate the role of the Warburg effect in influenza pathogenesis. By promoting apoptosis of IAV-infected cells, HIF-1 α inhibitors may constitute a novel treatment of IAV-induced ARDS.

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Student Support: R01 HL137090

Femoral remodeling in dogs with luxated dysplastic hips. A complicating factor for total hip replacement

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Hip dysplasia is one of the most common orthopedic diseases in dogs. If the condition is refractory to medical management, the preferred treatment for this condition can be total hip replacement (THR). In young dogs, chronic luxation of the dysplastic hip (luxoid conformation) is associated with remodeling changes within the femur. One characteristic of luxoid hip conformation is the lateral migration of the proximal medial femoral cortex. The resultant narrowing of the proximal femur encroaches on an appropriate envelop of femoral broaching. The goal of the study is to identify specific patterns of femoral remodeling in the luxoid situation. Radiographs of the last 200 cases of THR performed at the OSU Veterinary Hospital were grouped into luxoid and uncomplicated hip dysplasia. The age, weight, breed, sex of the patient was recorded. It was identified that 31% of dogs undergoing a THR had a luxoid hip. It was recognized that Golden Retrievers are 4 times more likely to have luxoid hips compared to German Shepherds (OR = 4.06, $P = 0.017$). German Shepherds are also less likely to have luxoid hips compared to the remaining breeds. CT Scans in record were categorized into luxoid and normal. These studies were used to create 3D models to study differences in cross sectional area (CSA) throughout the proximal femur in the region of stem insertion. To compare CSA in a normalized fashion, the femurs were calibrated by length. The goal is to prove that the CSA of the femoral canal of the luxoid hip is significantly different to that of a normal femur. Defining the pattern of femoral remodeling in the luxoid hip and publicizing this data should reduce the risk of surgical complication, in particular femoral fissure and fracture.

Research Grant: 29250 017000 29-J-Dyce Release time

Student Support: 29250 017000 29-J-Dyce Release time

Defining the impact of prenatal Zika Virus infection on neurodevelopment in a novel mouse model

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Zika virus (ZIKV) has been associated with serious human congenital defects including brain anomalies, pregnancy loss, and microcephaly, an otherwise rare birth defect. Animal models that accurately replicate human transmission are critically needed to understand how ZIKV impacts prenatal development but current mouse models lack contemporary ZIKV isolates or do not accurately recapitulate human infection. We injected the Puerto Rican ZIKV PRV-C6/36 strain of virus in the footpad of immunocompetent timed-pregnant C3H/HeJ mice at gestational day (GD) 6.5. Fetuses were harvested at GD 14.5 and trunks were tested for ZIKV by RT-qPCR, while heads were analyzed by gross morphometry. ZIKV was detected in the bodies and/or placentas of about 25% of fetuses, but no gross morphology differences were observed between treatment groups. These results indicate that ZIKV reaches the developing embryo but does not overtly affect fetal head growth by GD 14.5. This finding accords with our current understanding of human outcomes, where the vast majority of first trimester infections do not result in microcephaly. Therefore, detailed histological examination is being conducted to determine whether this novel mouse model of immunocompetent ZIKV transmission may predict subtle and more difficult to detect neurodevelopmental abnormalities and/or neurobehavioral outcomes following fetal ZIKV infection.

Research Grant: None

Student Support: NIH T35OD01107

Chemotherapeutic effects of the tyrosine kinase inhibitor, ponatinib, on canine mast cell tumours *in vitro*

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Ponatinib is a potent multi-target kinase inhibitor that has shown promise in the treatment of resistant human leukemias but has not yet been tested in dogs. It exerts antiangiogenic and antineoplastic activities by inhibiting the vascular endothelial growth factor and fibroblast growth factor receptors. This study evaluates the *in vitro* effects of varying concentrations of ponatinib on three canine mast cell tumour cell lines (CM-MC1, VI-MC1, and CoMS). Expression of the drug targets, fibroblast growth factor receptor-1, platelet-dependent growth factor receptor- α , and vascular endothelial growth factor receptor-2, were confirmed in the three mast cell tumor cell lines via Reverse Transcription-PCR and RNAscope. Cell viability was measured by the MTT assay. Ponatinib exhibited dose and time-dependent cytotoxicity, and the IC₅₀ at 24, 48, and 72 hours was determined for each cell line. Annexin V and Caspase Glo 3/7 assays were performed to evaluate the effect of Ponatinib on apoptosis. Annexin-V assay displayed substantial increase in apoptosis in each cell line treated with the corresponding IC₅₀. In contrast, caspase 3/7 assay showed no increase in apoptosis activity from 24 to 72 hours after treatment, suggesting that most apoptotic caspase activity occurs in the first 24 hours. Interestingly, western blot analysis showed increased expression of phosphorylated-ERK 1/2 pathway in the treated samples of VI-MC1 and CoMS. These results support the need for further study on the potential of ponatinib as an effective chemotherapeutic agent in the treatment of canine mast cell tumours.

Research Grant: None

Student Support: Florida Veterinary Scholars Program (Boehringer Ingelheim-NIH)

Comparing the pharmacokinetics and analgesic effect of a multi-dose methadone/fluconazole regimen

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Objective: To compare the pharmacokinetics and analgesia of a novel oral methadone formulation using two dosing regimens

Study Design: Prospective, randomized, blinded laboratory study

Animals: 12 pure bred beagles

Methods: The dogs were randomly assigned into two treatment groups: group 1 was administered 1 mg/kg of methadone and 5 mg/kg fluconazole orally at 6p (day 1), at 8a and 6p on Day 2 and 8a on day 3, while group 2 was dosed with 1 mg/kg of methadone and 5 mg/kg fluconazole orally at 8a followed by 0.5/2.5 mg/kg methadone/fluconazole at 12p, 6p and 8a the following day. A von Frey device (mechanical stimulus), a sedation score system ranging from none to heavy sedation, as well as rectal temperature and ocular temperature using a forward-looking infrared (FLIR) camera were used throughout the study.

Results: All dogs tolerated both dosing regimens with variable sedation from none to moderate. Opioid induced decreases in body temperatures (CNS mediated) were significant ($P < 0.05$) in Group 1 from 1-48 hours and from 2-36 hours in Group 2. Von Frey thresholds were quite variable, but significantly increased from 1-4 hours in Group 1 and in 10/13 assessments through 32 hours in Group 2. The difference in rectal temperature and ocular temperature was highly variable.

Conclusions and Clinical Relevance: The novel methadone formulation provided significant opioid effects after both dose regimens in dogs. This may be the first oral opioid formulation providing analgesic effects in dogs at relevant dosages.

Research Grant: Mark Derrick Canine Research Fund

Student Support: Boehringer Ingelheim Animal Health and Benjamin and Catherine Kurz Research Scholarship

Comparison of the host directed and antimicrobial effects of novel metal-based wound dressings

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Wounds are among the most common reasons pet owners seek veterinary attention and frequently these wounds become infected. Unfortunately, as in human medicine, there is increasing prevalence of antimicrobial resistance among bacteria isolated from these wounds. Thus, there is great clinical need to identify novel wound dressings that both enhance wound healing and treat/prevent wound infections including infection from antimicrobial resistant bacteria. The aim of this study was to examine several novel, metal-based wound dressings that are thought to exert both antimicrobial and host directed effects resulting in theoretical concurrent reduction of bacteria in cutaneous wounds and augmenting the inherent healing properties of the patient. We hypothesized that topical gallium maltolate (GaM) would significantly enhance healing (as quantified by change in wound size) and reduce the amount of bacteria (CFU/gram of tissue) as compared to two clinical controls in a porcine model of infected wounds. The outcome of this study will contribute to identifying novel antimicrobial wound dressings that would aid in reduction of the burden of chronic, infected wounds in people and animals. Heavy metal infused ointments, namely Gallium, consistently outperformed foams in contribution to wound healing and reduction of bacterial load. Cytokines were measured in skin samples from each pig, and histology sections were analyzed for each wound. This study suggests that gallium may enhance wound healing through host directed effects and simultaneously exerting antimicrobial effects.

Research Grant: Unknown

Student Support: NIH T35 Training Grant 5T35OD010991-14

PNAG hyperimmune plasma is superior to commercial plasmas for opsonizing and killing *Rhodococcus equi* in vitro

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Pneumonia in foals caused by the bacterium *Rhodococcus equi* has extensive clinical and economic consequences. A vaccine for *R. equi* is not available, and commercial *R. equi* hyperimmune plasma (REHIP) is only partially protective. These factors contribute to a great need for improved preventatives of this disease. We hypothesized that plasma hyperimmune to the capsular polysaccharide poly-*N*-acetyl glucosamine (PNAG) would be significantly more effective than REHIP at mediating complement deposition and opsonophagocytic killing (OPK) of *R. equi*. Plasma sources evaluated include 1) PNAG hyperimmune plasma (PHIP), 2) REHIP, 3) standard non-hyperimmune commercial plasma (SP), or 4) no plasma (media only). The capacity of each plasma source to deposit complement component 1q (C1q) onto PNAG- or *R. equi*-coated plates was determined by ELISA. Each plasma source was combined with *R. equi* (MOI of 1), equine complement, and neutrophils (1x10⁶/per well) isolated from horses (n = 9) to compare OPK. After 4 hours, the number of *R. equi* in each well was determined by quantitative culture. Results were analyzed using linear mixed-effects regression with significance set at $P < 0.05$. Neither SP nor REHIP deposited complement onto PNAG, whereas PHIP deposited significantly ($P < 0.05$) more C1q onto PNAG. Results of complement deposition onto *R. equi* are pending. The proportional survival of *R. equi* in the presence of SP (40%) was significantly ($P < 0.05$) greater than that for REHIP (32%), which was significantly greater than that for PHIP (18%). These data indicate that PHIP was functionally superior to the other plasma sources *in vitro*, and that *in vivo* evaluation of PNAG hyperimmune plasma is merited.

Research Grant: Link Equine Research Endowment and ALOPEXX Enterprises LLC; Hyperimmune and non-hyperimmune plasmas were provided by Mg Biologics, Inc

Student Support: Boehringer Ingelheim VSP, Texas A&M College of Veterinary Medicine & Biomedical Sciences

Antimicrobial-loaded poloxamer 407 hydrogel for the treatment of osteomyelitis

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The most common etiologic agent of osteomyelitis is *Staphylococcus aureus* (*S. aureus*), which often causes chronic biofilm formation in or around bone. Failure to resolve osteomyelitis may be due to an inability to reach effective antimicrobial concentration at the infection site or the resistance of pathogens to antimicrobials. Thus, the aim of this study was to evaluate vancomycin-, fosfomycin-, and bacteriophage-loaded hydrogels for localized treatment against *S. aureus*. Poloxamer 407 (P407) hydrogels loaded with 128 µg of fosfomycin or vancomycin were added to transwells, submerged in PBS, and incubated for 48 hours at 37°C. *S. aureus* (ATCC 6538-GFP) was treated with the eluents for growth curve analysis to estimate concentration and confirm bioactivity of antibiotics (in triplicate). Additionally, biofilms were treated with fosfomycin (0-128 µg/ml) in P407 or PBS (n=1-2). An in vivo pilot study establishing infection in the femur diaphysis of rats was used to compare the efficacy of fosfomycin and bacteriophage (n=1-2) in PBS or P407 for the treatment of chronic osteomyelitis. Approximately 8-16 µg of the loaded vancomycin eluted from P407 over 48 hours, while the fosfomycin concentration could not be determined. Vancomycin bioactivity was maintained, while fosfomycin efficacy and bioactivity was unclear. Treatment of biofilms with fosfomycin in P407 and PBS showed a decrease in colony counts as antibiotic concentration increased. The pilot in vivo results showed a transient reduction of bacteria in rats treated with bacteriophage, with no observable differences between delivery vehicles. Overall, the findings suggest P407 may serve as an effective local delivery vehicle for the treatment of chronic osteomyelitis.

Research Grant: Mississippi State University Start-Up Funds, USDA-ARS Biophotonics Initiative #58-6402-3-018

Student Support: NIH T35 Training Grant T35OD010432

Ticks & zoos: a subtle inquiry into their conquest

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Zoos fulfill an important role, educating the public about wildlife species and promoting wildlife conservation efforts. But they also present unique challenges, by bringing so many species into close proximity and putting them in contact with parasites often not encountered in their native habitat. One of the most important parasites in Michigan, the blacklegged tick (*Ixodes scapularis*), can carry a wide array of pathogens, including *Borrelia burgdorferi*, the causative agent of Lyme disease. In this project, we hope to better understand the spatial distribution of tick populations in and around one zoo in a Lyme-endemic county in Michigan. Host-seeking ticks were collected from different areas of the zoo likely to harbor *Ixodes* spp. Collection methods included dragging, flagging and heel flags based on terrain. Ticks were assayed by PCR for primary pathogens of concern, *B. burgdorferi*, *Borrelia miyamotoi*, and *Anaplasma phagocytophilum*. A total of 34 ticks were collected including all life stages of *I. scapularis* over the 19,849 meters sampled. Overall density along all transects combined was 0.0017 ticks per m², 10 times lower than 2 other public parks sampled in the same county during the same period. Ticks were ubiquitous throughout the park, but found in higher densities along staff transects (ST). Pathogen results are forthcoming. All life stages of blacklegged ticks were found within the perimeter fence, suggesting an established population within the zoo, likely sustained by a small wild deer population. With knowledge of spatial distribution of ticks and the pathogen prevalence, measures can be taken to ensure the safety of staff, visitors and exhibit animals.

Research Grant: Michigan State University College of Veterinary Medicine and Midwest Center of Excellence for Vector-Borne Disease

Student Support: NIH Grant T35OD016477 to Michigan State University

Detection of *Hepatozoon americanum* in Gulf Coast ticks from Oktibbeha County, MS

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American Canine Hepatozoonosis (ACH) is a protozoal disease caused by *Hepatozoon americanum*. This organism is transmitted to canines when they ingest the definitive host, the Gulf Coast tick (*Amblyomma maculatum*; GCT) or a paratenic host (rodents and rabbits). There is currently no treatment for eliminating protozoa in the infected canine. The main source of infection, tick or paratenic host, has not yet been identified for ACH making it difficult to develop preventative measures. Data on disease prevalence and distribution are reliant on detection in the diseased canine. Geographical distribution of ACH is assumed to overlap GCT distribution. Currently, there is no data detailing *H. americanum* prevalence in the tick vector. This study aimed to fill the gap in knowledge by investigating GCT infection prevalence. We collected 129 adult GCTs from 3 different sites in Oktibbeha Co., MS and extracted DNA from half or whole ticks. We used a TaqMan quantitative PCR assay to test for *H. americanum*. No half tick extracts were positive using conservative threshold levels; two whole tick extracts had low copy numbers but could not be confirmed. Thus, prevalence of *H. americanum* in these GCTs was 0% which supports the null hypothesis. To evaluate potential false negative extracts, ticks with amplifiable DNA were tested with conventional PCR. Thus far, results suggest GCTs in this area may not be a source of *H. americanum* infection. Future studies targeting areas with ACH cases and investigating infection rates of nymphal GCTs and various paratenic hosts will offer more insight on the main transmission route for ACH here. This information could be crucial for the development of improved methods for prevention.

Research Grant: American Kennel Club Canine Health Foundation

Student Support: NIH 5T35OD010432-17

Characterizing developmental lung disease related to neonatal mortality in the Norwich Terrier

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Although neonatal respiratory distress syndrome is attributed to over 60% of early life deaths in puppies, information regarding the specific mechanisms of disease remains sparse. This study focused on the most severe form of respiratory disease, that which arises due to abnormal development of the lung tissue. Previous work by this group had identified pathological similarities that suggested developmental lung disease (DLD) to be a common, yet unrecognized, cause of death during neonatal life - specifically in the Norwich Terrier (NT) breed. We hypothesized that DLD in NT puppies is a reflection of abnormal alveolar and vascular development, and is an important factor in these neonatal deaths. Working with the Norwich Terrier Club of America we collected lung tissues from NT puppies that did not survive past the first few weeks of life and performed histology, immunohistochemistry, and three-dimensional reconstruction of the lung vasculature. All submitted puppies were confirmed to have DLD and our diagnostic techniques revealed the underlying morphological abnormalities associated with this condition. A greater understanding of this disease is the first step in increased prevention and developing possible treatments. This information will be applicable to both other dog breeds as well as in relation to similar conditions observed in human medicine.

Research Grant: American Kennel Club, Canine Health Foundation

Student Support: NIH Grant T35OD016477 to Michigan State University

Whole genome sequencing of colony variants of *Burkholderia pseudomallei* for use in a chronic infection model

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Burkholderia pseudomallei is a recognized biothreat agent and the causative agent of melioidosis, an infectious disease endemic in tropical areas such as northern Australia and Thailand. Melioidosis can present as either an acute or chronic infection, and there is no preventative vaccine. Our lab is interested in studying the changes in immune response during a chronic infection in a murine model. However, during pilot acute and chronic infection studies with *Burkholderia pseudomallei* strain 1026b, we observed less robust disease than others have reported. Upon plating spleen homogenates from acutely infected mice, only two of the twelve animals harbored viable bacteria. Further, we observed a threefold reduction in bacterial titer on Ashdown's media, a selective media for *Burkholderia*, as compared to growth on Luria-Bertani media. In addition, colonies growing on the LB media displayed two distinct colony morphologies. From these observations, it was decided to use a replica plating technique to isolate colonies that lack the ability to grow on Ashdown's media and perform whole genome sequencing to compare the two variants. It has been previously documented that *Burkholderia pseudomallei* has a particular affinity for mutation and horizontal gene transfer. We hypothesize that these genetic changes could relate to the lack of virulence in our original *in vivo* model. Thus, any differences identified between the two populations could potentially result in identification of novel *in vivo* determinants of virulence.

Research Grant: University of Missouri Veterinary Pathobiology Start Up Fund

Student Support: Stipend for Leah Fray is supported by an endowment established by IDEXX-BioResearch

Investigating canine cancer-testis antigens to further cancer vaccine development

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Tumor-specific antigens (TSAs) are the protein fingerprints of malignant cells that allow immune defenses, in particular, T-cells, to target them for destruction. Unfortunately, in many cases, cancers eventually overwhelm T-cell defenses, leading to short survivals—just 6-14 months for dogs with hematologic cancers. If TSAs can be discovered, however, they can be used in immunotherapies, such as vaccines, to boost T-cell defenses and dramatically improve outcomes. The best TSAs are expressed highly in tumors, across individuals, but not in normal tissues, so that TSA-boosted T-cells do not inadvertently harm the patient. The largest category of TSAs fulfilling these criteria in humans are cancer-testis antigens (CTAs), expressed in gametogenic and fetal tissues, but silenced in adult tissues. Only a few CTAs have been identified in canine oncology, and we are evaluating additional candidates to determine their immunotherapy potential. Based on human data, we hypothesized that one established CTA, SYCP1, a protein involved in meiosis, would be expressed in canine hematologic cancers but not somatic tissues. To test this supposition, we evaluated SYCP1 mRNA levels in archived tumors and normal tissues by RT-PCR. Encouragingly, SYCP1 was present in 6/11 histiocytic sarcomas and 3/11 lymphomas. Almost all normal canine tissues were negative, but a slight signal was observed in two brain samples. The amplicon was confirmed to be SYCP1 by sequencing. Brain expression of SYCP1 would limit its usefulness in most immunotherapy approaches for canine lymphoma and HS; however, protein-level testing will be needed to uphold or refute these preliminary findings.

Research Grant: Charley & Hannah Collins Coat of Excellence Funds

Student Support: NC State CVM Boehringer Ingelheim Veterinary Scholars Program

Testing VSV recombinant vaccines expressing the F, G, M, and N proteins of HRSV in the cotton rat model

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Human Respiratory syncytial virus (HRSV) causes severe lower respiratory disease and even mortality in pediatric, geriatric and immunocompromised individuals. HRSV infection does not provide life-long immunity, and there is no licensed vaccine or antiviral therapeutic drugs available. Recent efforts in HRSV vaccine development are centered primarily on the fusion protein (F glycoprotein) of the virus. However, the focus on one protein may be suboptimal as a vaccine. This study utilized four HRSV proteins: the fusion protein (F) and the attachment protein (G glycoprotein) which are mainly antibody targets, in addition to the matrix protein (M2-1) and nucleocapsid protein (N) which are T-cell targets. The purpose was to see if immunizing with a combination of proteins provided better protection against HRSV than immunizing with the F protein alone. Vesicular stomatitis virus (VSV) was used as the vector system due to recent success with the recombinant VSV (rVSV) Ebola vaccine, and cotton rats were used as the animal model to better simulate infection in humans. Protection was seen after immunization with all rVSV candidates. The rVSV-M2-1 and rVSV-N showed reduced viral titer of 0.5-0.75 log, rVSV-F and rVSV-G showed reduced titer of about 2 log, and rVSV-G-F-M-N and rVSV-G-2A-F showed reduced titer over 2 log. All VSV recombinants induced T-cell and antibody responses, with the protection level correlating with the degree of the neutralizing antibody response. In conclusion, the rVSV-G2A-F yield higher protective capacity than using the F and G glycoproteins individually. While the rVSV-G-2A-F combination vaccine had better protection, the rVSV-G-F-M-N vaccine did not provide an added advantage in the cotton rat model.

Research Grant: None

Student Support: Boehringer Ingelheim

Susceptibility of bird and mosquito cells to African Usutu virus strains

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Usutu virus (USUV), a mosquito-borne flavivirus maintained in an avian host, is a member of the Japanese encephalitis virus (JEV) antigenic complex and closely related to West Nile Virus. While USUV is not currently in the United States like West Nile, in 1996 USUV spread from Africa to Europe and if it follows the same pattern as West Nile, could eventually come to the US. The purpose of this study is to evaluate the susceptibility of various birds and mosquitos to USUV. In order to evaluate this, poultry cell lines (chicken and duck) and mosquito cell lines (*Aedes aegypti*) were inoculated with three different African strains of USUV that were originally isolated in 1959, 1962, and 2010. For both poultry cell lines, the different African USUV strains followed a similar pattern of growth. The results showed that the viral titer in the chicken cell line peaked on day 3 at 7.5-7.8 log₁₀ PFU/mL, followed by a rapid decline. The titer in the duck cell line peaked on day 2 at 7.6-7.8 log₁₀ PFU/mL and was followed by a gradual decline. The USUV strains peaked in the *Aedes aegypti* cell line at 7.3-8.3 log₁₀ PFU/mL on day 5. These findings indicate that several poultry and mosquito cell lines are susceptible to African USUV strains. Future studies will test the susceptibility of local birds and mosquitoes to USUV infection.

Research Grant: None

Student Support: Boehringer Ingelheim Veterinary Scholar

Identifying the bioactive component of platelet-rich plasma lysate for the treatment of equine osteoarthritis

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Osteoarthritis (OA) is the most common cause of equine lameness and has a significant economic impact on the equine industry. Platelet-rich plasma (PRP) is increasingly being used to treat OA in humans and veterinary species. PRP lysate (PRP-L) preparations pooled from multiple donors capitalizes on individual variability and has greater benefit due to the acellular nature and higher concentration of bioactive factors. Our preliminary data revealed that PRP-L increased synovocyte proliferation and hyaluronic acid production and decreased chondrocyte toxicity in an *in vitro* synovocyte inflammatory model. Due to the allogenic nature of pooled PRP-L, we propose to fractionate PRP-L into its most bioactive component, allowing for removal of most of the immunogenic components. In a parallel study, we found that small, amphiphilic peptides within the < 10kDa, cationic fraction (BIO-PLY, patent pending) possessed antimicrobial and immunomodulatory activity. BIO-PLY is rich in host defense peptides, which have been found in wounds to reduce inflammation, promote angiogenesis, and increase collagen synthesis, thereby enhancing wound regeneration. Thus, we hypothesize that BIO-PLY will possess anti-inflammatory and chondroprotective properties in an *ex vivo* OA model. Given that degenerative joint disease is a common sequela to infectious arthritis, affecting > 50% of horses that survive the initial infection, this proposed multimodal function of BIO-PLY will enhance its therapeutic use. If BIO-PLY improves the regenerative capacity of both septic and non-septic osteoarthritic joints, lameness-related morbidity and mortality in equine patients may be significantly decreased, with opportunity for translation into other species.

Research Grant: Grayson Jockey Club Research Foundation; American Quarter Horse Association

Student Support: Boehringer Ingelheim Veterinary Scholars Research Program

Meloxicam sustained release injection site reactions in male and female mice

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The Mouse Biology Program at the University of California, Davis observed the development of lesions in rats and mice following treatment with meloxicam sustained release (MSR), some of which necessitated premature euthanasia. The lesions observed included skin reactions, granulomas, and open draining tracts. Standard meloxicam is a commonly used NSAID in research rodents that is effective for up to 12 hours of analgesia per dose, but MSR is an alternative that provides up to 72 hours of analgesia, which can reduce handling while providing analgesia of longer durations. A prospective study in Sprague Dawley rats resulted in 100% of animals developing lesions after treatment with MSR, and our study was designed as a follow up to evaluate injection site reactions in three commonly used research strains of mice; ICR(CD1), C57BL/6J, and Balb/cJ. Mice were injected with a single dose of MSR, or with a control dose of saline or standard meloxicam, and then observed for 7 or 14 day time courses. Each day following injection, animals were physically examined and lesions were scored for erythema and mass characteristics, and measured with digital calipers when possible. Animals were euthanized, necropsied, and samples were taken for histology. Data will be statistically analyzed for effect of treatment and differences in sex and strain, and histology from lesions will be interpreted by a pathologist blinded to treatment group. Preliminary evaluation of our results show trends of MSR treated mice of all three strains developing lesions with greater severity and duration than saline or meloxicam, and that the ICR(CD1) strain have more severe injection site reactions than the Balb/cJ and C57BL/6J strains.

Research Grant: NIH funded Mutant Mouse Resource and Research Center at UC Davis (3-U42 OD012210)

Student Support: Boehringer-Ingelheim Veterinary Scholars Program, through the UC Davis STAR program

Decorin modulation of corneal wound healing through autophagy

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Autophagy regulates cellular homeostasis and pathology. However, the role of autophagy in corneal wound healing is still unknown. Decorin, a key extracellular matrix protein, has been found to modulate autophagic signaling pathways in non-ocular tissues. We have previously shown that decorin modulates TGF- β /VEGF-induced cellular proliferation and differentiation in corneal wound healing. Additionally, we detected RNA expression of autophagic genes (LC3, Beclin1, Atg-5, Dram-1, Sqstm-1, and Prkn-2) in human corneal epithelial, stromal, and endothelial cells. This study sought to test the hypothesis that decorin regulates LC3 and Beclin1 gene function in response to TGF- β and VEGF during corneal wound healing. To test our hypothesis, human stromal fibroblast (HSF) cells were grown in 6-well plates to 60% confluence. Each well contained a control or treatment group with varied combinations of decorin (250 nM), VEGF (100 ng/mL), and TGF- β (5 ng/mL). Phase contrast microscopy of cells collected at 24, 48, and 72 hours after treatment shows morphological changes between control and treatment groups. The LC3 and Beclin1 immunocytochemistry and fluorescence microscopy are underway. In addition, cDNA preparation from RNA extraction has been completed, and qPCR analyses studying the role of LC3 and Beclin1 in corneal wound healing are pending.

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Student Support: University of Missouri College of Veterinary Medicine Office of Research

Talin expression markedly increases in myometrium during pregnancy with distinct detection in focal adhesions

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During pregnancy the uterus grows quickly through cell division in the proliferative phase and by hypertrophy in the synthetic phase, to become a powerful forceful tissue in the contractile phase prior to labor. In order for uterine muscle cells to transform into contractile cells, clusters of integrins and associated proteins called focal adhesions must form between cells and the extracellular matrix surrounding them. Talin plays a role in focal adhesion assembly by binding to integrins and changing their affinity to the extracellular matrix to modulate cell signaling. The objectives of the study were 1) to determine the spatiotemporal expression and distribution of talin using immunofluorescence and immunoblot analysis and 2) to assess the organization of talin-containing focal adhesions in myometrial cells throughout pregnancy. Both immunofluorescence and immunoblot analyses showed that talin expression increased dramatically in late pregnancy and labour. Talin expression was markedly increased at day (D) 22, 23 and post-partum compared to D12, D15 and D19. Immunofluorescence also indicated that talin co-localized with another integrin activator named kindlin-2, and that talin-containing focal adhesions became more prominent during late pregnancy. We conclude that talin is important in promoting phenotypic changes of uterine muscle cells by aiding assembling of focal adhesions to prepare the uterus for labor. By studying proteins involved in myometrial contractile activation such as talin, we increased our basic knowledge of myometrial biology during pregnancy and such an approach could identify therapeutic targets that could be used to regulate labour initiation in humans and domestic species.

Research Grant: Natural Sciences and Engineering Research Council of Canada

Student Support: Meril Veterinary Research Scholars Program

Evaluation of recombinant attenuated *Salmonella* vaccine (RASV) vectors for induction of immune response

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Recombinant *Salmonella Typhimurium* strains have been developed to exhibit the same or better attributes than their wild-type parental strains in their ability to colonize internal lymphoid tissues and deliver heterologous antigens, to induce both mucosal and systemic immune responses. Additionally, *Salmonella* has exceptional adjuvant qualities and can even induce mucosal and systemic immune response. In this study we evaluate different recombinant attenuated *Salmonella* vaccine (RASV) constructs with differing genotypes to activate production of NF κ B in HEK cells expressing individual TLR and Nod receptors (NOD1, NOD2, TLR4, and TLR5). The ultimate objective is to identify a genotype to exhibit maximal adjuvant activity. Live RASV was incubated at a multiplicity of infection (MOI) of 10 with the NF- κ B/SEAP reporter HEK293-Blue (HEK-Blue) cell lines expressing either TLR4/MD-2/CD14, TLR5, NOD-1 (HEK-Blue-NOD-1) or NOD-2 (HEK-BlueNOD2). All cell lines contained an NF κ B-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. Pattern recognition receptor (PRR) signaling leads to the expression of SEAP, which was detected by HEK-Blue Detection kit. HEK-BlueNOD2 showed strong NF κ B activity in response to χ 12341 strain. Our findings demonstrate that χ 12341 has maximum adjuvant activity and can be used as an adjuvant to induce the host immunity.

Research Grant: University of Florida Foundation - F022583

Student Support: Merial Veterinary Research Scholars Program

Evaluation of a commercial probiotic marketed for use in captive hermit crabs (*Coenobita* spp.)

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Hermit crabs (*Coenobita* spp.) are popular in the pet trade, being wild-caught and sold wholesale by commercial distributors. The product labels of some probiotics marketed for use in captive hermit crabs do not disclose the cultures present nor the proposed mechanism of action of those cultures for the health of the animals. The goals of this study were to identify live cultures present in a commercial probiotic and investigate their interaction in vitro with select cultures of commensal flora of captive hermit crabs. 3 samples from each of 2 lots of a probiotic (All Living Things Hermit Crab Probiotic Spray, Petsmart, Philadelphia, PA) were cultured on MacConkey agar, blood agar, and Columbia-CNA agar under aerobic and anaerobic conditions at ambient temperature and 37° C. Growth was observed on blood agar and Columbia-CNA at both temperatures under aerobic conditions. *Bacillus pumilus* and *B. licheniformis* were identified via MALDI-TOF analysis of four morphologically distinct colonies. Sterile swabs were collected from 6 captive hermit crabs each from a different private collection and plated on blood agar and CNA at room temperature and 37° C. From the resulting colonies, select bacterial cultures will then be speciated using MALDI-TOF analysis and plated uniformly on duplicate blood agar along with *B. pumilus* and *B. licheniformis*, respectively, and the subsequent cultures will be characterized. This preliminary investigation will demonstrate the characteristics a commercial probiotic culture and its in vitro effects on select commensal flora of captive hermit crabs.

Research Grant: NIH grant T35OD010919

Student Support: Boehringer Ingelheim

Guiding antimicrobial therapy: prevalence of bacteremia in dairy calves with diarrhea

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Calfhood diarrhea is the most common cause of mortality in dairy calves. Septicemia is an important sequela of diarrhea, and the primary justification for high rates of antimicrobial use for calf diarrhea. The prevalence of bacteremia in diarrheic calves has been estimated to be 30%; however, this estimate was not based on data from a commercial dairy farms, and may not reflect the true prevalence in this population. Farm workers make routine decisions on the initiation of antimicrobial therapy based on observable clinical signs. Therefore, there's a need to define the set of observable signs most associated with bacteremia. We hypothesized that the prevalence of bacteremia is less than 30% in calves with diarrhea, and that observable clinical signs are accurate predictors of bacteremia. Calves were enrolled across three commercial dairy farms, 1-21 days old, presented with watery diarrhea, dehydration or depression, and were not previously treated with antibiotics. Health assessments were performed and aseptic blood samples were collected to immediately inoculate blood culture bottles (BCBs). After incubation, all positive BCBs were cultured to determine bacterial species present. Preliminary results reveal that out of 99 diarrheic dairy calves enrolled, the prevalence of bacteremia was 14.14%. This indicates there may be an opportunity to decrease antibiotic treatment of calves with diarrhea that are not septicemic. Analyses of clinical predictors of bacteremia are ongoing. Having a practical, straightforward protocol for farm workers to help determine when antimicrobial use is permitted is vital to calves suffering from diarrhea, antibiotic stewardship, and the future efficacy of antimicrobials in the herd.

Research Grant: USDA-Agriculture and Food Research Initiative

Student Support: NIH T35 Training Grant OD010977

Relationship between endoscopic and histopathologic findings of the gastric glandular mucosa in horses

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Equine glandular gastric disease is a common disorder of horses which is diagnosed by gastroscopy. However, little is known about the relationship between endoscopic appearance and histopathology. We hypothesized that mild lesions (endoscopic grade 1, or hyperemia) were associated with lymphoplasmacytic gastritis, while endoscopic grade ≥ 2 lesions (ulcers or fibrinosuppurative lesions) were associated with mucosal disruption and neutrophilic infiltration. For this study, 17 horses presenting for elective euthanasia underwent gastric endoscopy. Each horse was scored using three established endoscopic scoring systems. Following euthanasia, samples were collected from the dorsal and ventral pylorus, and from any visible glandular lesions. All samples were formalin fixed and stained with hematoxylin and eosin. Glandular inflammation was characterized using an established grading system. Relationships between endoscopic severity and inflammation of the glandular mucosa were evaluated with a Spearman's correlation coefficient. Fourteen horses had EGGD. Fifteen horses had evidence of lymphoplasmacytic inflammation. There was no association between lesion severity and inflammation severity ($P > 0.1$). There was a strong association between hyperemia and lymphoplasmacytic inflammation ($r=0.67$, $P=0.01$). There was no association between presence of endoscopic ulceration ($r=0.17$, $P=0.4$) or fibrinosuppurative appearance ($r=0.32$, $P=0.6$) and presence of neutrophils. These findings suggest that hyperemia may predict lymphoplasmacytic inflammation. However, more severe endoscopic lesions (i.e., ulcers) are not strongly associated with any specific histopathologic findings.

Research Grant: None

Student Support: Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University

Essentiality and functional analysis of Calcium Dependent Protein Kinase 1 (CDPK1) in *Cryptosporidium parvum*

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Cryptosporidium parvum, a protozoan parasite, is a leading cause of diarrheal disease in young children and agricultural animals. There is no effective drug to treat cryptosporidiosis, which makes *C. parvum* highly relevant to veterinary medicine and research. The development of novel therapeutics against *C. parvum* has been challenging since there is limited understanding of the parasite biology. With new molecular genetic tools now available for *C. parvum*, it is possible to dissect gene function. One of the leading drug targets in *C. parvum* is the Calcium Dependent Protein Kinase-1 (CDPK1). The ortholog of CpCDPK1 in other protozoan parasites is essential for parasite growth. However, there is no information available regarding the essentiality of CDPK1 for *C. parvum*. To determine essentiality of CDPK1, we used the genome-editing CRISPR/Cas9 tool and immunocompromised mouse model system to create a transgenic CDPK1 knockout strain. To create this strain, we attempted to delete the *cdpk1* gene and replace it with a sensitive luciferase reporter fused to a drug-resistance marker. Fecal luminescence measurements were performed to monitor the emergence of drug-resistant parasites. When compared to our positive control, there was no rise in luciferase readings for the CDPK1 knockout strain, indicating that the CDPK1 was essential for *C. parvum*. We are repeating our experiments to confirm our results. We also created another transgenic strain to append an epitope tag to the *cdpk1* gene. Efforts are underway to purify these tagged transgenic oocysts and determine the localization of CDPK1 in the parasite. Discovery and validation of new drug targets will aid in development of effective treatments against cryptosporidiosis.

Research Grant: Research Grant: Bill and Melinda Gates Foundation, Grand Challenges Exploration award number OPP1171934 & University of Illinois College of Veterinary Medicine

Student Support: Student Support: Office of the Director, NIH, T35 OD011145

Collaboration between veterinarians and honey bee researchers through exploration of the bee gut microbiome

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The ongoing challenges to the honey bee population has prompted research efforts to determine the cause and a method of mitigation. Recent regulation changes placing honey bees under the jurisdiction of veterinarians allows for a medical lens that honey bee research and management has previously lacked. With this, it is essential to collaborate between these fields and develop tools that can act as health markers for effective management of honey bees. A major concern facing honey bees relates to depletion of nutritional resources and habitat loss. Ongoing studies confirm increases in longevity and pathogen tolerance when bees receive dietary supplementation from phytochemicals found in certain floral nectars, which include caffeine, gallic acid, p-coumaric acid, and kaempferol. The aim of the present study is to determine the effect of these phytochemicals on the bee gut microbiome. Human and veterinary medicine research provides significant evidence for the impact of diet on the gut microbiome in healthy and diseased states. Similar research on the honey bee gut microbiome is now gaining momentum. 8d-old worker bees (n=550), collected from two foster colonies were allowed to feed on a solution of 20% sucrose + 25ppm of one of the phytochemicals, for 3 and 6 days. Entire GI tracts were excised, genetic material extracted and sequenced using Next-Gen sequencing. Analyses of the sequences will allow us to understand the impact of these floral chemicals on the composition and diversity of the gut microbiota, and how this relates to the known benefits. Our findings will be significant as they provide preliminary evidence of tools that can alter the honey bee gut microbiome to promote bee health and sustainability.

Research Grant: Project Apism Healthy Hives 2020, Foundation for Food and Agriculture Research Pollinator Health Fund

Student Support: USDA Animal Health & Disease Program NI17AHDRXXXXG018

Prevalence of *Leptospira* spp. in mongoose (*Herpestes auropunctatus*) in Grenada

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Leptospirosis is a bacterial infection of public health significance in Grenada. The mongoose (*Herpestes auropunctatus*) is considered a potential reservoir population on Grenada. In 1976, seroprevalence levels in mongoose were around ~35%; however, prevalence has not been published since. The aim of this study is to collect current prevalence levels by PCR among the mongoose populations in Grenada which is expected to be at a similar level to what was seen in 1976 (~35%). 73 mongoose samples were previously collected from several parishes of Grenada. DNA was extracted from liver, spleen, and mesenteric lymph node tissues. The extracted DNA was tested via PCR and gel electrophoresis using *rpoB* primers used in previous studies. Positive bands were to be sequenced at MCLAB (San Francisco, CA, USA) with Sanger sequencing based upon the *rpoB* primers. Of the 73 samples tested, gel electrophoresis detected no positive bands. The findings suggest the need to collect new kidney samples from mongoose as none were available at the time of the study. Future studies should repeat the same protocols using fresh samples to determine if the *rpoB* primers used in these protocols can detect *Leptospira* infection in mongoose and more adequately assess whether Grenadian mongoose are indeed *Leptospira*-positive.

Research Grant: None

Student Support: Research Grant: BI Summer Scholars Research Award

Effects of Early Life Stress and Biological Sex on Histamine N-Methyltransferase

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Background: Biological sex and early life stress are major risk factors influencing the prevalence of functional gastrointestinal disorders and immune-mediated diseases. Research has shown mast cells, located in the neuroimmune and peripheral immune systems, play a critical role in these disorders. The relationship between biological sex, stress, and mast cell activity has many variables. One that has yet to be addressed is the role of histamine catabolism. Histamine N-methyltransferase (HMT) is highly expressed in gastrointestinal epithelium and is the only histamine catabolizing enzyme of the central nervous system. We hypothesize that sex specific differences exist in the expression of HMT in the brain and gastrointestinal mucosa and that early life stressed animals exhibit reduced basal expression of HMT which may contribute to the pathophysiology observed in stressed females.

Methods: We will use western blots and ELISAs to quantify the expression of HMT in non-peyers patch ileal mucosa of male and female pigs, as well as in the colonic mucosa and hippocampus of male and female mice exposed to early life stress. We will utilize immunofluorescence to determine the localization and intensity of staining of HMT in the intestinal mucosa. We will use t-tests and 1 or 2-way ANOVAs where appropriate.

Results: We expect that males will have higher expression of HMT compared with females of the same group. Further, we expect that early life stressed animals will exhibit diminished expression of HMT compared with non-stressed controls.

Conclusion: Understanding the relationship between stress, biological sex, and histamine metabolism may lead to new approaches and individualized therapy for inflammatory diseases.

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Student Support: NIH Grant T35OD016477 to Michigan State University

Characterization of the canine aural microbiome

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Ear infections are consistently ranked as a top health concern for dogs in veterinary clinics and shelters. Prior studies have suggested that standard diagnostic methods for directing treatment of ear infections (cytological staining, culture and sensitivity testing, or isolation of specific pathogens) are often inaccurate, and therefore clinically misleading. Calls for a more reliable method of characterization are widespread. Consequently, an understanding of the canine aural microbiome and its role in ear health and disease has potential to greatly improve canine welfare. To this end, the present study seeks to compare established methods of DNA preparation in regard to their utility for sequencing the canine aural microbiome and to use the results of these experiments to characterize these populations. We hypothesized that different extraction methods would differ in ability to adequately prepare such samples. We swabbed clinically normal and diseased ears of research dogs at the University of Missouri and local shelter dogs using common clinical methods, extracted DNA utilizing two different established methods, and used targeted 16s rDNA next generation sequencing to characterize bacterial species present. We found that manual nucleic acid precipitation followed by Qiagen DNeasy kit (n=56) more often provided quantifiable amounts of extracted DNA compared to sole use of the Qiagen PowerFecal kit (n=14), (42.9% vs. 21.4% of samples, respectively). These results establish an appropriate technique to employ in aural microbiome analysis. We also predict that sequencing results will illustrate differences in the aural microbiome on an individual and environmental basis and in relation to health and disease status.

Research Grant: University of Missouri Metagenomics Center

Student Support: PetSmart Charities

Development of a mouse model to study the impact of radiation-induced pain on metastatic propensity of cancer

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For cancer patients, chronic pain results from both the presence and treatment of cancer. It has been shown that pain from tumor excision surgery can contribute to cancer metastasis. The effect from radiation therapy-induced pain on cancer progression remains unknown. This study aimed to determine the effect of radiation-associated pain (RAP) on cancer progression using a syngeneic murine breast cancer metastasis model. We hypothesized that pain associated with radiation-induced glossitis will increase pulmonary metastatic tumor burden. We established the model by intravenously injecting 2×10^5 4T1-Luc2 murine mammary carcinoma cells into female Balb/c mice, monitoring cancer progression with in vivo bioluminescence imaging (BLI) and manually counting pulmonary metastases ex vivo. Metastatic progression and burden were compared between irradiated mice and a control group. Two pilot studies of 8 mice each determined optimal injection route, imaging protocol, and lung staining procedure. The first pilot validated the cancer model using retro-orbital injection, but found exophthalmia as a complication. A second pilot validated the tail vein injection model and BLI protocol. These studies informed a larger 40-mouse study with half receiving 27 Gy to the rostral tongue using a clinical linear accelerator, the other half undergoing sham irradiation, and all injected with tumor cells into the tail vein at the peak of glossitis severity. Experiments and data analysis are ongoing. Determining the impact of RAP on the metastatic propensity of tumors could help inform future developments in pain management, thereby increasing the therapeutic benefits of radiation therapy while improving both quality of life and longevity.

Research Grant: NC State CVM Intramural Grant

Student Support: NIH T-35 Interdisciplinary Biomedical Research Training Program (IBRTP)

Novel cellular tools to study HIV replication in tissue reservoirs and improve standard viral outgrowth assays

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Human immunodeficiency virus (HIV) remains a dominant global health crisis, affecting tens of millions of people worldwide. Viral reservoirs are established early during acute infection, and despite years of fully suppressive antiretroviral therapy (ART), they persist for the lifetime of the patient in an unknown diversity of cells. Our prior work demonstrated the presence of HIV mRNA in tissue-resident alveolar macrophages in the lungs of Malawian adults in Malawi, Africa, suggesting these cells may be viral reservoirs. Aided by cell-level HIV readouts, we have recovered infectious HIV from bronchoalveolar lavage and are studying the human transcripts and viral adaptations that facilitate this persistence. New reporter cell lines we have developed have shown that standard protocols of viral outgrowth likely miss many replication competent viruses within the context of gold-standard assays, underestimating the true HIV burden during HIV or SIV cure or treatment interruption trials. Our findings suggest that rare HIV proviruses within tissue reservoirs may be poorly adapted to replicate in standardized, homogeneous outgrowth assays. Furthermore, comparing human monocyte-derived macrophages with other cells in the tissue-resident macrophage lineage, we have uncovered opportunities to pursue novel restriction factors that may affect HIV persistence in vivo. Our overarching objective is to contribute to new HIV therapies targeted to specific tissue reservoirs.

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Student Support: NIAID (AI118582), Russell; NIH OD (T32OD011182), Gludish

The role of type III collagen in the progression of post-traumatic osteoarthritis

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Osteoarthritis (OA) is a multifactorial joint disease, primarily characterized by the destruction of articular cartilage. The irreversible breakdown of the cartilage extracellular matrix, which is composed mostly of collagens and proteoglycans, causes weakened mechanical properties of the joint. Conservation of articular cartilage is key to joint health as it has limited capacity for intrinsic repair and healing. While there is increasing interest in the role of type III collagen (Col3) in joint health, it has yet to be determined how the loss of Col3 impacts joint health and the mechanism by which Col3 may prevent arthritis. Although Col3 is a small constituent of articular cartilage, there is an increase in Col3 deposition in the articular cartilage of mature joints in patients with OA. Also, the covalent bonding of Col3 and type II collagen in mature articular cartilage may occur to add structure to a weakened fibril network as a response to the matrix damage. This suggests Col3 is an important structural constituent of cartilage ECM that promotes resiliency of articular cartilage. To elucidate the role of Col3 in maintaining cartilage mechanical properties, we induced OA in 6 Col3 wild-type(+/+) and 6 Col3-deficient(+/-) mice using destabilization of the medial meniscus (DMM), a standard post-traumatic OA model. We hypothesize that following DMM, Col3 plays a chondroprotective role in maintaining cartilage mechanical properties and attenuating OA. The projected results are that Col3+/- mice will develop OA faster and to a greater extent than Col3+/+ littermates. This will be measured by examining aggrecan loss and OA score via histology, surface collagen fibrillation via SEM and molecule degradation products via IHC.

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Expression analysis of mu, delta and kappa opioid receptor genes in pigeons and cockatiels

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Little is known about the pharmacogenetics of birds, particularly in the area of opioid receptor expression and its relation to pain management. In some avian species, mu opioid agonists are the drugs of choice based on the current evidence available, while in others kappa opioid agonist drugs like butorphanol are recommended. Despite their popularity, cockatiels (*Nymphicus hollandicus*) and pigeons (*Columba livia*) are also included in this pharmacogenetic paucity. We hypothesize that cockatiels and pigeons have varied expression of kappa, mu, and delta opioid receptors, which may alter their response to common opioid drugs. Samples from the cerebrum, brain stem, spinal cord, and footpad were harvested from twenty birds (10 cockatiels, 10 pigeons). RNA was extracted from each tissue and converted to cDNA. Kappa, mu, and delta opioid receptor primers were used for qPCR per tissue type for each species. Cycle threshold (Ct) values were recorded and gene expression was analyzed using the $\Delta\Delta\text{CT}$ method. High quality cDNA was generated for each tissue type in both species. Kappa $\Delta\Delta\text{CT}$ and fold-change difference between cockatiels and pigeons were 2.2 and 0.23 respectively with cockatiels having higher expression values. However, Kappa ΔCT values were not significantly different for brainstem tissue between cockatiels and pigeons ($P > 0.71$). Continued data generation for all remaining tissue types is underway. Species differences in opioid receptor expression remains a plausible explanation for variable response to opioid analgesics between cockatiels and pigeons. Continued evaluation of the data generated from this study aims to identify the pharmacogenetic impact on these species.

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Student Support: This research was supported by the Boehringer-Ingelheim Veterinary Scholars Program

Analysis of changes in stem cell biomarkers to determine tissue viability in horses with strangulating colic

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Horses suffering from small intestinal strangulating lesions often have poor outcomes and increased complication rates due to the loss of epithelial barrier function and cell death. Cellular regeneration and viability following injury depends on intestinal epithelial stem cells (ISC). Currently, intestinal tissue viability and extent of resection is determined by subjective intra-operative evaluation. An objective measure of intestinal regenerative potential is needed. We hypothesized that ISC biomarker expression would be decreased at the proximal resection margin and that ISC loss would be associated with increased post-operative complications. We aimed to (1) evaluate changes in ISC biomarker expression at resection margins from cases of small intestinal strangulation and (2) associate the number of post-operative complications with ISC biomarker expression. Mucosal biopsies were obtained intra-operatively from the proximal and distal resection sites of 15 clinical cases. Protein and gene expression for ISC biomarkers and cells undergoing apoptosis (Caspase 3) were measured by co-immunofluorescence and qRT-PCR, respectively. One-way ANOVA was used to determine statistical significance. The number of ISC (SOX9⁺) cells and ISC gene expression (HopX) were both decreased in the proximal site ($P < 0.05$). Evaluation of ISC apoptosis and association of gene expression with complication rate is pending. Decreased ISCs at resection margins suggests delayed cellular regeneration and possible association with increased complications. Results from this study may determine a biomarker associated with intestinal viability in horses to aid in surgeon decisions regarding resection thereby decreasing morbidity and improving prognosis.

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Student Support: NIH T-35 Interdisciplinary Biomedical Research Training Program (IBRTP)

Cryopreservation of body cavity fluids for diagnostic cytology

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Cytologic examination of body cavity fluids is a frequent diagnostic procedure used in veterinary medicine. Ideally, fluids should be fresh when prepared for cytology; however, this can be problematic when samples must be shipped to a diagnostic laboratory. Freezing samples is contraindicated due to the irreparable damage to cellular morphology that may occur, which subsequently compromises an accurate diagnosis. The goal of this study was to examine the effects of freezing various biological fluids in a commercially available cryopreservative medium, C80EZ (CryoCrate LLC, USA), on the maintenance of cellular morphology for clinical diagnosis. Using a cytocentrifuge or direct smear, slides were prepared of freshly submitted bodily fluid samples, including urine, peritoneal, pericardial, and bronchoalveolar lavage fluids. The samples were chilled to 4°C: one part of the sample was prepared by mixing the sample with C80EZ in a 1:1 (v/v) ratio and the other was untreated. The samples were then frozen at -20°C for 24-48 hours, thawed at 37°C, and slides prepared using the same methodology. Each slide was examined microscopically for cellular morphologic changes against the unfrozen samples. In samples frozen with C80EZ, cellular morphology was maintained in comparison to the unfrozen samples. The untreated frozen samples showed loss of cellular morphology, including absent cellular aggregates and indistinct nuclear and cell borders. In conclusion, C80EZ-treated frozen samples were not as adversely affected by the freezing process as untreated frozen samples. Results from the study encourage the viability of freezing diagnostic fluid samples with C80EZ for shipment to a laboratory without sacrificing cell integrity.

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Molecular characterization of avian influenza virus H9N2 isolated from Algerian poultry flocks

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The avian influenza virus (AIV) H9N2 subtype is the most widespread and is known to infect both humans and poultry, causing economic losses to the industry. AIV H9N2 can undergo reassortment with highly pathogenic avian influenza viruses (HPAIVs). Reassortment events can create novel HPAIVs, causing further economic loss and impact on public health. Consequently, the detection and characterization of AIV H9N2 is of significance. The purpose of this study was to determine the molecular composition of Algerian AIV H9N2 poultry isolates, their phylogenetic relatedness to other H9N2 AIVs, and to indicate their origins. Viral RNA was extracted from isolates. Quantitative PCR (RTq-PCR) was used to confirm the presence of AIV. Complementary DNA (cDNA) was created using reverse-transcription PCR (RT-PCR). Genomic segments (PB2, PB1, PA HA, NP, NA, M and NS) were amplified from the cDNA with conventional PCR. Amplified segments were purified and sequenced. A consensus sequence for each of the isolates' segments was created. Consensus sequences were phylogenetically analyzed, and a phylogenetic tree was constructed for each segment. To date, 10 viral isolates have undergone sequencing and phylogenetic analyses. A portion of the HA and NA segments was sequenced for all 10 isolates. The NP, PA (10 isolates), and PB1 (8 isolates) segments were sequenced with minor portions missing. The complete PB2 segment was sequenced for 7 isolates. Early phylogenetic analyses show the Algerian isolates being closely related and clustering with Moroccan strains in the Middle East group, part of the Lineage A. Future studies would be beneficial in determining means of control and viral spread between the Moroccan and Algerian poultry industries.

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Student Support: 2018 Boehringer Ingelheim Veterinary Scholar Program Europe

The olfactory mirror test: testing self-recognition in dogs using anogenital odor

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The mirror self-recognition test is an important cognitive test used widely to assay self-recognition and self-awareness among non-human animals. While dogs have not passed the traditional test, some argue that canine self-recognition would be better assessed through an “olfactory mirror” test, given dogs’ heavy reliance on olfaction. Using this concept, previous research has found that dogs appear to recognize the scent of their own urine, suggesting self-recognition, but this result has not been replicated. Consequently, our study aims to examine canine self-recognition but in a novel olfactory context, specifically one that has received very little research attention. In particular, based on observations that dogs frequently sniff conspecifics’ anogenital region, we ask if dogs recognize their own anogenital scent and differentiate it from others’. To answer these questions, we obtained swabs from the anogenital regions of 28 dogs and presented each dog with swabs from their own and other dogs’ anogenital area, including some swabs that are modified with an added scent. If dogs recognize their own odor, we expect they will investigate their own modified swabs longer than their own scent alone or the modified scent alone. We also expect dogs to sniff swabs from unfamiliar dogs longer than their own swabs. This study will further validate the “olfactory mirror” test as a method to detect self-recognition in animals. In addition, it may also provide a valuable assay for dog-handlers to predict olfactory capabilities in working dogs and for veterinarians to detect changing olfactory capabilities in aging patients.

Research Grant: None

Student Support: NC State CVM Boehringer Ingelheim Veterinary Scholars Program

Efficacy of Cryptotanshinone against human and canine melanoma

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Cryptotanshinone (CTS) is the major constituent of the plant *Salvia miltiorrhiza*. It has been used in ancient Chinese medicine for hundreds of years for its potent cardiovascular effects. In recent years, CTS has been discovered to have significant apoptotic and anti-tumor properties against a variety of cancer cell lines. In this study, we investigated the in vitro and in vivo effects of CTS, alone and in conjunction with carboplatin, on human (UACC 647) and canine (KMSA) melanoma cell lines. CTS alone and in combination was shown to significantly reduce viability and invasion in both cell lines. There is no evidence of synergistic activity between CTS and carboplatin in either assay. Caspase activity was also increased in the CTS alone and combination groups. Western blot to be completed. PCR is underway to analyze the genetic expression of STAT3 and its downstream targets. A xenograft study using a nude mice model is in progress with data to come. Once data is complete, this study will hopefully lay the grounds for follow up investigation of CTS in a phase 1 canine study, potentially making a permanent home in clinical practice one day.

Research Grant: Fred and Vola Palmer chair for comparative oncology

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Polymorphisms in glutathione-S-transferase genes in Boxers with lymphoma and unaffected geriatric controls

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Glutathione-S-transferases (GSTs) conjugate reactive xenobiotics, including pro-carcinogenic chemicals. Human GST genes are polymorphic, and low functioning variants in human *GSTT1*, *GSTM1*, and *GSTP1* have been associated with cancer risk, including lymphoma. Boxer dogs are at a higher risk of developing lymphoma, but the underlying cause for this breed risk is not understood. We have found variants in 4 GST genes expressed in canine liver with shown or predicted deleterious effects. The aim of this study is to determine whether these canine GST alleles are over-represented in boxers with lymphoma compared to unaffected geriatric boxers (10 years of age or older). Four major canine GST genes are being studied: *GSTT1*, *GSTM1*, *GSTP1*, and a novel canine GST, *GSTT5*. To date, we have recruited 32 boxers with lymphoma and 89 unaffected geriatric controls. Genomic DNA was isolated from cheek swabs, and owners completed a questionnaire on environmental exposures to herbicides, pesticides, urban air pollution, and household chemicals. Multiple GST polymorphisms are being screened: one *GSTT1* 3'UTR haplotype (containing *101-102 ins T) leads to a 50% reduction in gene expression in luciferase constructs; a 6bp coding deletion in *GSTT5* leads to virtual loss of enzyme activity in recombinant expression studies; *GSTP1* polymorphisms with predicted deleterious effects in the promoter, exon 2, exon 5 and the 3'UTR and *GSTM1* variants in the promoter, exon 8, and the 3'UTR. Ongoing work is comparing allele frequencies for these variants in the two groups of boxers. Follow-up work will examine gene-environment interactions and the relationship between peripheral blood DNA damage and GST genotype.

Research Grant: AKC Canine Health Foundation

Student Support: AKC Canine Health Foundation

Effect of acupuncture on recovery from general anesthesia in canines

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In modern western medicine, surgery requires general anesthesia, which upon recovery may lead to dysphoria, resulting in the need for further sedation while others experience extreme delay to normal physiological status. The objective of this study is to evaluate the effectiveness of stimulating acupuncture point GV-26 alone or in conjunction with Wei-Jian after anesthesia to improve recovery and lessen any negative side effects in canines. Included in the study were thirty dogs with an American Association of Anesthesia physical status classification of I or II. Animals were randomly assigned to one of five groups. Three of the five groups used acupuncture points by themselves or in combination. The points stimulated were GV-26 (middle point of the philtrum) and Wei-Jian (tip of the tail). The other two groups were either sham acupuncture or no acupuncture. Sham acupuncture still uses acupuncture needles, but they are placed in locations on the body not attributed to an "acupoint". The recovery of the animals was video recorded for later scoring of the recovery quality by an evaluator unaware of the treatments. Following times were recorded: time from discontinuing anesthesia until first movement, time to extubation, to sternal recumbency, and to standing. Analysis of variance with Wilcoxon signed rank test will be used for the non-parametric data; significance set at $P < 0.05$. There are four more dogs to take place in the study, and data will not be submitted until 17 July 2018. Therefore, results are still pending.

Research Grant: None

Student Support: Boehringer Ingelheim

An immunohistochemical evaluation of integrin and cadherin expression within mesenchymal stem cell spheroids

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Mesenchymal stem cells (MSCs) formed into three-dimensional spheroids have increased therapeutic potential compared to dissociated MSCs demonstrated by their increased trophic factor secretion and cell survival in harsh conditions. Although widely suggested in the literature that these effects are due to a hypoxic gradient that stabilizes various protein signaling pathways, the Leach lab demonstrated that large hypoxic gradients do not exist in these MSC spheroids. Alternatively, we hypothesize that the improved function of MSC spheroids is due to spatial differences in cell-cell and cell-matrix adhesion as a function of cell position in the spheroid. This study aims to characterize the spatial differences in integrin and cadherin expression within MSC spheroids to better understand the differentiation potential, and thereby optimize spheroid synthesis. Bone marrow-derived MSCs were formed into 15,000-cell spheroids, incubated for 48 hours, and then formalin-fixed and sectioned with a microtome. Immunohistochemistry (IHC) will be used to evaluate the expression of N-cadherin, E-cadherin, Cadherin-11, and integrin subunits $\alpha 2$, $\alpha 5$, the vitronectin receptor of the α subunit (αv), and $\beta 1$. We anticipate that MSCs within spheroids exhibit increased cadherin expression at the periphery of the spheroid and increased integrin expression towards the center.

Research Grant: NIH R13; University of California, Davis, School of Veterinary Medicine

Student Support: NIH R13; University of California, Davis, School of Veterinary Medicine

The potential of transmission of *Ureaplasma diversum* by bovine embryo transfer

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Ureaplasma diversum is a bacterium that can be found in the lower urogenital tract of bovines. If present, it may lead to decreased fertility, endometritis, vulvitis, abortions, stillbirths and weak calves. It is of concern with embryo transfer as the routine embryo washing procedure is unable to remove the bacterium due to its strong adherence to the zona pellucida. The aim of this study is to determine if there is a potential for *U. diversum* to create a natural infection when an embryo is transferred from donor to recipient. Approximately 87 embryo recovery fluid samples, collected since January 2017, from both dairy and beef donors, were tested for *U. diversum* using PCR. Bacterial load was analyzed by semi-quantitative estimation. This retrospective cohort study will analyze producer reproduction records to determine the relative risk of pregnancy, pregnancy loss and stillbirths in recipients who received embryos from a positive flush compared to recipients who received embryos from a negative flush. This research is still in progress. It was found that the overall 30 day pregnancy rates was approximately 56%.

Research Grant: None

Student Support: AVC Veterinary Student Research Award (VetSRA)

The impact of supplemental heat in rodent housing units on behavior, breeding, and anesthetic recovery

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Critics of animal research emphasize that laboratory rodents are under chronic ‘cold stress’ in their standard housing environments. The term ‘cold stress’ is applied to rodents because the national guideline sets housing room temperatures at 20-26°C, despite the rodent thermoneutral zone (TNZ) occurring at 26-32°C. Physiological variances are noted in ‘cold stressed’ mice which may result in poor translation of rodent data for models of human disease and therapeutic agents. In this study, we evaluate differences in behavior, breeding, and anesthetic recovery in mice when supplemental heat is provided to OptiMouse cages (Animal Care Systems). A prototype Smart System Rack, containing heat plates, warmed a quadrant of each OptiMouse cage to ~30°C. Mice were microchipped for body temperature collection and mice (n=12) were placed into breeding pair arrangements. Cage-side videography (GoPro Cameras) captured mouse and pup behavior and nest location. Mice (n=16) were anesthetized with injectable agents. Body temperature and length of time until recovery was evaluated in mice who recovered on the heated rack compared to a warmed static cage. It was expected that during the inactive phase, rodents would occupy space directly over the heating panel to aid in increasing body temperatures toward TNZ. Due to the higher ambient temperature of the cage environment, it was suspected that nest building would decrease, anesthetic recovery would be expedited, and pups would thrive. Housing rodents in an environment that gives them the ability to regulate their body temperature across the circadian cycle will decrease overall physiological stressors, resulting in improved animal models for human disease.

Research Grant: None

Student Support: Boehringer Ingelheim Veterinary Scholars Grant, MSU; Nathan Brewer Fund, MSU CAR

Pangolins in peril: unraveling reproductive mystery in a race to save a species

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All eight species of pangolin are under threat of extinction and a captive breeding program is critical to their survival. Endocrine data are useful in characterizing cycles to establish captive breeding protocols. An ambassador population of the African tree pangolin (*Phataginus tricuspis*) is the only species in managed care in the U.S. We sought to establish the reproductive cycle of this species through non-invasive hormone monitoring. We validated the use of group specific enzyme immunoassays to measure estrogen and progestagen metabolites voided in pangolin feces. The serially diluted fecal extract displaced labeled hormone like that of standard in both estrone glucuronide (E_1G) and progesterone (P_4) assays. Additionally, added standard to sample substrate yielded significant recovery in the E_1G and P_4 . These results are the first to demonstrate the use of pangolin feces in measuring the two most important steroids produced by the ovary, estrogen and progestagens. Based on these results we can characterize the reproductive cycle of the pangolin. Such knowledge will be instrumental in guiding the captive breeding program and establishing a template to study the reproductive cycle of other pangolin species.

Research Grant: Chicago Zoological Society

Student Support: Boehringer Ingelheim

The efficacy of mannosylation of chitosan derivatives as a nanoparticle DNA carrier

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DNA vaccines are a fairly new technology to help combat evolving infectious diseases as they have proven to have better manufacture rates and stability in ambient temperatures. Furthermore, the vaccines would be a viable and safer option since the DNA plasmids are nonliving and nonreplicating. This means the risk for the vaccines to reverse to a pathogenic state is eliminated. The goal of second-generation DNA vaccines is to optimize humoral and cellular immunity response. Currently, there is a focus in improving immunogenicity by optimizing DNA vaccine formulation. The DNA plasmids are coated into the surface of polymers, such as liposomes and microparticles, and are taken up by dendritic cells triggering the innate immune system. Chitosan is a polymeric microparticle that has shown potential to improve DNA vaccines. It is an abundant biodegradable, biocompatible, and non-toxic polysaccharide, making it a good candidate for drug delivery. To improve uptake, mannosylation of chitosan has been investigated as mannose receptors have proven to increase endocytosis into dendritic cells. This study aims to compare the efficacy of mannosylated chitosan against the transfection reagent Lipofectamine 3000. Two molecular weight derivatives of chitosan will be mannosylated. Once resuspended, mannosylated chitosan will be complexed with pCMV dsRed DNA. Following the complexing of DNA, HEK 293 cells seeded into a 24-well plate will be transfected. After 48 hours, the fluorescent marker of the DNA will allow us to quantify the transfection efficiency. The comparison of mannosylated chitosan, un-mannosylated chitosan and Lipofectamine 3000 will allow a better understanding of which method provides better carriage for the DNA.

Research Grant: AG-2019-LIFT 003 and Boehringer Ingelheim

Student Support: Boehringer Ingelheim

Assessing differences in the human-animal bond among service dogs and pet dogs

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Introduction: Service dogs play an important role in assisting individuals with disabilities. Often, a strong bond is formed between the service dog and handler. Pet dogs also form a bond with their owner, but how this relationship may differ from the bond with a service dog is not well researched. The purpose of this study was to compare the strength of the human-animal bond among individuals with disabilities who have service dogs or pet dogs. **Methods:** Study participants were recruited through Canine Assistants, an organization that places mobility and medical service dogs across the United States. Participants had either already received a service dog ($n=96$) or were on the waitlist ($n=38$) to receive one in the future but had a pet dog in the home. The strength of the human-animal bond was assessed with The Monash Dog-Owner Relationship Scale (MDORS) subscales of Emotional Closeness and Dog-Owner Interaction. **Results:** Results indicated that a stronger human-animal bond existed between service dogs and their handlers when compared to pet dogs and their owners. Specifically, service dog handlers reported higher Emotional Closeness scores ($t=4.31$, $P < 0.001$) and higher Dog-Owner Interaction scores ($t=4.14$, $P < 0.001$) than pet dog owners. Analyses of individual questions also revealed that specific aspects of the human-animal bond were stronger in the service dog-handler relationship. **Discussion:** Results show that in addition to the physical assistance they provide, service dogs have a unique relationship with their handlers that elevate their role beyond that of a pet dog. Future research should examine how bonding with service dogs varies by age and disability severity and explore potential drawbacks of this relationship.

Research Grant: Elanco Animal Health

Student Support: PetSmart Charities and Purdue Veterinary Medicine

Assessment of basal behaviors in genetic mouse models of mild and severe hydrocephalus

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Hydrocephalus is a devastating neurodevelopmental disorder with variable and mostly unknown etiologies. It is a complex multifactorial disorder which is caused by genetic mutations that result in severe and mild forms. We compared two mouse models that mimic these forms of hydrocephalus in two distinct genetic backgrounds to better understand behavioral deficits in relation to high and low-grade hydrocephalus. In one model (Foxj1-cKO), ependymal cells which steer the cerebrospinal fluid within the cerebral ventricles, lack motile cilia causing severe early onset hydrocephalus with devastating effects on neuronal integrity throughout the brain. In the second model (EGFR-cKO), gliogenesis is genetically disrupted during perinatal stages resulting in a forebrain inflammation-induced transient hydrocephalus. Thus, the objective of this study is to characterize basal behaviors of 3-week-old Foxj1-cKO mice (n=5) exhibiting a severe hydrocephalic phenotype and EGFR-cKO mice (n=6) exhibiting a transient hydrocephalus that is specific to the olfactory bulbs during early postnatal life. We hypothesized that Foxj1-cKO and EGFR-cKO mice would display behavioral deficits compared to controls. Differences in locomotive responses were assessed over a 30-minute period in a novel environment. A two tailed Mann-Whitney test showed that FoxJ1-cKO mice spent significantly more time rearing ($P=0.0127$) and mobile ($P=0.0117$) compared to FoxJ1 controls. Whereas, EGFR-cKO mice spent significantly more time mobile ($P=0.0043$) compared to EGFR controls. Hence, our preliminary findings indicate that both severe and mild models of hydrocephalus exert significant effects on basal behaviors, compared to control groups.

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Student Support: NIH T-35 Interdisciplinary Biomedical Research Training Program (IBRTP)

Use of preference testing to establish reinforcer value in rats and influence perception of handlers

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Routine handling in the laboratory environment can provoke anxiety in animals, activating behavioral and physiologic stress responses that affect animal wellbeing and confound research results. Preference testing was conducted in 12 female Lewis rats using food rewards to determine primary reinforcer value in the context of a novel stimulus (delivery apparatus). The highest scoring food reward was considered the most valuable and subsequently used in a subset of 6 rats undergoing the counter-conditioning paradigm. Behavioral and physiologic responses were recorded in rats undergoing three different commonly used methods of restraint. An ethogram was collected prior to, during, and after restraint, and blood glucose was measured as a stress sensitive physiologic parameter. We hypothesized that pairing a high value positive reinforcer with anxiety provoking stimuli (restraint) could positively shift the valence towards a more favorable experience for the animals. We determined the influence of reinforcement on behavioral response is affected by the strength of the reinforcer. Although restraint methods were similar from the handler perspective, they provoked substantially different behavioral responses suggesting rats perceive certain methods to be more aversive than others. Rats were generally motivated by primary reinforcers (e.g. food) but some demonstrated motivation towards more abstract reinforcers (e.g. physical enrichment and sociality). Mitigating aversive stimuli in combination with a high value positive reinforcer reduces anxiety and promotes a favorable perception of handlers. Operant conditioning is an important refinement in optimizing experimental handling and supporting the bond between animal and handlers.

Research Grant: The Witowski Integrative Medicine and Surgery Fund

Student Support: National Institutes of Health, Award Number T35OD011118

Investigation of genetic risk factors for squamous cell carcinoma in the equine eye

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Squamous cell carcinoma (SCC), the most common cancer of the equine eye, has a high prevalence in Haflinger horses indicating a genetic predisposition. A missense variant on chromosome 12 (c.103 C>T p.Thr338Met) in damage specific DNA binding protein 2 (DDB2) can explain 76% of ocular SCC-affected Haflingers. It is hypothesized that genetic heterogeneity contributes to cancer risk. To investigate this hypothesis, whole genome Illumina sequencing data from four Haflingers (two ocular SCC affected horses without the DDB2 risk variant and two clinically confirmed unaffected horses) were evaluated to identify variants segregating with disease status. Sequencing data were aligned to EquCab2 reference genome, variants were called with Freebayes and annotated using SNPeff. Previous genome-wide association studies identified ocular SCC associated loci on chromosomes 6, 11, 12 and 23 and thus our analysis focused on these loci. XX variants were selected based on location, concordance with phenotype, gene function, and predicted effect of variant. Assessing these variants in a sample set of 76 Haflinger horses (14 ocular SCC affected horses without the DDB2 risk variant and 62 unaffected controls) will determine if any of these are associated with SCC risk.

Research Grant: Center for Equine Health (UC Davis)

Student Support: Boehringer-Ingelheim Animal Health

Progression and persistence of nasal turbinate pathology in F344 rats exposed episodically to ozone

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Epidemiological studies suggest that elevated ambient concentrations of ozone, a common gaseous component of photochemical smog, are associated with nasal eosinophilic inflammation (rhinitis) in children. Laboratory rodents exposed to high ambient levels of ozone develop a similar rhinitis as well as other nasal lesions. The present study was designed to determine the progression and persistence of the principal lesions in the nasal turbinates of rats episodically exposed by inhalation to ozone. Male rats were exposed to 0 or 0.8 ppm ozone (4 h/day) for 9 consecutive weekdays (2-week, episodic exposure). Other rats received three 2-week inhalation exposures separated by 2 weeks of no exposure (6-week, episodic exposure). All rats were sacrificed 1d or 2 weeks after the last exposure day. Nasal tissues were processed for light microscopy, immunohistochemistry, and morphometry. Eosinophilic inflammation, mucous cell metaplasia, and loss of turbinate bone (atrophy) were significantly greater in the nasal turbinates of rats exposed for three 2-week ozone exposures, as compared to those exposed for a single 2-wk exposure. All of these nasal lesions persisted for 2 weeks post-exposure with little change in severity. The results of this study suggest that ozone-induced nasal pathology may be a long-lasting adverse health effect of children episodically exposed to high ambient levels of ozone.

Research Grant: ACC 6148 and EPA RD83479701

Student Support: NIH Grant No. R25HL103156

Hepatic concentrations of copper and other metals in dogs with hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the most common primary liver tumor in dogs and humans. Abnormalities in hepatic trace metal concentrations have been associated with increased risk for HCC development in humans. While trace mineral concentrations are frequently evaluated in dogs with hepatitis, they have not been studied in dogs with HCC. Our objective was to investigate hepatic trace metal concentrations in dogs with HCC. A histopathology database was searched to identify dogs with hepatocellular carcinoma (test population) and an age matched control population. Cases were reviewed and classified based on tumor grade, underlying hepatic pathology, and qualitative copper assessment. Concentrations of copper, iron, zinc, and selenium in non-neoplastic liver tissue as well as in HCC tissue were determined using inductively coupled plasma mass spectrometry. Fourteen of 47 (29.8%) dogs with HCC had hepatic copper concentrations exceeding the normal reference interval (> 400 parts per million). In dogs with HCC, copper, iron, zinc, and selenium concentrations were greater in the non-neoplastic tissue as compared to the neoplastic tissue ($P < 0.01$ for all comparisons). Analyses of Rhodanine stained slides in all dogs and mineral concentrations in control population dogs are ongoing. These findings suggest that abnormal copper concentrations are common in dogs with hepatocellular carcinoma. Additional studies are needed to determine if trace mineral abnormalities contribute to the risk of HCC in dogs, and whether treatment of these abnormalities could decrease risk or alter progression.

Research Grant: Michigan State University College of Veterinary Medicine

Student Support: NIH Grant T35OD016477 to Michigan State University

Contact networks and season: a longitudinal analysis of the Florida panther population

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Disease transmission in wildlife is in large part influenced by patterns of contact between individuals in a population. Contact networks, graphical representations of interactions, can provide information about a given population, with important consequences for disease transmission. This is especially true for threatened and endangered species, which may be more vulnerable to the consequences of a major disease epidemic. One such species is the Florida panther, a previously critically endangered species which experienced a serious outbreak of feline leukemia virus in 2002-2005. Florida panther habitat is highly dynamic, with seasonal rains causing usable dry land one month to become swamp the next. This habitat variation may impact connectivity within Florida panther populations, with subsequent impacts on disease transmission and epidemic size. Using a decade's worth of existing telemetry data, we created and analyzed panther contact networks, examining the relationship between network connectivity and Florida's seasons. Networks were based on spatial overlap between panthers, with each panther's home range determined by 95% kernel density estimation. Utilization distribution overlap index was then used to determine potential contacts between individual panthers. Networks were analyzed using social network analysis metrics such as degree, closeness, betweenness, and modularity. Statistical analysis was completed using Kruskal-Wallis tests. Preliminary analysis has revealed a mixed relationship between some contact network metrics and season. This information will be useful for future investigations and for conservation and disease prevention in the Florida Panther.

Research Grant: National Science Foundation (DEB-1654609)

Student Support: The Department of Veterinary Population Medicine, University of Minnesota CVM

Role of the OVLT-gut microbiome axis on the development of DOCA-salt hypertension

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Studies suggest an association of gut microbiome changes with certain forms of hypertension, but a cause/effect relationship has yet to be fully defined. The deoxycorticosterone acetate (DOCA)-salt model of hypertension in rats is known to have a neurogenic component linked to increased sympathetic nervous system activity. We have shown the hypertensive response in DOCA treated rats requires the organum vasculosum of the lamina terminalis (OVLT). Furthermore, celiac ganglionectomy supports a role of splanchnic sympathetic innervation to the gut in the hypertensive response to DOCA. Taken together, we hypothesize an OVLT-sympathetic-gut (OSG) microbiome axis is involved in the development of hypertension. In uninephrectomized OVLT lesioned (OVLTx; n=5) and SHAM (n=2) Sprague-Dawley rats consuming a 2% NaCl diet and 0.9% NaCl drinking solution, 24-hour mean arterial pressure (MAP) was recorded telemetrically 5 days before and 21 days after DOCA implantation (100mg/rat SQ). Microbiome analyses were performed on regional gut samples at the end of the study and compared to normal rats (n=2). The chronic pressor response to DOCA was attenuated in OVLTx rats. Moreover, DOCA hypertension in SHAM rats was associated with decreased levels of *Lactobacillus* species in the jejunum and ileum, and increased *Escherichia* levels in the cecum compared with normal rats. However, these changes in bacterial genera were mitigated and offset by lesion of the OVLT. We conclude that DOCA-salt hypertension is associated with specific microbiome changes in the gut, and the attenuated hypertensive effects of DOCA-salt in OVLT lesioned rats is mediated in part through counteracting changes in these bacterial populations.

Research Grant: Merial Veterinary Scholars Research Grant and University of Minnesota Grant In Aid

Student Support: Boehringer Ingelheim Veterinary Scholars Grant

Readability as a source of measurement error in veterinary medical education assessments

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Background: Readability is a measure of the accessibility of a text to a reader. As a general rule, text should not exceed the readability levels of the intended audience. Readability scores routinely are used to inform the text appearing in medical resources, educational materials, newspaper articles, and more. **Purpose:** The purpose of this study was to investigate the role of readability as a potential source of measurement error in the context of veterinary education assessment.

Methods: Two readability formulas, FOG and FORCAST, were selected for their intended purposes and requirements for shorter texts. These formulas were utilized to produce readability scores for multiple-choice items appearing on major exams in five first-year DVM courses. The association between readability scores and item difficulty was explored to determine if higher readability scores correlated with more difficult items.

Results: Results indicate the most difficult items (as determined by student performance) share a moderate correlation with higher grade level readability scores.

Discussion: There is evidence to suggest an item's readability level may introduce a source of measurement error that can threaten the validity of students' examination scores.

Conclusion: Items that are poorly targeted due to excessively high grade level readability scores are candidates for potential revision and/or exclusion from future examinations as these items may introduce measurement error that distorts score quality and meaning.

Research Grant: None

Student Support: Department of Academic Affairs, College of Veterinary Medicine, NCSU, Raleigh, NC

The role of childhood obesity-associated adipose tissue fibrosis in adulthood cancer development

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Background: Childhood obesity has been linked to adult cancer development. In obesity, extracellular matrix (ECM) remodeling (fibrosis) mediated by adipose stromal cells (ASCs) occurs in response to rapid fat expansion. Fibrosis is associated with both aging and cancer development. Understanding the role of childhood obesity-associated fibrosis in adulthood cancer may inform strategies for cancer prevention.

Objective: Characterize similarities in ASC growth and differentiation and ECM assembly between young obese and aged individuals relative to young lean individuals using mouse models of obesity.

Methods and Results: Subcutaneous fat pads were harvested from young and aged C57/Bl6 WT mice and young *Lep ob/ob* (obese) mice. Fat pads were fixed for histology or underwent collagenase digestion for *in vitro* ASC culture. Differences in ASC growth rates were assessed by growth curves and BrdU incorporation. Increased population doubling was observed and BrdU incorporation was supportive, but not statistically significant. Alpha-smooth muscle actin immunofluorescence imaging was completed to assess for myofibroblast differentiation. No differences were present between groups. ECM composition was assessed via an *in vitro* decellularized ECM model. Immunofluorescence imaging for fibronectin and collagen VI revealed that young obese and aged WT had greater average area of Collagen VI, but young WT had greater average fluorescent intensity. Collagen I content was assessed via histology and picrosirius red staining. Results for fibronectin and collagen I are pending.

Conclusions: Young obese and aged adipose tissue have similar Collagen VI content, but this change does not appear to be mediated by ASC myofibroblast differentiation.

Research Grant: Kansas State University Department of Diagnostic Medicine and Pathobiology

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Evaluation of antimicrobial resistance to Chlortetracycline in *Mannheimia haemolytica* and *Pasturella multocida*

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The goal of this study was to determine if a 14 day course of chlortetracycline (CTC); dosed at 350 mg/day, promoted antimicrobial resistance to tetracyclines in *Mannheimia haemolytica* and *Pasturella multocida* collected from sheep with naturally occurring ovine respiratory disease (ORD). Recent pharmacokinetic studies suggest that the FDA approved dose of 80 mg/hd/day, as well as a 500 mg/hd/day dose may fail to reach sufficient plasma concentrations for the prevention of ORD, and thus potentially select for the development of resistant strains. *M. haemolytica* and *P. multocida* isolates were collected from sheep with naturally occurring ORD while either receiving a 14 day course of oral CTC or no CTC. Sheep that developed evidence of ORD were also treated with injectable tetracycline, resulting in clinical improvement in most cases. 92 of 94 isolates collected had a MIC of $\geq 0.5 \mu\text{g/mL}$ to $1.0 \mu\text{g/mL}$, indicating they were still susceptible to CTC at the end of the study. When CTC plasma concentrations were taken prior to necropsy, a range $0.0628 \mu\text{g/mL}$ to $0.425 \mu\text{g/mL}$ was observed. Although the expected penetration of CTC into lung tissue is moderate, it is questionable if even the highest concentration observed would result in sufficient tissue drug levels to achieve the MIC in the majority of animals. These results demonstrate that, while not effective at preventing ORD, daily dosing with 350 mg/hd/day of oral CTC did not select for the development of significant tetracycline resistance and is more likely associated with an inability to achieve sufficient drug concentrations in lung tissue to inhibit bacterial growth. Given the importance of antibiotic stewardship, use of oral CTC to prevent ORD is likely not warranted.

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Effect of the commercially available probiotic *Enterococcus faecium* SF-68 on canine giardiasis

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Enterococcus faecium SF-68 is an available probiotic (FortiFlora; Purina PetCare, St. Louis, Missouri) that is an immune modulator in dogs, cats, and rodents with anti-*Giardia* effects in mice. The primary hypothesis of this study is that dogs with giardiasis that are treated with SF-68, diet, and an anti-*Giardia* drug will have improved clinical outcomes compared to dogs treated with diet and an anti-*Giardia* drug. In this ongoing study, dogs with normal attitude and appetite that develop small or large bowel diarrhea while housed in a local shelter and test positive with a commercially available *Giardia* antigen assay (SNAP Giardia; IDEXX Laboratories, Portland ME) are randomized into the 2 treatment groups. Each dog is fed a standardized diet (EN Fiber Balance; Purina PetCare) and administered an anti-*Giardia* drug (Drontal Plus; Bayer Animal Health, Merriam, Kansas) daily PO for 3 days and randomized dogs are fed the probiotic daily for 7 days. Trained observers masked to the treatment groups assign a fecal score using a standardized system to feces passed each morning. The dogs are also evaluated by fecal flotation, *Giardia*/*Cryptosporidium* fluorescent antibody staining (Meridian Diagnostics, Indianapolis, IN) and *Giardia* assemblage testing. To date, 29 dogs have been screened for *Giardia* antigen, of which 7 dogs (24.1%) were positive and entered into the study. Fecal results to date for 5 dogs showed 1 false negative result for *Giardia* cysts, *Cryptosporidium* coinfection in 3 dogs, *Cystoisospora* coinfection in 1 dog, and that the 4 dogs with adequate *Giardia* spp. DNA for assemblage testing were all positive for Assemblage D, the non-zoonotic dog genotype. Clinical evaluations in this masked study are ongoing.

Research Grant: Research support: Center for Companion Animal Studies and Nestle Purina PetCare, St. Louis, MO

Student Support: Student support: Nestle Purina PetCare

Sexual dimorphism in the metabolic response to dietary protein restriction

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Dietary protein restriction elicits an increase in production and circulation of the hormone fibroblast growth factor 21 (FGF21). Interaction of FGF21 with its target tissues results in metabolic improvements and behavioral changes as compensation for a diet low in protein. This response has been studied in the male mouse model but little is known about the metabolic response in females. Recent literature suggests that there is a relationship between sex and response to dietary protein restriction, with females having minimal response. High and low fat feed studies were conducted to test this theory. Male and female mice were fed isocaloric feeds differing in protein/carbohydrate ratios. Body weights, feed weights, and metabolic processes were measured regularly. Tissue samples were harvested for RNA extraction at euthanasia. As has been proven in previous studies, male mice maintained on a PR diet showed marked increases in FGF21 levels in both serum and tissues, as well as marked improvements in metabolism when compared to their control counterparts. Female PR response resulted in blunted levels of FGF21 levels and only marginal improvements in glucose tolerance when compared to control groups. Data supports that female mice are less responsive than their male counterparts when dietary protein is restricted.

Research Grant: Unknown

Student Support: NIH 5T35 OD011151-15

Development of clinically relevant chronic rotator cuff models in sheep: a pilot study

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Various animal models have been used to investigate rotator cuff tears (RCT), each model has inherent challenges, thus limiting some aspects of their translatability. The goal of animal RCT models is to recapitulate, at least in some part, biomechanical and cellular changes observed following RCT degeneration in humans. Therefore, the objective of this study was to evaluate the histopathologic and biomechanical changes of two novel ovine partial infraspinatus tendon (IFT) transection methods: 1st) “combed” fenestrations along the long axis of the IFT, and 2nd) sharp partial transection of the IFT perpendicular to the tendon fibers at the humeral attachment site. Six animals underwent surgery (n=3 per group). At 6-weeks IFT samples were collected. Non-destructive biomechanical analysis and histopathological scoring was performed on all samples utilizing established methods. Comparisons were made between the ovine models and to human tissues collected from similar regions of interest (ROI). Both models were less stiff compared to healthy ovine IFT, with the 1st model demonstrating higher mechanical stiffness at 6-weeks compared to the 2nd model. All ovine ROI's demonstrated histological changes within the tendinous portion of the IFT, similar to changes observed in the tissues collected from people with chronic RCT. This study confirms that partial disruption of the IFT via combed or sharp transection resulted in translatable degenerative changes over a 6-week period. Future work will examine RNA changes secondary to degeneration of these methods to further validate translatability. Validation of these models will help improve basic science research, and advanced development of repair devices or biologic treatments.

Research Grant: Orthopaedic Bioengineering Research Laboratory (OBRL) and Preclinical Surgical Research Laboratory (PSRL), Colorado State University, Fort Collins, CO

Student Support: Boehringer Ingelheim Veterinary Scholars Program

The potential use of MALDI Sepsityper™ technology for rapid diagnosis of bacteremia in dairy calves

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Culture-based diagnosis of septicemia in veterinary patients can require a week or more and is therefore impractical to use when rapid treatment is needed. In most cases, antibiotics are given empirically without confirmation of sepsis or identification of the causative agent, raising concerns over unnecessary or suboptimal antibiotic administration. A recent alternative method for diagnosing bacteremia involves utilization of a Sepsityper™ kit (Bruker Daltonics, Billerica, MA) in conjunction with MALDI (matrix-assisted laser desorption/ionization) mass spectrometry, which is capable of detecting and identifying bacteria from a blood culture sample in as little as 30 minutes. Here, we investigated the use of Sepsityper with MALDI, hypothesizing that it would demonstrate more rapid and sensitive results for detecting bacteremia than traditional culture-based methods. Aseptic blood samples were collected from diarrheic calves, and 10mL of blood sample was inoculated in a blood culture bottle (BCB) with indicator top. Bottles were incubated at 35°C and checked for five days following inoculation for turbidity and fluid translocation into the indicator top, which defined a positive BCB. Blood culture fluid from positive bottles was analyzed by Sepsityper with MALDI and regular isolation and identification methods. Preliminary results show that the Sepsityper method is capable of identifying bacteria in 56% (n=10/18) of cases positive on BCB, while the culture-based method has identified bacteria in 82% (n=14/17) of these cases. Despite a potentially lower sensitivity, the Sepsityper method is at least 24 hours quicker than traditional methods, and thus may play an important role when rapid treatment decisions are required.

Research Grant: USDA-AFRI (Agriculture and Food Research Initiative)

Student Support: Dr. Boyd and Anne Epperson Summer Research Fellowship

Understanding the role of avian hosts in the sylvatic transmission of Mayaro virus

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Mayaro virus (MAYV) is a mosquito-borne alphavirus first discovered from infected Trinidadian forest workers in 1954. Since its discovery, MAYV has caused several relatively small-scale outbreaks in South America and the Caribbean, although the true breadth of its impact is unknown due to similarities in disease presentation to more well-characterized arboviruses like dengue and chikungunya. Currently, the major sylvatic transmission cycle is thought to occur between South American non-human primates and mosquitoes of the genus *Haemagogus*, but MAYV has been isolated from several other species in the field, including birds, small mammals, and various mosquitoes. The role of these other vectors and hosts in maintaining MAYV transmission has not been elucidated. The aim of this study is to test the ability of various avian hosts to replicate and transmit MAYV, and determine whether these can contribute to MAYV transmission in nature. Since several species of birds are migratory, with multiple major flyways between the North and South American continents, the ability of birds to replicate and transmit MAYV would be a significant finding and cause for concern. If an infected bird were to be fed upon by a competent vector in the North American continent, MAYV could be spread well beyond its current range. Chicken (DF-1), duck (PDE), and quail (QNR/K2) cell lines were infected with MAYV strain 12A at an MOI of 1 and 0.1, and viral growth was quantified over 3 days. While the virus did not replicate well in DF-1 cells, it reached significantly high titers in PDE cells (6.18 Log₁₀ PFU), suggesting future *in vivo* studies in ducks and other waterfowl are warranted.

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Student Support: Boehringer Ingelheim Veterinary Scholars Program

A selective BCL-2 inhibitor impairs canine B-cell lymphoma cell survival, *in vitro*

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Canine Lymphoma (cL) is a leading cause of cancer-related death in dogs. Current gold standard treatment is a multi-agent chemotherapy protocol centered around doxorubicin, one of the most potent single agent drug against cL. Despite initial favorable responses to treatment, the majority of dogs with cL succumb to their disease indicating the need for alternative treatment strategies. BCL-2 is an anti-apoptotic protein and critical for cell survival. Venetoclax (ABT-199), an oral selective BCL-2 inhibitor, was recently approved for use in humans with chronic lymphocytic leukemia. The focus of this study was to evaluate the effects of venetoclax on cell growth, viability, apoptosis and cell cycle in a canine B-cell lymphoma (17-71) cell line. Cells were cultured for 72h in the presence of venetoclax or doxorubicin at increasing concentrations to establish half-maximal inhibitory concentrations (IC₅₀). Cells grown with IC₂₀ and IC₅₀ concentrations for 72h were stained with 7-Aminoactinomycin D (7-AAD) and Annexin V to measure early and late apoptotic and necrotic cells, or with propidium iodide (PI) for cell cycle analysis and then analyzed by flow cytometry. Venetoclax inhibited cell proliferation, while cell enumeration analysis indicated venetoclax IC₅₀ was more cytotoxic than doxorubicin. Further, a numerically higher proportion of venetoclax-treated cells were in the early and late stages of apoptosis compared to either untreated or doxorubicin-treated cells. Venetoclax disrupted the G0/G1, S and G2/M phases of the cell cycle. Overall, these preliminary *in vitro* findings suggest that venetoclax represents a promising treatment approach to improve outcome in dogs with B-cell lymphoma.

Research Grant: VMES 2926GR207002

Student Support: Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Non-steroidal anti-inflammatory drugs reduce vas deferens epithelial MRP4 expression and prostaglandin export

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Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit prostaglandin (PG) synthesis and can compromise epithelial barriers. The present study attempted to characterize whether NSAID-induced alterations occurred in the male reproductive tract, which typically expresses high levels of PG synthase 2 (PTGS2). Immortalized porcine vas deferens epithelial cells (PVD9902) were grown on Snapwell permeable supports in the absence or presence of NSAIDs (Ibuprofen, Celecoxib, Indomethacin) and transepithelial electrical resistance (R_{te}) was measured daily. On day 14, NSAIDs were withdrawn, and tests were performed on days 14, 16 and 18. Mucosal and serosal media were harvested to determine PGE_2 concentrations. Electrophysiological assessments were conducted followed by harvesting total cell protein and RNA. Monolayers in all conditions developed a 'tight' epithelial barrier with $R_{te} > 4000 \Omega cm^2$. NSAID exposure increased time to half-maximal barrier formation by 13-35 hours, and reduced the overall barrier development rate. Absent stimulation, PG accumulates preferentially in the mucosal medium, although serosal accumulation can be detected. NSAIDs reduced PGE_2 in both mucosal and serosal media to undetectable levels. We speculated whether PG export from the cell was due to the multidrug resistance-like protein (MRP4). Immunoblot and RT-PCR confirmed MRP4 expression, which was reversibly reduced by NSAID exposure. Our results show that NSAID exposed male reproductive duct epithelia exhibit decreased PG secretion (> 95%), delayed barrier formation and reduced expression of a major PG export protein. These results suggest that therapeutic NSAID treatments may have a negative effect on male reproductive tracts, thus affecting fertility.

Research Grant: Kansas State University College of Veterinary Medicine (SMILE)

Student Support: Boehringer Ingelheim Veterinary Scholars Program; KSU-CVM Dean Fund

Surveillance of flaviviruses by use of LAMP and qRT-PCR in insect vectors

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Zika virus (ZIKV), a member of the *Flaviviridae* family, has demonstrated vector-borne transmission primarily by *Aedes spp.* mosquitoes. Following ZIKV infection, an infected individual may present asymptotically or with clinical symptoms such as a rash, febrile illness, joint pain, or conjunctivitis. Similarly, other viruses within the *Flavivirus* genus such as Dengue (DENV), West Nile (WNV), and Yellow Fever (YFV) can elicit a similar clinical response, so it is essential to detect flaviviral RNA in biofluids and insect vectors. A highly sensitive assay known as loop-mediated isothermal amplification (LAMP) has been developed for the Puerto Rican ZIKV strain, PRVABC59. This assay utilizes *Bacillus stearothermophilus* (Bst) DNA polymerase to amplify viral RNA from mosquitoes and human biofluids without prior RNA isolation or reverse transcription. Furthermore, quantitative RT-PCR (qRT-PCR) has been developed for the general detection of flaviviruses with the use of pan-flavivirus primers that hybridize to the highly conserved NS5 gene. Crude homogenates and isolated RNA obtained from mosquitoes collected in Lajas, Puerto Rico, were screened using the ZIKV LAMP, ZIKV qRT-PCR, and the pan-flavivirus qRT-PCR assays for the detection of flaviviruses in this sample pool. Additionally, pan-flavivirus PCR amplicon sequences were used to identify flaviviruses present in the mosquito samples.

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Macrophages facilitate angiogenesis in mammary tumors of obese mice

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Obese women diagnosed with breast cancer, on average, have larger and more aggressive tumors. Obesity induces a state of chronic inflammation within mammary adipose tissue, and we observed that tumors in obese mammary tissue contain a greater number of macrophages than tumors from lean tissue. The function of these tumor-associated macrophages has not been well-characterized. We hypothesize that obesity-activated macrophages stimulate angiogenesis within mammary tumors to facilitate tumor growth and progression. To examine this hypothesis, mice were fed either a high fat diet (60% kcal from fat) or a control diet (10% kcal from fat) for 16 weeks, then Met-1 tumor cells were transplanted into the inguinal mammary glands. Mouse weight and tumor diameter were measured weekly. At end stage, mice were euthanized, and histological sections of the tumors were analyzed for the presence of CD31, an endothelial cell marker, via immunofluorescence and areas of tumor necrosis were quantified. We collected macrophages from the primary tumors, isolated RNA and examined gene expression for angiogenic promoters and inhibitors using qPCR arrays. Obese mice had significantly larger tumors with smaller areas of necrosis on tissue sections and expressed higher levels of CD31. These data suggest that the microenvironment of obese tumors promotes tumor growth and angiogenesis. Gene expression analyses suggest that macrophages from tumors of obese mice had increased expression of angiogenesis promoting factors with decreased expression of angiogenic inhibitors compared with lean mice. Due to their association with angiogenesis and tumor proliferation, macrophages may be an important target for treatment of obesity-associated breast cancer.

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Student Support: NIH T35OD01107

Increased expression of a marker of endoplasmic reticulum stress in an experimental equine laminitis model

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Laminitis is a debilitating disease characterized by tissue damage and loss of mechanical integrity of the dermal lamellae of the distal phalanx and the epidermal lamellae of the inner hoof wall. Hyperinsulinemia (HI) is a major risk factor for most naturally-occurring cases of laminitis and has been used to induce experimental laminitis. We hypothesize that HI overstimulates protein synthesis, which causes Endoplasmic Reticulum (ER) stress and activation of the Unfolded Protein Response, resulting in increased expression of the ER chaperone protein Grp78/BiP (BIP). Moreover, we hypothesize that cooling the foot and reduced weight-bearing in hind (relative to fore) feet reduce both BIP expression and laminitis pathology. Lamellar tissue from a fore foot (HI fore), a hind foot (HI hind), and a cooled fore foot (ICE) from eight Standard-bred horses that had undergone HI-induced laminitis were evaluated for BIP expression by indirect immunofluorescence (IIF) and confocal microscopy. Serial sections from the same samples used for IIF were stained with periodic acid-Schiff and hematoxylin (PASH), imaged, and the displacement of the secondary dermal lamella (SDL) relative to the keratinized axis of the epidermal lamella (KA) was determined as a quantitative measure of laminitis severity. Increased BIP expression was localized to epidermal and dermal cells in the lamellar regions from HI fore and HI hind feet that also displayed the most tissue damage and increased KA-SDL displacement. These changes were reduced in the ICE feet. We conclude that ER stress, as detected by BIP expression, is associated with laminitis histopathological severity in acute HI-induced laminitis, and that both are prevented by cooling.

Research Grant: American Association of Equine Practitioners Foundation

Student Support: Boehringer Ingelheim, NIH T35 Training Grant T35OD010919

Comparative hematology in a One Health approach - inappropriate metarubricytosis in canine and human diseases

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While inappropriate metarubricytosis is documented in humans, this condition is not well-defined in veterinary medicine. In dogs, inappropriate metarubricytosis has been reported in lead toxicity, heat stroke, and chemotherapy, and suspected to be associated with bone marrow injury, sepsis/endotoxemia, and pancreatitis. Prognostication of metarubricytosis in dogs has been restricted to heatstroke. Our retrospective study of canine inappropriate metarubricytosis examined if similar diseases contribute to the occurrence of nucleated red blood cells (nRBCs) in dogs as in people. Also, nRBCs/100 WBCs were analyzed for morbidity outcomes. From 2013-2018, a review of 1759 canine patients resulted in a selection of 75 patients with 122 laboratory reports based on a reported hematocrit $\geq 37\%$ (reference interval 37-55%) and > 5 nRBCs/100 WBCs at clinical presentation. Our analysis determined that canine inappropriate metarubricytosis is associated with heat stroke, lead poisoning, bone marrow injury/dyserythropoiesis, pancreatitis, sepsis/endotoxemia, abnormal splenic function with a subcategory of hemangiosarcoma, neoplasia (with or without chemotherapy), disseminated intravascular coagulation/thromboemboli, hypoxia, intervertebral disc disease, hepatic dysfunction, and diabetes (with or without ketosis). Mortality data did not support a significant difference between deceased or alive patients. The breeds listed as mixed breed (N=12), beagles (N=7), and corgis (N=6) were overrepresented, which requires further investigation. This first phase of our study indicates while humans and dogs share similarities in diseases associated with inappropriate metarubricytosis, certain conditions may be unique to canines.

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CCK-58 prolongs the intermeal interval, whereas CCK-8 reduces this interval

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It has been accepted for decades that “all forms of cholecystokinin (CCK) have equal bioactivity,” despite accumulating evidence to the contrary. To challenge this concept, we compared two feeding responses, meal size (MS, 10% sucrose) and intermeal interval (IMI), in response to CCK-58, which is the major endocrine form of CCK, and CCK-8, which is the most abundantly utilized form. Doses (0, 0.1, 0.5, 0.75, 1, 3 and 5 nmol/kg) were administered intraperitoneally over a 210-min test to Sprague Dawley rats that had been food-deprived overnight. We found that (1) all doses of CCK-58, except the lowest dose, and all doses of CCK-8, except the lowest two doses, reduced food intake more than vehicle did; (2) at two doses, 0.75 and 3 nmol/kg, CCK-58 increased the IMI, while CCK-8 failed to alter this feeding response; and (3) CCK-58, at all but the lowest two doses, increased the satiety ratio (IMI between first and second meals (min) divided by first MS (ml)) relative to vehicle, while CCK-8 did not affect this value. These findings demonstrate that the only circulating form of CCK in rats, CCK-58, prolongs the IMI more than CCK-8, the peptide generally utilized in feeding studies. Taken together, these results add to a growing list of functions where CCK-8 and CCK-58 express qualitatively different bioactivities. In conclusion, the hypothesis that “all forms of cholecystokinin (CCK) have equal bioactivity” is not supported.

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Phenotypic and transcriptomic characterization of canine myeloid-derived suppressor cells

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Myeloid-derived suppressor cells (MDSCs) are key players in immune evasion, tumor progression, and metastasis. MDSCs accumulate under various pathological states and fall into two functionally and phenotypically distinct subsets in humans: polymorphonuclear (PMN)-MDSCs and monocytic (M)-MDSCs. Increased MDSC frequencies in the peripheral blood of cancer patients correlate with poor prognosis, suggesting that these cells may represent a novel therapeutic target in cancer. Dogs develop spontaneous tumors that share many features of human cancers, serving as a large animal cancer model. We characterized the phenotypic and transcriptomic signatures of MDSCs in the dog as a prelude to the future therapeutic manipulation of these cells. We identified MDSCs as hypodense MHC class II-CD5-CD21-CD11b+ cells that can be subdivided into PMN-MDSC (CADO48A+CD14-) and M-MDSC (CADO48A-CD14+) subsets. The transcriptomic signatures of PMN-MDSCs and M-MDSCs were distinct from each other, and from those of conventional PMNs and monocytes respectively. Peripheral blood frequencies of PMN-MDSCs and M-MDSCs were significantly different in dogs with cancer compared to healthy control dogs. Furthermore, a comparison of our canine MDSC RNA-Seq data with previously published human and murine MDSC transcriptomic data highlighted five commonly upregulated genes in PMN-MDSCs in all species, suggesting that these genes are evolutionarily conserved and functionally important. Our findings therefore demonstrate for the first time that dogs have two distinct populations of MDSCs, characterized by specific phenotypic and transcriptomic signatures that share key features of human MDSC subsets.

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Prevalence of *Aelurostrongylus abstrusus* and *Toxoplasma gondii* in feral cats in the Willamette Valley

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There has been a recent increase in lungworm diagnoses at the Oregon Veterinary Diagnostic Laboratory (OVDL), and it is of interest to determine infection prevalence and to confirm that the feline lungworm species *Aelurostrongylus abstrusus* is responsible. Similarly, the prevalence of active *Toxoplasma gondii* infections in the cat population of the Willamette Valley is unknown. The hypothesis of this study is twofold, stating that all feline lungworm infections that are being presented to the OVDL are due to *A. abstrusus*, and that the prevalence of active *Toxoplasma* infections in the Willamette Valley will be less than the 6% prevalence found in a similar study performed in Virginia (Lilly & Wortham 2013). Fecal samples will be collected from shelters in Willamette Valley, mainly targeting cats known to have outdoor access. Sex and approximate age of cats will be recorded. Samples will be collected as soon as possible after cats are admitted to the facility. These samples will be held a maximum of 2 days at 4C before processing. This will involve running both a fecal flotation and a Baermann. Any larvae recovered by Baermann will be tested by PCR to confirm the identity of *A. abstrusus* using published techniques (Annoscia et al 2014). Similarly, products from flotation will be tested by PCR for the presence of *Toxoplasma* oocysts (Kompalic-Cristo et al 2007).

Research Grant: Boehringer-Ingelheim Graduate Summer Program

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Expression and secretion of high mobility group box-1 in lipopolysaccharide-activated canine platelets

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High mobility group box 1 (HMGB1) is a pro-inflammatory and pro-thrombotic alarmin released during inflammation and sepsis. Human and murine platelets are shown to express and secrete HMGB1 in sepsis augmenting neutrophil and platelet function. Expression of platelet-derived HMGB1 has never been investigated in dogs. This project aims to characterize the expression of HMGB1 in canine platelets stimulated by lipopolysaccharide (LPS). Washed platelets, isolated from 7 healthy dogs, were activated with 5 µg/ml LPS in the presence or absence of 10 µM adenosine diphosphate (ADP). Platelets were labelled with 3 monoclonal antibodies to identify platelet integrin β3a (CD61), P-selectin (CD62p) and HMGB1 on the platelet surface using flow cytometry. Surface expression of HMGB1 was assessed by fold-change in mean fluorescence intensity (MFI) and proportion of platelets expressing HMGB1. Thrombin-activated platelets had significantly higher MFI fold-change compared to ADP-stimulated platelets (0.26 vs 0.017, $P=0.0041$). ADP- or LPS-stimulated platelets had no significant increase in surface expression of HMGB1 compared to un-stimulated (resting) platelets ($P=0.85$, $P=0.41$) while ADP-priming prior to LPS stimulation resulted in increased HMGB1 expression when compared to resting platelets ($P=0.02$). Currently, additional experiments are being conducted measuring platelet HMGB1 secretion using a commercially available ELISA kit. Our current data suggests that ADP-priming in the presence of LPS stimulation increases HMGB1 surface expression.

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Student Support: NIH T35 Training Grant

True colors: the frequency of hidden alleles in canine coat color

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Few species match the physical diversity of *Canis lupus familiaris*, the domestic dog. There are hundreds of recognized breeds, defined by genetically-determined physical characteristics. Epistasis, the masking of a gene's expression by the presence of a different gene variant, results in some alleles being "hidden" or not phenotypically visible, despite being present. Data from over 12,500 purebred dogs, representing over 250 breeds, was evaluated at 7 coat color (full and dilute expression of pheomelanin and eumelanin, white spotting, and color patterns) and 5 physical characteristic (ear set, skull shape, tail length and coat type) genes. Allele frequencies at each locus were calculated by breed and related breed groups, revealing hidden variation. For example, American Eskimo Dogs, UK Schipperkes, and Flat Coated Retrievers carry the "e" allele (yellow) 92.2, 41.7 and 7.3 percent of the time, respectively. In American Eskimo Dogs, yellow is masked by other genes and persists because it is not under selection pressure. In both Schipperkes and Flat Coated Retrievers, yellow is undesirable, so may go unreported in litter records. Unexpected alleles were occasionally observed; for instance, based on physical appearance, the Polish Greyhound was expected to be more closely related to UK sighthound breeds. However, we found it more likely developed from Mediterranean sighthounds due to the high allele frequency of a coat color trait that has higher frequency in those breeds. Hidden allele frequencies provide information about the history and relatedness of breeds, and allows estimation of when specific coat color variants were first observed.

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Safety/efficacy of novel isonitrile compounds as candidates for treatment of resistant *Staph. pseudintermedius*

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This study tested the hypothesis that aryl isonitrile compounds will significantly reduce *Staphylococcus pseudintermedius* replication and survival in planktonic culture and biofilm, and are not toxic to mammalian cells. Aryl isonitrile compounds were recently shown to have potent activity against drug-resistant *Staphylococcus aureus*. A panel of 38 compounds synthesized in Dr. Mingji Dai's laboratory at the Purdue Institute for Drug Discovery was screened against two *S. pseudintermedius* isolates; 13 compounds with minimum inhibitory concentration (MIC) ≤ 4 μ M were evaluated further against a panel of 13 isolates. *Staphylococcus pseudintermedius* isolates from animals treated at Purdue University's Veterinary Teaching Hospital were obtained from the Indiana Animal Disease Diagnostic Laboratory. Minimum bactericidal concentration (MBC) and MIC were tested using Clinical and Laboratory Standards Institute methods. Efficacy against biofilm was tested using the 96 well plate method. Toxicity for mammalian cells (Madin-Darby canine kidney (MDCK) cells) was assessed using MTS (3-[4,5-dimethylthiazol-2-yl]-5-[3-carboxymethoxy-phenyl]-2-[4-sulfophenyl]-2H-tetrazolium, inner salt, Promega) assay. MBC data suggest that the compounds are bacteriostatic. Time kill assays are underway to further address this issue. Compounds were not toxic to MDCK cells at concentrations above MIC (256 μ M) and showed good ability to disrupt established biofilm. In summary, aryl isonitrile compounds had potent activity against planktonic *S. pseudintermedius*, were not toxic for mammalian cells, and effectively disrupted established biofilms. Further analysis is currently being conducted to investigate structure-activity relationships of the compounds.

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Student Support: Meriel Veterinary Research Scholars Program, Purdue University College of Veterinary Medicine

The role of exocrine pancreatic inflammation in the pathogenesis of canine diabetes mellitus

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Diabetes Mellitus (DM) is a chronic and debilitating metabolic disease that affects hundreds of thousands of dogs worldwide. Canine DM is characterized by pancreatic β -cell loss, leading to insulin deficiency and persistent hyperglycemia. DM and exocrine pancreatic inflammation (pancreatitis) are closely associated, and spillover inflammation from pancreatitis to neighboring endocrine tissue and collateral pancreatic endocrine tissue injury has been proposed as a potential etiology for canine DM. This project aims to determine if direct 'collateral' damage to endocrine islets due to concurrent pancreatitis may be driving DM in dogs. We have collected pancreatic tissues from dogs that had confirmed DM only (n=10), pancreatitis only (n=10) and DM with concurrent pancreatitis (n=10), along with healthy controls (n=5). We investigated these tissues via 4-color confocal microscopy and determined inflammatory cell infiltrate (CD45) distribution and islet cell composition (insulin and glucagon). To avoid bias we have imaged the entire pancreas tissue on each slide, and have further collected 10-15 high resolution images of individual islets. Number of islets, islet size, CD45+ cell distribution, and β -cell (insulin+) to α -cell (glucagon+) ratio were analyzed. Preliminary results confirm that dogs with DM only have marked islet hypoplasia characterized by a marked β -cell loss. Thus far, we did not see any evidence of insulinitis (islet infiltration by CD45+ cells) in diabetic pancreata. Information from this project will shed light on the role of pancreatitis in the pathogenesis of DM in dogs and may aid in designing novel bio-markers, prognostic markers, and therapeutic approaches.

Research Grant: Center for Companion Animal Health, UC Davis

Student Support: NIH, STAR (Students Training in Advanced Research) Program

Establishment of a drug screen to repurpose drugs targeting the induction of DNMT3B in human breast cancer

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Metastasis accounts for up to 90% of cancer-related mortalities. In human breast cancer, the 5-year survival rate drops from 90% to 27% if the cancer has spread to a distant part of the body. Triple-negative breast cancer (TNBC) is a subtype of breast cancer that currently has no targeted therapies, often leading to a poorer prognosis compared to other breast cancer subtypes. Recent work in the lab revealed that induction of DNMT3B, a member of the DNA methyltransferases, promotes metastatic colonization through epigenetic reprogramming. Therefore, DNMT3B provides an attractive therapeutic target to reduce metastatic lesions in patients. The aim of this project is to establish TNBC cell lines that report expression of DNMT3B to screen for drugs that can be repurposed for the inhibition of DNMT3B induction. Cell lines used include MDA-MB231-LM2 and 4T1, which are human and mouse TNBC cell lines respectively. NanoLuc with a 5' bicistronic P2A-peptide was knocked in behind the terminal exon of the endogenous *Dnmt3b* gene using the CRISPR/Cas9 system. Potential positive clones will be verified by sequencing amplified PCR products around the knock-in region and functionally validated with qPCR, western blots, and luciferase assays to ensure that luciferase activity faithfully reflects changes in DNMT3B transcription. Successfully established cell lines can then be used to screen over 2500 FDA-approved compounds from the Pharmaceutical Collection drug library at the National Center for Advancing Translational Sciences (NCATS). This process would be valuable in identifying potential drug candidates that can be repurposed for reducing the likelihood of metastatic colonization, thus improving overall prognosis in TNBC patients.

Research Grant: Center for Cancer Research, NCI, NIH

Student Support: NIH Summer Internship Program in Biomedical Research

A retrospective clinicopathological study of myocarditis in dogs: 70 cases (2007-2017)

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Myocarditis is a rare, but life-threatening clinical syndrome in people and animals. It is typically part of a systemic inflammatory process, although it can occur alone. Regardless, the etiology of myocarditis is often unknown and its diagnosis is often an unexpected finding at necropsy. The goal of this study was to characterize the clinicopathological trends from patients with necropsy-confirmed myocarditis, and to investigate the utility of PCR to identify the etiology of myocarditis in previously unknown samples. Medical records (2007-2017) were screened for time of year; location; patient breed, age, and gender; type of lesion; and etiology (if known). Sample DNA from cases with unknown etiology was extracted from formalin-fixed paraffin-embedded (FFPE) tissues and amplified by PCR. Clinically, there were no trends in seasonality, geographic location, age or gender. German Shepherd Dogs (GSD) were over-represented in this cohort. Etiologies were confirmed at necropsy in 22/70 cases. Of the remaining unknown samples, all tested (27) by PCR for prokaryotic 16s ribosomal subunit were negative and PCR specific for trypanosomal satellite DNA was positive for 1/70 samples. In summary, GSD were overrepresented and identifying bacterial etiologies from FFPE tissues by PCR was unsuccessful. GSD are prone to systemic fungal infections, which is often accompanied by myocarditis.

Research Grant: Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University

Student Support: Oklahoma State University CVHS 2018 Summer Research Training Program

Common practices in cold sterile use in veterinary clinics and prevalence of bacterial contamination

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Cold sterilization is commonly used in veterinary practices, but the frequency and manner of use is not described in the literature. The efficacy of cold sterilization is unknown. There is concern that use of cold sterile promotes bacterial growth and emergence of bacterial resistance. The objectives of this study were to describe the prevalence of cold sterile use among veterinary practices, determine prevalence of bacterial contamination, and describe the knowledge attitudes and practices (KAP) of instrumentation sterilization. A sampling frame was generated using web-based searches and a grid system within Google Maps™. A total of 1,464 veterinary clinics and shelters in WA, ID, and OR were identified. Of these, 451 were randomly selected for enrollment. At each clinic a 20-minute oral KAP survey was conducted with a veterinarian and veterinary technician. At locations where cold sterile was in use, a 15ml aliquot of sterilant was collected from each cold tray. Samples were concentrated then cultured on selective media in both aerobic and anaerobic conditions. Bacterial isolates were identified using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF). Upon study completion, data will be stratified by practice type and urban/non-urban setting. Prevalence of bacterial contamination will be determined and the 95% confidence interval will be calculated as the exact binomial around the point estimate. Using survey data and culture results, logistic regression will be used to identify risk factors for bacterial contamination of cold sterile trays. Based on study findings, Best Practices guidelines for the use of cold sterile in veterinary medicine will be developed.

Research Grant: None

Student Support: PetSmart Charities and the Washington State University College of Veterinary Medicine

Meniscal sizing based on morphometric measurements in canines: a cadaveric study

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Information regarding the utility of meniscal allograft transplantation is sparse in veterinary literature. In humans, meniscal transplantation is frequently performed with good outcome and a correlation between height, weight, and gender and meniscal dimensions has been documented. To our knowledge there is no protocol available to predict meniscal allograft size in dogs. Therefore, the purpose of our study was to determine if there is a correlation between morphometric measurements and meniscal dimensions in dogs. Eighteen mix-breed skeletally mature canine cadavers were obtained which provided 36 of each medial and lateral menisci. The following morphometric measurements were obtained in duplicate: body weight, gender, BCS, shoulder height, vertebral height at shoulder, pelvic height, vertebral height at pelvis, height at greater trochanter, hock to stifle length, chest circumference, pelvic circumference, occiput to tail base length, and body fat percentage was calculated. The meniscal length and width measurements were obtained in duplicates using photographs taken of the menisci with a ruler on the same sagittal plane as the meniscus. Hock to stifle length (HSL) had the highest correlation value at 0.77, 0.76 and 0.85 for medial meniscal length, width and lateral meniscal width respectively. Vertebral height at pelvis (VHP) had the highest correlation value at 0.73 for lateral meniscal length. When normalized to body-weight, medial meniscal width was significantly larger in males. HSL and VHP were found to be proportional to meniscal dimensions and should be considered by tissue banks and veterinary surgeons as quick and cost-effective variables when predicting meniscal dimensions.

Research Grant: Summer Scholars Program, Louisiana State University School of Veterinary Medicine

Student Support: Dr. Kenneth F. Burns Lectureship and Clinical Clerkship Foundation

Is cardiac cachexia an independent risk factor for mortality in dogs with congestive heart failure?

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Congestive heart failure (CHF) is a common condition in both human and veterinary medicine with effects extending beyond the cardiovascular system. One important systemic effect of CHF is muscle loss, or cardiac cachexia, which is associated with significant morbidity in humans and dogs. More importantly, in humans, cardiac cachexia is an independent risk factor for mortality. In dogs, however, an association between cardiac cachexia and survival has not been reported. This information is important for advancing the diagnosis, prevention, and treatment of cardiac cachexia in companion animals. In order to study associations with survival, we first need to determine the optimal definition for cardiac cachexia. Most human studies have relied upon the loss of body weight to diagnose cardiac cachexia. However, this method is considered to be insensitive as muscle is the primary tissue affected by cachexia and total body weight may not be affected until late in the disease, particularly in patients with fluid retention. Therefore, in order to optimize the definition for cardiac cachexia, we are first retrospectively reviewing the medical records of 300 dogs with CHF to compare muscle condition score to percent weight loss as the criterion for diagnosing cardiac cachexia. Our hypothesis is that there will be a greater prevalence of dogs with cardiac cachexia based on muscle condition score compared to the use of weight loss. Once we determine the optimal definition, our second aim is to evaluate the relationship between cardiac cachexia and survival in dogs with CHF, with the hypothesis that dogs with cardiac cachexia will have shorter survival times than those without cardiac cachexia.

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Student Support: NIH

Interaction between intestinal microbiota, tissue regeneration, and immune response following nerve injury

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Dysbiosis can alter the host immune response to injury and affect tissue repair and regeneration. However, the interaction between antibiotic-induced alterations in the intestinal microbiota, the immune response, and regeneration after nerve injury have not been examined. Macrophages are the major cell type in the first few days after nerve injury. They phagocytose myelin debris and encourage the proliferation and transdifferentiation of other cell types such as Schwann cells (SC). Macrophages also sense hypoxia at the injury site and secrete vascular endothelial growth factor (VEGF-A) which promotes development of a polarized microvasculature formed by endothelial cells that directs SC migration at the injury site. Without macrophages, regeneration does not occur. The purpose of this study is to determine the effects of antibiotic-induced alterations in the microbiome on macrophage function and the immune response to nerve injury. The intestinal microbiome was altered in C57BL/6J mice by oral gavage with an antibiotic cocktail for seven days or left unaltered (saline gavage, control). Experimental groups were matched for sex and litter. We isolated peritoneal macrophages and compared phagocytosis between dysbiotic and control animals following sciatic nerve injury using green fluorescent protein (GFP) bioparticles and flow cytometry. The supernatant from the peritoneal cells and inflammation at the injury site were evaluated for protein expression using a Luminex assay. Fecal samples were collected every two days and analyzed using 16S rRNA sequencing to confirm alteration of the microbiome. The results of this study will give insight into the interaction between the microbiome and the immune response following injury.

Research Grant: Boehringer Ingelheim Veterinary Scholars Program

Student Support: Cornell University Veterinary Investigators Program

Pharmacokinetic profile of insecticide synergist piperonyl butoxide in mice

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Piperonyl butoxide (PBO) is an insecticide synergist that entered mass production in 1952 and is currently found in over 1,500 household products. PBO was long thought to be an arthropod-specific inhibitor of enzymatic inactivation of insecticides but was recently discovered to be a Sonic hedgehog (Shh) signaling inhibitor in mammalian systems. Shh signaling is required for development of the forebrain and face, and genetic and chemical disruptions of this pathway are linked to etiologically complex human birth defects such as holoprosencephaly and orofacial clefts. Despite the long-standing use of PBO and its potential developmental toxicity, little work has been done to develop a pharmacokinetic profile in mammalian systems. Building from dose-response correlations for PBO-induced developmental toxicity in the mouse, the purpose of this project was to determine potentially harmful PBO concentrations following oral exposure. We assessed PBO levels in naive female C57BL/6J mice following oral gavage of 22.22mg/kg, 66.67mg/kg, and 200mg/kg doses of PBO. Serum samples were collected via maxillary bleed at various time points and PBO was quantitated by LC-MS/MS. Establishment of pharmacokinetic profiles of PBO exposure that causes developmental toxicity in the mouse is a necessary first step to understanding the potential of this environmental toxicant in contributing to human birth defect risk.

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Effects of vaccination on chlamydial load in *Chlamydia suis*-inoculated gilts

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Chlamydia (C.) trachomatis infections are the most prominent bacterial sexually transmitted disease, but the research to develop an effective vaccine is ongoing. Pigs have been demonstrated as a useful model for *C. trachomatis* vaccine development due to similarities to humans, including in the reproductive cycle and immune response of the female genital tract. In this study a porcine strain of chlamydia, *C. suis*, was used to investigate chlamydia infection due to it having similar disease outcomes to *C. trachomatis*, a high prevalence in pigs, and zoonotic potential. Previous research has shown that chlamydia particles are consistently detected in the vagina of gilts inoculated with *C. suis*. Our goal is to characterize the pathological and immunological responses caused by *C. suis* infections in vaccinated and unvaccinated gilts by inoculating them with *C. suis*, taking vaginal swabs at set days post infection (dpi) and following the infection by detecting chlamydial DNA via qPCR and characterizing viability through culture. Preliminary qPCR results show that in gilts inoculated with *C. suis*, the vaccinated groups had lower median levels of chlamydia at 2-3 dpi than the unvaccinated group. Additionally, by 7 dpi all 12 vaccinated gilts had cleared the infection, compared to only 3 out of the 6 unvaccinated gilts. The next step is to culture the swab samples to determine the viability of the detected chlamydial load. Future qPCR analysis of samples from the genital tracts extracted at necropsy will reveal whether or not the live chlamydia detected from the swabs produced an active infection.

Research Grant: NC TraCS Translational Research Grant 17-029-B

Student Support: NCSU CVM Veterinary Scholars Program

The impact of parasite infection on the canine microbiome

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Enteric parasitic infections, including protozoa (*Cryptosporidium* and *Giardia*) and helminths (roundworms and hookworms), are among the most prevalent infections in the developing world and have a tremendous impact on global public health. The microbiome is increasingly recognized as a key organ system that can have major impacts on host metabolism, development and immunity. A balanced and stable microbiome is critical for maintaining gastrointestinal health; disruptions in the microbiome due to external influences, such as antibiotics or infections, can significantly impact the health of patients, and can also lead to diarrheal disease as well as decreased capacity for energy harvest from dietary intake. Dogs and cats in the United States are routinely infected with gastrointestinal parasites, some of which are zoonotic, and their fecal samples are annually screened. In this study, we compared the composition and diversity of the bacterial microbiome in a cohort of 80 dogs. Fecal samples of dogs infected with single parasites, consisting of 39% helminth species, 37% yeast, and 24% protozoa, and healthy controls were obtained from patients at Ryan Hospital at the School of Veterinary Medicine of the University of Pennsylvania. The 16S rRNA V4 region was sequenced at an average of fifty-five-thousand reads per sample. We hypothesize that perturbation of the microbiome during enteric parasite infection will vary depending on the type of parasite, and that this perturbation worsens gastrointestinal disease during parasite infection.

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Diversity of marine apicomplexans

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Apicomplexans are protozoan parasites that cause disease in a wide variety of hosts both on land and in our oceans. The diversity of marine apicomplexans is severely under-studied, and many of these organisms can cause disease in commercially important species of fish, crustaceans, and mollusks. Phylogenetic analyses suggest that *Eimeria* spp. in terrestrial animals has evolved from *Eimeria* spp. infecting fish. The evolutionary history of marine Eimeriidae including *Eimeria* and *Goussia* spp. is unclear, and it is possible these have evolved from coccidians infecting marine invertebrates. The aim of this study is to investigate the diversity of marine apicomplexan parasites to determine the evolutionary history of marine coccidians including those that infect fish, mollusks, and corals. Our preliminary phylogenetic analyses demonstrate that *Goussia* and *Eimeria* are polyphyletic and tissue tropism could be more important than sporocyst morphology in determining phylogenetic clades. Analyses also suggest a link between coral-infecting apicomplexans and fish associated with coral reefs. This may indicate that fish were the first vertebrates to acquire these infections and it is possible that they acquired them from corals.

Research Grant: None

Student Support: 2018 Merial Veterinary Scholarship Award

Effects of short-chain fatty acids on murine intestinal epithelial cell cytokine gene expression *in vitro*

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The intestinal microbiota produce a variety of metabolites from prebiotics that are absorbed by the intestinal epithelium. Butyrate and propionate, short-chain fatty acids, are end products of prebiotic fiber digestion by microbes. These short-chain fatty acids are present in the intestinal lumen in nearly equivalent molar ratios, and are metabolized by epithelial cells to varying degrees. Both have been shown to exert anti-inflammatory effects and may have direct or indirect immunologic effects, influencing differentiation and modulation of T-cells in the gut-associated lymphoid tissue. The goal of this study was to determine the influence of short-chain fatty acids butyrate and propionate on intestinal epithelial cell cytokine expression. The MODE-K murine epithelial duodenal cell line was used. Cell culture was performed in RPMI+GlutMAX media supplemented with 10% FBS, 1% sodium pyruvate, 1% non-essential amino acids, 1% HEPES, and 0.1% β -mercaptoethanol and incubated in 5% CO₂ at 37° C. Effects of butyrate and propionate alone and in combination (0, 0.5, 1, 2, and 4mM) on cell proliferation and viability were measured over 12, 24, and 48 hours using the methyl-thiazolyldiphenyl-tetrazolium (MTT) and trypan blue exclusion assays. Significant decreases in cell proliferation were observed with doses higher than 2 mM butyrate alone or in combination. No significant differences in cell viability at any time point were observed for any dose. Cells were treated with propionate, butyrate, or both combined (0, 0.5, 1, and 1.5mM) for 4 hours. Cells were then challenged with TNF α (5ng/mL), and RNA was extracted after 2 hours. Further efforts will examine changes in gene expression of various cytokines using q-RT PCR.

Research Grant: None

Student Support: Oklahoma State University CVHS 2018 Summer Research Training Program

Antinociception in ball pythons (*Python regius*) using a clinically relevant model of pain

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Snakes are increasing in popularity as pets with veterinarians consequently treating more snakes in primary care practices. However, information regarding effective pain management in snakes is limited. Our lab recently showed that dexmedetomidine (alpha-2 adrenergic receptor agonist) appears to provide effective analgesia in ball pythons in response to noxious thermal stimuli, but also causes respiratory depression. To evaluate the use of dexmedetomidine (0.1 mg/kg) in a more clinically relevant pain model, we are performing coeliotomies on a group of ball pythons, and evaluating their behavior before and after surgery. Observed behavior includes tongue flicking, movement, posture, righting reflex, and aggressiveness. We hypothesize that ball pythons administered dexmedetomidine prior to surgery, will demonstrate fewer behavioral changes than ball pythons administered isovolumetric saline. In addition, to evaluate ball python respiration, we are testing whether doxapram (10 mg/kg; respiratory stimulant), co-administered with dexmedetomidine, stimulates breathing while still providing analgesia. Respiratory data are collected using closed chamber plethysmography to measure respiratory frequency and estimate tidal volume after drug administration. Analgesic efficacy is assessed using a Hargreaves apparatus, which measures withdrawal latencies in response to a noxious thermal stimulus. Initial results suggest that doxapram increases respiratory frequency and tidal volume, but does not interfere with the analgesic efficacy of dexmedetomidine. Depending upon the final results, the combination of dexmedetomidine and doxapram may provide a novel analgesic protocol for snakes, while minimizing concurrent respiratory depression.

Research Grant: Companion Animal Fund, School of Veterinary Medicine, University of Wisconsin – Madison

Student Support: School of Veterinary Medicine, University of Wisconsin – Madison

Investigating the ecology of mobile genetic elements in beef feedlot cattle using high-throughput sequencing

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Mobile genetic elements are of interest because they facilitate horizontal transfer of genetic features between unrelated species. The bulk of research on these elements has focused on investigating single elements in a single genome. This approach makes studying the broader relationships between mobile genetic elements and antimicrobial resistance difficult as it does not consider the more ecological, metagenomic considerations. It is possible to evaluate changes in microbiomes by evaluating shotgun sequencing data for both mobile genetic elements and antimicrobial resistance. We hypothesize that evaluating mobile elements in a metagenomic context will provide greater insight into factors affecting the resistome. In order to evaluate this hypothesis, we created a comprehensive genetic database of mobile genetic elements named MEG-Mobile from published genetic sequences. MEGMobile is a comprehensive annotated database which utilizes a taxonomic structure designed for high throughput data analysis. MEGMobile sequences were obtained from online searches of all previously published sequencing data for insertion sequences, phages, transposons, integrons, plasmids and plasmid incompatibility markers. These major groups are shown in current literature to be elements of primary interest. We used MEGMobile to investigate the composition of mobile elements in beef feedlot cattle, and evaluated the response to mass medication for disease prevention. Results show future use of this bioinformatics tool will allow a broader understanding of the ecological drivers of antimicrobial resistance.

Research Grant: Supported by funding provided by Beef Checkoff and USDA NIFA grant 2015-68003-23048

Student Support: USDA Animal Health & Disease Program NI17AHDRXXXXG018

Intravenous contrast-enhanced computed tomography: dosing and pharmacokinetics of iopamidol in *Cyprinus carpio*

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Koi carp (*Cyprinus carpio*) are a variety of common carp and are a species that has gained popularity as an ornamental fish. In a preliminary study, iopamidol, a contrast agent used for computed tomography (CT), was shown to be safe and effective for enhancing the visualization of vasculature and organs at a dose of 480mg of iodine per kg (mgI/kg). However, the efficacy of lower doses, distribution and elimination of the contrast agent in fish is unknown. The goals of this study were to determine if IV administration of iopamidol at a dose of 150mgI/kg is effective for CT imaging of adult carp, and to evaluate the pharmacokinetics and elimination of iopamidol at the effective dose(s). CT of carp (n=3) at doses of 150mgI/kg and 480mgI/kg were performed at ~5min and 1hr post-injection. Images were evaluated for objective parameters, including contrast distribution within organs using Hounsfield units, and qualitative parameters such as organ recognition and the ability to identify margins. The 150mgI/kg dose was not clinically effective, nor were the 1hr scans at either dose. Blood, bile, gills and kidney were collected after carp (n=6) were injected with 480mgI/kg IV at 5min, 1hr, 6hr, 24hr, 48hr. Samples were analyzed by liquid chromatography-mass spectrometry. Noncompartmental analysis for sparse data was performed on plasma iopamidol concentration using commercially available software. The total systemic clearance = 0.051mL/min/kg, volume of distribution at steady state = 79.9mL/kg, and the terminal phase half-life = 20.4hr. Our results suggest that diagnostic CT scanning using iopamidol as a contrast agent in carp is safe and effective and could be used to evaluate internal organs and diagnose coelomic diseases.

Research Grant: UC Davis Center for Companion Animal Health and Department of Medicine and Epidemiology, UC Davis-School of Veterinary Medicine

Student Support: Boehringer-Ingelheim Animal Health funding (Students Training in Advanced Research Fellowship)

Genetic diversity of feline foamy virus (FFV) in Florida panther (*Puma concolor coryi*)

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Feline Foamy Virus (FFV) is a spumaretrovirus that produces putatively apathogenic infections in feline hosts including puma and domestic cats. FFV is directly transmitted, causes lifelong infection, and does not produce measurable behavioral changes in its host. As such, the phylogeny of FFV can be used to infer contacts within free-ranging felid populations, including the endangered Florida panther (*Puma concolor coryi*). While the ecology of FFV in domestic cats has been studied, the genetic diversity and evolutionary rates have not been fully characterized in free-ranging puma populations. In this preliminary study, we adapted and optimized a multiplex PCR enrichment protocol to generate short, overlapping amplicons spanning the FFV genome. We demonstrate that this protocol is a sensitive screening tool for FFV and efficiently produces nearly whole genome sequence data for phylogenetic analyses. Using this approach, we screened 87 Florida panthers and 43 domestic cats to reveal a prevalence of 54% and 32% respectively. 30 samples from Florida panthers and 3 from domestic cats were sequenced on an Illumina MiSeq. The data will reveal the diversity of FFV within the sampled population as well as provide instruction for future employment of the multiplex PCR enrichment protocol. Our future direction is to use these preliminary results to compare evolutionary rates of FFV with those of FIV in the Florida panther and produce empirical viral phylogenies that can inform modeling of Florida panther population vulnerability to infectious disease outbreaks.

Research Grant: NSF EF-0723676; EF-1413925

Student Support: Georgia Norris Foundation

Evaluating contributions of amniotic fluid sterility, maternal and infant factors on primate infant mortality

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In a closed breeding colony of captive African green monkeys, infant mortality rates are consistently above 30%, with the majority of infant deaths occurring on day 0. Stillbirth and infant mortality accounts for about 1% of pregnancies in humans. In both populations, causes of stillbirth or early death remain unknown. However, the sterility of the intra-amniotic environment during pregnancy has come into question in regards to pregnancy outcome. We hypothesized that the amniotic fluid (AF) is not sterile and that older, heavier females would have bacteria present, correlating to increased likelihood of infant mortality. Two cohorts of AF samples (n=20) were collected via ultrasound-guided amniocentesis under clean conditions during the second or third trimester of gestation. After DNA extraction, quantitative PCR was performed using 16s bacterial DNA forward and reverse primers. Based on a Ct threshold of 30.5 cycles, 8/20 samples were positive for bacterial DNA (avg Ct=29.2±0.32). A gastrointestinal source was a positive control (Ct=27.7) and storing AF did not affect PCR values. Samples were evaluated in relation to numerous factors to determine potential correlations including age, weight, and waist circumference as a measure of obesity (maternal) and birth outcome, sex, birth weight, and head circumference (infant). Lighter ($P=0.013$) and thinner ($P=0.012$) mothers were significantly more likely to have bacterial DNA present in AF compared to heavier and obese mothers, countering our hypothesis. Bacterial presence also correlated with a better reproductive history. Because pregnancy is immunosuppressing, it is possible that a higher bacterial translocation signifies proper immunosuppression and pregnancy maintenance.

Research Grant: P40 OD010956, UL1TR001420, Wake Forest School of Medicine

Student Support: NIH T35 Training Grant T35OD010946

Comparison of two serological tests for detection of *Leptospira*-specific antibodies in dogs from Mexico

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Leptospirosis is a worldwide zoonotic disease most prevalent in areas with a wetter climate but cases have also been found in arid cities like Phoenix and Los Angeles most likely due to presence of reservoir hosts and irrigation. There are 3 serological tests in the United States used to aid in the diagnosis of leptospirosis. The microscopic agglutination test (MAT) is considered the gold standard as titers can be measured against multiple *Leptospira* spp. and serovars. However, MAT is complex and is only available in diagnostic laboratories which resulted in the development of 2 qualitative (positive or negative) point of care kits. The objectives of this study were to determine whether dogs in Baja Sur Mexico have antibodies against *Leptospira* spp. and to compare the results of 2 *Leptospira* spp. serological assays. In this study, sera from 100 dogs from an ongoing zoonotic disease study of client owned dogs in Todos Los Santos, Baja Sur, Mexico were selected. The samples are to be assayed by one rapid point of care test (SNAP Lepto, IDEXX Laboratories, Westbrook, ME, USA) and by MAT and compared. To date, the point of care assay results are complete and were all negative. MAT test results are pending. In previous studies in Mexico City and the Yucatan region using MAT, *Leptospira* spp. prevalence rates were estimated at 54% and 45%, respectively. Both of these regions are more humid than Todos Los Santos which may explain the differences in seroprevalence rates to date. In addition, the Mexico City samples were from shelter dogs in contrast to the client owned dogs in Todos Los Santos; the shelter dogs may have been at greater risk for exposure.

Research Grant: Center for Companion Animal Studies

Student Support: Young Investigator Grant, Center for Companion Animal Studies

Hypoglycemia in dogs after ingestion of metformin: a retrospective evaluation (2001 - 2017)

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Metformin is a biguanide oral antihyperglycemic agent used in the treatment of type 2 diabetes. Metformin intoxication can occur in pet dogs that ingest the prescription drug that is intended for human use. Most cases will resolve with fluid therapy and the prescription of gastrointestinal (GI) protectants. The Animal Poison Control Center (APCC) is a 24-hour consultation service that provides support to poisoned animals through advising both animal owners and licensed veterinarians across North America and collecting case information to be stored in the AnTox database. In this study, the AnTox database was searched for calls regarding metformin monointoxications in dogs to specifically search for common characteristics in previous cases that led to the development of hypoglycemia. Between the years 2001 and 2017, the APCC received 1,833 calls regarding metformin monointoxication and 4,281 regarding multiple-point intoxications with metformin. Twenty-four dogs ingested only metformin and developed hypoglycemia. Doses ranged from 29.76 mg/kg to 859.80 mg/kg, with a mean of 259.02 mg/kg. The other 1,809 dogs developed signs of GI upset, lactic acidosis, or no clinical signs. In these cases, the average metformin dose was 202.67 mg/kg, with a range of 1 to 3,000 mg/kg. Dogs that developed metformin-induced hypoglycemia were generally young, smaller breeds (0.2 - 2 years of age). There did not appear to be a link between metformin dosage and the development of hypoglycemia. Identifying the variables associated with metformin-induced hypoglycemia would improve monitoring of cases. This information is important because severe hypoglycemia can lead to a worse prognosis and more lethal sequelae, such as seizure and death.

Research Grant: None

Student Support: University of Illinois College of Veterinary Medicine

The effects of early life stress on diamine oxidase levels in swine and mice

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Rational: Early life stress (ELS) has been shown to have effects on the development of the gastrointestinal system, with patients who experienced early life stress being at higher risk of gastrointestinal disorders like irritable bowel syndrome (IBS) later in life. These developmental alterations to the gastrointestinal tract in turn affect the gut-brain axis, influencing neurobehavioural comorbidities such as anxiety or depression. Studies that have examined these effects in swine models have shown increased levels of histamine in comparison to control groups, with increased levels being linked to issues like gastrointestinal pain, depression, anxiety, or allergic inflammation. The mechanism by which ELS results in increased histamine levels is poorly understood. We hypothesized that diamine oxidase (DAO), an enzymes that help catabolize histamine, will be seen in reduced amounts in ELS models.

Methods: DAO and histamine will be compared between early weaning stress (EWS) and late weaning stress (LWS) swine as well as between maternal separation with early weaning (MSEW) and non-MSEW mice. Serum and tissue levels for DAO for the swine and mice will be assessed using ELISAs. Data analysis will be performed using t tests and 1 or 2 way ANOVA where appropriate.

Results: It is predicted that the EWS swine and the MSEW mice will show decreased levels of DAO in comparison to the LWS swine and non-restraint stress mice models.

Conclusion: Understanding the role of altered DAO expression in the elevated histamine levels and brain-gut axis dysfunction caused by ELS could reveal a new therapeutic target for the treatment of gastrointestinal and neurobehavioral disorders linked with ELS including IBS and depression/anxiety.

Research Grant: National Institutes of Health Grants NIH R01 HD072968 (to AJM) and NIH R21 AI140413 (to AJM) USDA-NIFA #2017-67015-26673 (to AJM)

Student Support: NIH Grant T35OD016477 to Michigan State University

MicroRNA regulation of development of corticospinal motor neurons

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Corticospinal motor neurons (CSMN) are a subtype of projection neurons that control precise voluntary movement in mammals. Paralysis results when their axons are injured and do not regenerate. Understanding CSMN development may help inform regenerative therapies for spinal cord injury. CSMN and a subset of callosal projection neurons (CPN) share common characteristics, including peak birth occurring on E13.5 and residing in the layer V of the cortex. Transcriptional regulators LMO4 and CITED2 are critical for CPN development. One mechanism by which these transcription factors may be regulated is by microRNA (miRNA) control. Tharin lab has identified a number of miRNAs enriched in CSMN. Based on bioinformatic analysis, miR-CSMN1 is predicted to inhibit LMO4 and CITED2, while miR-CSMN2 and CSMN3 target CITED2. Furthermore, Tharin lab has demonstrated that miR-CSMN1 regulates CSMN specification via LMO4 repression in embryonic cortical cultures. In this study, these 3 miRNAs were pooled and transfected early in mouse-ES cell differentiation and LMO4 expression was measured. Because multiple miRNAs can converge on a single target, we hypothesize that a pool of 3 miR-CSMNs will have enhanced repression of LMO4 expression and enhanced effects on the final neuronal fate of embryonic stem cells differentiated into CSMN than a single miR-CSMN1 alone. Our preliminary data shows a sharper decrease in LMO4 expression by a pool of 3 miRNAs in ES cells-derived-neurons compared to a single miR-CSMN1 alone. However, more data is required before we can conclude synergistic regulation of miRNAs on LMO4. Better understanding the role of these miRNAs in cortical cell fates may help develop novel miRNA based therapies for spinal cord injury.

Research Grant: Unknown

Student Support: NIH/ORIP T35 OD010989

Pain assessment via consumption of yogurt treats in rats treated with sustained-release meloxicam

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Minimizing pain in laboratory rats and its undesirable physiologic and behavioral impacts on biomedical research are necessary in science and medicine. However, the appearance and degree of pain is frequently ambiguous in rats. Recent advancements in pain assessment (e.g. rat grimace scale, gait, and automated analyses) have limitations including altered behaviors in response to humans and considerable financial investments. The aim of this study was to evaluate food motivation using high-value yogurt treats (Yogies), in association with a cage-side behavioral ethogram, to enhance pain assessment in rats. We hypothesized that rats treated with sustained-release (SR) meloxicam (4 mg/kg SC) will improve approaching and consuming Yogies compared to rats treated with saline post-surgically. Twelve female Sprague Dawley rats were separated into 3 groups of 4 rats each: bilateral ovariectomy with SR meloxicam, bilateral ovariectomy with sterile saline (equivalent SR meloxicam SC volume), and anesthesia with SR meloxicam. Ethogram data were collected 48 and 24 hours pre-operatively, and 3, 6, 12, 24, and 48 hours post-operatively. The rats took longer to approach and consume their Yogies 6 and 3 hours post-operatively, respectively. Overall, with the exception of orbital tightening, there was no statistical difference in any behavior within the ethogram between the study and control groups. The few differences observed might be a result of small sample sizes, but this study nonetheless demonstrates that treat consumption is not a dependable measure of pain. Notably, the limited behavioral disparity highlights the necessity for reliable and accessible pain measurements in laboratory rats.

Research Grant: Department of Molecular, Immunology, and Pathology, Office of the Vice President for Research; The American Society of Laboratory Animal Practitioners

Student Support: Pfizer

Using CRISPR-Cas9 to knockout the sterile alpha motif domain in the bicaudal C gene of zebrafish (*Danio rerio*)

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Genetic alterations in the Bicaudal C (*Bicc1*) gene have been shown to cause renal cysts in humans, mice, and zebrafish. The Bicc1 protein has two types of functional domains: a Sterile Alpha Motif (SAM) and multiple tandem K Homology (KH) domains. The KH domains are important for RNA binding and the SAM domain is involved in protein-protein interactions. We have shown previously that morpholino knockdown of the zebrafish bicaudal c gene, *zbicc2*, causes renal cysts, however, point mutations expected to alter the SAM domain only, did not result in a discernible phenotype. These same point mutations do cause renal cysts in both humans and mice. Our hypothesis is that the function of the *zbicc2* SAM domain in the kidney has not been evolutionarily conserved. In order to test this, the region coding for the SAM domain of *zbicc2* was selectively knocked out using CRISPR-Cas9 genome editing in zebrafish embryos. Embryo development was monitored and gross morphology was observed until 5 days post-fertilization, a time point at which the fish kidney is fully developed and functional. DNA was extracted from the fish and will be analyzed by PCR to identify fish with a deletion of the region of *zbicc2* that codes for the SAM domain. Correlation between any genetic alterations and renal phenotype will be noted. The results of these studies will guide the appropriateness of future use of zebrafish as a model organism to study Bicc1 function in the kidney.

Research Grant: None

Student Support: Boehringer Ingelheim

Computed tomography to evaluate hepatic attenuation in three species of freshwater turtles

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Freshwater turtle species are suffering from anthropocentric-caused population declines, making preservation of managed populations increasingly important. Turtles under human care have an increased risk to develop hepatic lipidosis, potentially resulting in early death. Computed tomography (CT) provides a more reliable and detailed approach to screen disease and provide antemortem diagnosis of increased fatty liver composition. Hepatic attenuation values (measured as Hounsfield units (HU)) of Vietnamese Pond turtles (10) and Northern Snake-Necked turtles (6) under human care and wild Blanding's turtles (95) were measured and recorded using CT studies. There were significant differences in hepatic attenuation between Vietnamese Pond, Northern Snake-Necked, and Blanding's turtles, with median HU values (95% confidence interval) of 5.39 HU (-6.45-61.50), 71.74 HU (59.44-94.49) and 95.42 HU (78.55-116.37), respectively. Bloodwork was performed in the two species under human care. The AST values were significantly higher in Vietnamese Pond turtles, and the HU were significantly lower. The lower attenuation values negatively correlated to higher AST. Blanding's turtles undergoing folliculogenesis presented with higher HU, suggesting a potential use of hepatic lipid stores for energy. Overall, the findings demonstrate species variation, with CT analysis that showed a negative correlation of HU and AST in two managed species; CT can therefore be used as a screening tool for hepatic lipidosis. Moreover, the results indicate further need to assess natural species variation on CT, which will aid in more accurate diagnosis.

Research Grant: None

Student Support: The Ohio State University College of Veterinary Medicine

Mammalian orthoreovirus as an oncolytic therapy and vaccine candidate for HER2 positive breast cancer

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Human epidermal growth factor receptor 2 positive (HER2+) breast cancer (BC) is a highly aggressive BC subtype diagnosed in 15% to 20% of reported cases. Recent advances in passive immunotherapy have improved HER2+ disease prognosis, however many patients are unresponsive or develop resistance to current therapies, highlighting the need for the development of additional methods of treatment and prevention of this disease. Mammalian orthoreovirus (MRV) is a clinically benign oncolytic virus that is being studied as a therapeutic option for cancer treatment. Pre-clinical and clinical studies have demonstrated its efficacy at preferentially infecting and killing tumor cells and aiding in tumor regression. MRV attacks cancer cells by inducing apoptosis, activating the adaptive immune system, and regulating cellular pathways that aid in tumor growth and therapy resistance. The aims of this research are to 1) measure the potential of MRV as an oncolytic therapy for HER2+ breast cancer and 2) develop a replicating recombinant MRV that infects and expresses HER2 peptide antigens on tumor cells as a vaccine candidate against HER2+ BC. Replication, apoptosis, and immunoblot assays have been performed to delineate the impact of T3D^C infection on HER2+ and HER2- cell lines. In addition, DNA plasmids encoding HER2 peptides from intact MRV gene segments have been created and confirmed via sequencing. Plasmids will be used in a reverse genetics system in attempts to recover a viable recombinant virus that expresses HER2 peptides and induces a response from the adaptive immune system. With greater understanding of the effects of MRV on HER2+ breast cancer we hope to contribute to the battle against this disease.

Research Grant: Margaret Barry Cancer Research Award

Student Support: Margaret Barry Cancer Research Award

Leptospirosis in shelter dogs and cats in the tristate area of Kentucky, Tennessee, and Virginia

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Leptospirosis, a worldwide zoonotic infection that affects dogs and many other mammalian species, including man, is caused by infection with pathogenic members of the genus *Leptospira*. Pathogenic leptospires live in the proximal renal tubules of asymptomatic carrier animals and are shed in the urine. Virtually any mammalian species can act as asymptomatic reservoir, characterized by chronic renal carriage and shedding of a host-adapted leptospiral serovar. Environmental contamination by these chronic shedders results in acquisition of infection by susceptible animals. In this study, we investigated if clinically normal shelter dogs and cats harbor leptospires in their kidneys by screening urine samples for the presence of *Leptospira* spp. Additionally, we measured *Leptospira*-specific serum antibodies to test correlation between seroprevalence and urinary shedding. The results showed that approximately 10.6% of the 244 shelter dogs and cats screened by qPCR were positive for leptospiral DNA in urine and 38 of the 254 animals screened with microscopic agglutination test (MAT) had a titer level greater than 1:100 across five tested *Leptospira* serovars. Twelve animals in the study showed positive results for both qPCR and MAT. Fourteen animals were positive with qPCR but not with MAT. qPCR-positive samples were genotyped to identify infecting leptospiral species. These findings have significant implications regarding animal and public health in the area and possibly outside where these animals may be adopted.

Research Grant: LMU-CVM Intramural Grant

Student Support: Pet Smart Charities Student Summer Research Program

Variability in home cage CO₂ euthanasia of the laboratory mouse

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Carbon dioxide inhalation is one of the most commonly used methods of euthanasia for mice in a research setting, but this technique lacks standardization. The Guide for the Care and Use of Laboratory Animals states that further study is needed to optimize methods for CO₂ euthanasia in rodents. At our institution, two methods are most commonly used to administer CO₂ for the euthanasia of laboratory mice. The first method utilizes a CO₂ tube, which is placed into the lixit port of an Individually Ventilated Cage (IVC). The second method replaces the IVC lid with a solid metal lid that has a central port for the CO₂ tube to enter the cage. The present study offers a comparison of two different points of entry of CO₂ gas, side entry vs. top entry, and two different lids, standard cage lid with filter vs. solid metal lid. The time to loss of consciousness and time to death between all study groups were measured. Behavioral markers of acute stress were monitored. It is hypothesized that due to CO₂ being heavier than air, the side entry will lead to a quicker time to unconsciousness compared to the top entry CO₂ and that the type of lid will have little impact on the time differences. This study and further research can help refine euthanasia practices for the common laboratory mouse.

Research Grant: None

Student Support: American Society of Laboratory Animal Practitioners Foundation

Lipin-1: a novel target for metabolic reprogramming of triple negative breast cancer cells

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Triple negative breast cancer (TNBC) accounts for 10-20% of all breast carcinomas. Because these cancers do not express HER2, progesterone, or estrogen receptors, standard receptor targeting therapies are ineffective. Propranolol, a commonly used beta adrenergic receptor (β -AR) antagonist, was found to increase recurrence-free survival and a reduce risk of metastasis in women with TNBC. Propranolol inhibits lipin-1, an enzyme that catalyzes the conversion of phosphatidic acid (PA) to diacylglycerol. A key target of PA is mTOR, which integrates nutrient and growth factor signals to control cell growth and proliferation. Lipin-1 is highly expressed in TNBC, and knockdown of lipin-1 induced apoptosis in the TNBC cell line MDA-MB-231. Propranolol has also been found to increase infiltrating lymphocytes in the tumors of treated mice and we predicted that while detrimental to breast cancer cells, inhibition of lipin-1 by propranolol may favor the proliferation of T cells. To test this hypothesis, we compared the effects of propranolol and desipramine, a tricyclic antidepressant and potent lipin-1 inhibitor with no β -AR activity, on viability, lipin-1 content and localization, and gene expression in MDA-MB-231 cells. We also assessed the effects of these drugs on T cell activation and proliferation. We found that desipramine killed tumor cells more effectively than propranolol and more profoundly altered the expression of genes associated with cholesterol and lipid synthesis. Propranolol increased T cell activation and proliferation in vitro, while desipramine did not alter these parameters compared with a vehicle control. Drugs that inhibit lipin-1 may provide a more effective way to inhibit TNBC while sparing T cell function.

Research Grant: Brainstorm Award, Masonic Cancer Center, University of Minnesota; Morris Animal Foundation D18CA-017

Student Support: Skadron Family Fund, College of Veterinary Medicine, University of Minnesota

Inducible *hGlp-1r* knockout in the mouse hypothalamus promotes feeding and body weight gain

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Obesity and its associated co-morbidities, such as type II diabetes mellitus, represent widespread and costly public health epidemics. The current lack of effective, long-lasting anti-obesity treatments necessitates a better understanding of the neurobiological systems that regulate feeding behavior and energy balance. Due to its established role in reducing food intake and promoting glycemic homeostasis, the glucagon-like peptide-1 (GLP-1) system has been a promising target for pharmaceutical interventions. However, the mechanisms through which this incretin hormone acts in the central nervous system remain unclear. Conditional knockout studies offer an elegant technique to investigate the effects of endogenous receptor signaling in a targeted tissue without compromising normal ontogenetic development. Using the Cre/loxP system, we generated an inducible, site-specific knockout model of the GLP-1 receptor (GLP-1R) in the mouse hypothalamus. Adult male C57BL/6 mice expressing floxed human *Glp-1r* (*hGlp-1r*) were maintained on a 60% high fat diet to achieve diet-induced obesity. Subsequently, mice were stereotactically injected with a Cre-expressing or control adeno-associated virus (AAV-Cre / AAV-Ctrl). Viral knockout of hypothalamic *hGlp-1r* caused increased food intake and body weight gain throughout the 14 days post-injection (Final 24h food intake: AAV-Ctrl = 3.06 ± 2.16 g, AAV-Cre = 4.30 ± 3.04 g, $P = 0.02$; Final body weight: AAV-Ctrl = 27.20 ± 0.10 g, AAV-Cre = 32.89 ± 1.04 g, $P = 0.06$). Altogether, these data demonstrate that endogenous GLP-1R signaling in the mouse hypothalamus is necessary to regulate energy balance.

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Student Support: Cornell University College of Veterinary Medicine Veterinary Investigator Program

Acupuncture at Stomach 36/Pericardium 6 does not reduce nausea and sedation during lidocaine infusion in dogs

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Intravenous lidocaine constant rate infusions (CRIs) provide many patient benefits such as reduced post-operative opioid use, visceral inflammation, and sympathetic nervous system stimulation. However, significant sedation and nausea/vomiting are associated with lidocaine CRIs. We hypothesized that stimulation of visceral acupoints Pericardium 6 (PC6) and Stomach 36 (ST36) would reduce nausea/vomiting but would not mitigate increases in gastrointestinal motility also associated with lidocaine CRIs. Barium infused polyethylene spheres (BIPs) were fed to healthy dogs in a small meal. Three view abdominal radiographs were obtained immediately, followed promptly by: 1) bilateral targeted acupuncture at PC6 and ST36, 2) bilateral sham acupuncture at Lung 5 and Bladder 55 and 3) no acupuncture for 30 minutes in a randomized, crossover design. At the beginning of treatment, lidocaine was immediately administered IV as a bolus (1 mg/kg) followed by 50 mcg/kg/min for 11 hours. BIPs were radiographically tracked and sedation/nausea scoring was performed every hour. Nausea scores were significantly higher at 6 hours post-treatment compared with other time points (all $P < 0.05$). Sedation scores were greater at baseline and 1 hour post-treatment compared to other time points (all $P < 0.05$). No differences between treatments were present (all $P > 0.05$). BIPs data remain to be analyzed. Our preliminary results do not indicate that acupuncture at PC6 and ST36 significantly reduced nausea or sedation associated with lidocaine CRIs in healthy dogs. However, additional studies are required to assess the usefulness of acupoints in dogs with other causes of nausea or in those with altered physiological states (i.e. painful or arrhythmic patients).

Research Grant: Companion Animal Foundation, University of Wisconsin, Madison, WI

Student Support: Boehringer Ingelheim Veterinary Research Scholars Program

Histotripsy effects on primary tumor and metastasis in a 4T1 breast cancer model

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Histotripsy, an emerging and non-invasive tumor ablation technology, uses high intensity focused ultrasound (HIFU) to mechanically ablate tissue. Current treatments, such as radiation therapy and surgery, are often associated with significant side effects compared to histotripsy. Additionally, treatments like HIFU are thought to act as immunotherapeutics by activating a systemic immune response to tumor associated antigens (TAAs) released after treatment. We applied histotripsy to a 4T1 breast cancer model that mimics the aggressive nature of human breast cancer metastasis, the leading cause of death from breast cancer. We hypothesized that treatment of primary mammary carcinomas with histotripsy will retard primary tumor growth as well as reduce the incidence of metastasis through activation of the immune system. To test this, we utilized the 4T1 model in vivo. Primary mammary tumors were treated with histotripsy 7 days post tumor engraftment. Half of the animals were sacrificed 24 hours post treatment to evaluate acute treatment effects while the remaining mice were monitored for the remainder of the 4T1 model to assess primary tumor growth, weight change, and metastasis. To evaluate immune activation, we extracted RNA from primary tumors and evaluated the expression of pro- and anti-inflammatory genes, including IL21, IL6, IFN γ , IL-1 β , IL-10, TSLP and CCL2. Our results showed that treated mice had a 15% decrease in tumor size over the course of the model compared to untreated mice. In addition, mice with mammary tumors that received histotripsy had exhibited increases in expression of pro-inflammatory genes and decreases in anti-inflammatory genes 24 hours post treatment.

Research Grant: VMCVM IRC

Student Support: NIH T35OD01887

Evaluation for novel causes of chronic kidney disease in cats

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Chronic kidney disease (CKD) is a leading cause of death in cats, however the etiology is multifactorial and further research into causation is needed. *Bartonella* species, feline foamy virus (FFV), and *Leptospira* spp., have been implicated as causes but not studied extensively. The objective was to assess for associations amongst serum antibodies against these agents and azotemia suspected to be from CKD. Feline sera from geographically and age-matched cats with ($n = 13$) or without ($n = 41$) renal azotemia were collected from IDEXX Laboratories. Serum antibodies were measured by validated assays and were classified as positive or negative (FFV and *Leptospira* spp.) or by titer (*Bartonella* spp.). Each of the cats lived in California or Nevada when sampled. *Leptospira* spp. antibodies were not detected. FFV antibodies were detected in 92.3% of the azotemic cats and 90.2% of the non-azotemic cats ($P = 1$). *Bartonella* spp. antibody titers of $> 1:64$ were detected in 84.6% of the azotemic cats and 51.2% of non-azotemic cats ($P = 0.0511$). Titers of $> 1:128$, $> 1:256$, and $> 1:512$, were all more common in the azotemic cats ($P < 0.05$). *Leptospira* spp. prevalence is low in these states compared to other regions of the United States, such as the Northeast and so the negative results are not surprising. *Bartonella* spp. have been detected in renal tissues of cats and the data from this study support a potential role of this agent as a cause of CKD. This study is ongoing, with a prospective final sample size of 2,000 matched cat sera, encompassing all major geographic locations in the United States and will also study several other potential causes of CKD.

Research Grant: Research support from the Center for Companion Animal Studies and the Sue Vandewoude Research Group

Student Support: Student support from Morris Animal Foundation

Quantifying error in measurement of the angle of lateral opening using the cup position assessment device

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Hip dysplasia, coxofermoral joint laxity or instability, is a common finding in large breed dogs. Total hip replacement (THR) is a surgical procedure that replaces the coxofemoral joint with an artificial joint. THRs are performed for a variety of conditions that affect the coxofemoral joint. Improper placement of the acetabular cup can lead to post-operative complications such as luxation. When angle of lateral opening (ALO) is out of the clinically acceptable range in an open or closed position, (open $< 30^\circ$, closed $> 60^\circ$), it can lead to increased risk of dorsal or ventral luxation respectively. Currently there is no quantitative method of intra-operative evaluation of the acetabular cup position. The acetabular cup must be surgically emplaced during a THR and the ALO is assessed post-operatively via radiograph. If the cup is placed in a suboptimal position, correction requires a revision surgery. A novel device, the cup position assessment device (CPAD), would allow for assessment of the ALO with intraoperative imaging. The goal of this study is to quantify inter- and intra-observational accuracy in measuring the ALO and version with the CPAD. The study hypothesizes there will be minimal variability in both intra-observational measurements and inter-observational ALO and version measurements. Data collection is ongoing at this time.

Research Grant: None

Student Support: Meriel Veterinary Research Scholars Program

Amphibians as a possible new reservoir for Dengue virus

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Dengue virus is the world's most important mosquito-borne human pathogen. Four genotypes- designated DEN 1-4- have been characterized. Dengue has a sylvatic maintenance cycle involving blood-feeding arboreal mosquitoes of the genus *Aedes*, and non-human primates. Little is known about whether ectothermic vertebrates are permissive to infection. The understanding of their role in the sylvatic cycle could provide valuable insights for control of several arthropod-borne viruses. In this study, members of the species *Lithobates sphenoccephalus* (leopard frog), and *Anaxyrus speciosus* (Texas toad) were inoculated with Dengue virus 2, and blood samples were tested to characterize viremia and seroconversion. Plaque assays were performed to determine viral titer prior to inoculation. Plaque reduction neutralization tests will be performed to quantify the titer of neutralizing antibody for the virus. If high virus titers are detected in serum, it may suggest that amphibians play a role in the maintenance and transmission of Dengue viruses in nature. Future directions of the study include an introduction of mosquitoes to determine if the magnitude of viremia in the amphibian hosts is sufficient enough to transmit via mosquito bite.

Research Grant: Funded by the Animal Models Core at Colorado State University

Student Support: NIH Grant T35OD015130-06

Positive reinforcement of a foundation behavior to reduce perceived anxiety of kennel dogs: a pilot study

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Measurement of animal welfare and establishment of protocols to reduce fear, anxiety and stress have become a research priority in recent years. Anxiety and stress in research dogs can impact lifespan, life quality and physiological parameters that may be significant to experiments, in addition to damaging the bond between dogs and their caretakers. The purpose of this pilot study was to evaluate the use of positive reinforcement training in the open kennel environment of research dogs and determine if training would decrease observed clinical signs of stress in dogs with a history of fearful behavior. Five purpose-bred research dogs with history of fearful behavior in the kennel environment were taught "nose touch targeting" using positive reinforcement clicker training over the course of two to four weeks. Dog behavior in response to a stranger was recorded before, immediately after, and two weeks after the training period to observe possible changes in stress signs and determine whether these changes were retained two weeks post-training. Three observers were instructed to use a standardized list of stress-related behaviors produced in a previous study to conduct a randomized, blind-order video review. All five dogs met the target mastery level for "nose touch targeting" during the training period. Weaknesses in the methods for analyzing dog behaviors were identified, as there was only moderate agreement between observers and no significant relationship was found between successful training and changes in observed stress behavior. This study serves as a foundation for future positive reinforcement and cooperative care training with dogs in the laboratory and other open kennel environments, including animal shelters.

Research Grant: Boehringer Ingelheim Veterinary Scholars Program

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Characterization of circulating exosomal microRNA expression in canine osteosarcoma

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Osteosarcoma (OS) is the most common primary bone tumor in children and dogs. Despite aggressive therapy, 90% of dogs and 30% of children die of chemotherapy-resistant metastatic disease. MicroRNAs (miRs) are noncoding RNAs that post-transcriptionally regulate gene expression and are frequently dysregulated in OS. Exosomes are 40-150nm vesicles that contain proteins and nucleic acids, including miRs. They are secreted by several cell types, including tumor cells, and are thought to mediate cell-to-cell communication through transfer of their cargo. The objective of this study is to characterize the expression of exosomal miRs in serum from dogs with OS and determine the prognostic significance of circulating miRs in canine OS. We hypothesize that dogs with OS will possess a unique serum exosome miR expression signature distinct from that found in healthy dogs. We further predict that circulating miRs detected in canine OS patients will be associated with disease outcome. Exosomes were isolated from the serum of dogs with OS (N=12) and age-matched healthy dogs (N=12) and exosome purity was validated using NanoSight imaging and Western blotting. RNA was isolated and global miRNA expression was performed using the NanoString platform. The expression of candidate miRs associated with OS was validated using real-time PCR. Studies are ongoing to identify circulating miRs associated with outcome in dogs with OS and determine their utility as noninvasive biomarkers to predict prognosis. This study will generate important new data characterizing circulating miR expression in canine OS. These data will lay the foundation for the identification of potential therapeutic targets and prognostic biomarkers in both human and canine OS.

Research Grant: Research Grant: Harley's Canine Cancer Support Fund

Student Support: Student Support: Rohovsky Fund

Investigating the effect of p53 loss on folate metabolism in the development of neural tube defects

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Neural tube defects (NTDs) are birth defects that result in herniation and exposure of nervous tissue during embryogenesis when the neural tube fails to close. Folate deficiency causes failure of neural tube closure and it is estimated that 70% of all NTDs are folate responsive. However, the mechanism of this rescue effect remains to be elucidated. The tumor suppressor protein p53 plays an important role in development and both increased and decreased expression and/or function can lead to NTDs in mouse models. Studies in vitro demonstrate interactions among p53 and folate one-carbon metabolism pathways, specifically the *de novo* thymidylate (dTMP) biosynthesis pathway. Mouse models with impaired *de novo* dTMP synthesis have increased rates of NTDs that are responsive to folate supplementation. The aim of this project was to determine if folate supplementation could rescue p53^{-/-} induced NTDs. Interestingly, p53^{-/-} mouse embryonic fibroblasts have higher rates of folate-dependent *de novo* dTMP synthesis, purine biosynthesis, uracil accumulation in DNA, and proliferation. These results suggest that loss of p53 causes increased growth and thus increased nucleotide synthesis to accommodate, however not at levels that are sufficient to ameliorate uracil accumulation in DNA. Folate supplementation is also not adequate to prevent p53^{-/-} NTD incidence.

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Student Support: NIH National Institute of Child Health and Human Development F30 HD093288

Efficacy of the feline temperament profile in evaluating shelter cats for adoption

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The most common reason for relinquishment of cats to shelters in the U.S. is behavior issues, and cats' temperament is not broadly assessed before adoption from shelters. Reliable and valid cat temperament assessment instruments are needed. The proposed study is part of the Feline Friends Project, which investigates stress in shelter cats adopted by families of children with Autism Spectrum Disorder (ASD). Cats are screened for calm temperament using the Feline Temperament Profile (FTP). This standardized test is used to score the cat's general levels of sociability, aggressiveness, and adaptability in new situations, based on a model developed by Lee et. al. (1983). It has been subjected to reliability and validity testing only once (Siegford et. al., 2003). The FTP comprises ten items, with a list of "acceptable" and "questionable" behaviors under each item. The rater carries out a series of interactions and observations with the cat and notes occurrence (1) or absence (0) of each behavior. The purpose of the present study is to answer the following research questions: What items of the FTP are most predictive of a calm temperament score in cats? What are similarities and differences between cats who qualified and those who didn't? We will study a group of cats that were rejected for placement (n=44) and a group that qualified (n=26). Descriptive statistics and analysis of variance will be used to compare these two groups on demographic characteristics and various components in the FTP scores. We expect to find what behaviors to look for in matching the cat with the expectations of the adopting family. The findings have implications for increasing cat adoption and reducing their return to shelters.

Research Grant: Winn Feline Foundation, Human Animal Bond Research Initiative Foundation

Student Support: Ecole Nationale Veterinaire de Toulouse, France

Examination of *Anaplasma marginale* genetic diversity in Kansas beef cattle

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Anaplasma marginale, a tick-borne rickettsial pathogen and the causative agent of clinical bovine anaplasmosis, poses a substantial economic burden to the global cattle industry. Multiple strains of *A. marginale* have been reported from the United States and can vary in virulence, transmissibility, and drug susceptibility. Kansas is the third largest beef producing state in the U.S. and is endemic for bovine anaplasmosis. This study was conducted to determine which *A. marginale* strains were present in cattle samples submitted to the Kansas State Veterinary Diagnostic Laboratory (KSVDL) in 2016. A subset of samples, identified 'positive' for *A. marginale* during KSVDL screening, were selected for *A. marginale* strain-typing analysis. Strain-typing analysis was conducted by PCR amplification, cloning and sequencing the variable repeat region of the Major Surface Protein 1a gene (*msp1α*). Resulting sequences were translated and the type, number, and arrangement of the repeats present in the N-terminal tandem repeat region of the Msp1a protein were identified to determine the *A. marginale* strain(s) present in each sample. Of the total 91 *A. marginale*-infected samples selected for strain-typing analysis, 72 displayed adequate amplification of the *msp1α* variable region for subsequent cloning and sequencing. Over 60 *A. marginale* Msp1a genotypes, comprised of various arrangements of 32 distinct repeats, have been identified so far. Of interest, several identified strains and repeats have not previously been reported. Knowledge of *A. marginale* genetic diversity could inform the development and implementation of effective control measures, including vaccine development, to mitigate the economic cost of bovine anaplasmosis.

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Student Support: KSU-CVM Startup

Hmga2 inactivation suppresses the development of a melanoma phenotype in skin exposed to UVB light

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Hmga2 is an architectural chromatin-remodeling factor that is upregulated in many cancers, including melanoma. Previous findings suggest that Hmga2 inactivation may prevent ultraviolet B (UVB)-mediated melanoma development, potentially through changes in inflammatory infiltration. Using murine models, we investigate the role of Hmga2 in skin exposed to UVB radiation to characterize environmental changes in Hmga2 ^{-/-} skin that may inhibit early melanomagenesis. We demonstrate that moderate acanthosis occurs in wild type skin within three days following UVB exposure, and loss of Hmga2 significantly attenuates this phenotype. Specifically, time course experiments demonstrate that expansion of the basal Keratin 5 (K5) and suprabasal Keratin 10 (K10) epidermal populations are markedly reduced in Hmga2 ^{-/-} skin. These findings delineate the role of Hmga2 in modifying the cutaneous microenvironment in response to UVB exposure and in early melanoma formation.

Research Grant: NIH Training Grant

Student Support: Veterinary Investigators Program, Cornell University College of Veterinary Medicine

Modulatory effects of JAK 1,2 inhibitor Oclacitinib on a canine T-cell lymphoma cell line

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Lymphoma is one of the most common diagnosed neoplasms in dogs. A majority of dogs with T-cell lymphoma experience shorter remission durations and survival than dogs with B-cell lymphoma when treated with a standard multi-chemotherapy protocol. Thus, new therapeutic strategies tailored for T-cell neoplasms are indicated. Janus kinase (JAK) signaling plays a major role in T cell functions including activation and proliferation. Moreover, dysregulation of the JAK pathway promotes growth and survival of neoplastic cells. In this study, the cytotoxic effects of oclacitinib, a JAK 1/JAK 2 inhibitor licensed to treat canine atopic dermatitis, were evaluated in a canine T-cell lymphoma cell line (OSW) and compared to doxorubicin. Cells were cultured with decreasing concentrations of the drugs to measure effects on cell proliferation and to determine the IC₅₀ and IC₂₀. Annexin V/ 7AAD was used to assess cell viability and apoptosis and propidium iodide for cell cycle analysis via flow cytometry. Oclacitinib inhibited cell proliferation at IC₅₀ concentrations higher than doxorubicin and increased cell death. Following treatment with oclacitinib, there was a reduced total number of viable cells and fewer cells in the S phase of the cell cycle compared to untreated control. These preliminary results demonstrate that oclacitinib is effective in inhibiting proliferation and cell cycle, while promoting enhanced cell death in neoplastic T cells *in vitro*, but to a lesser degree than doxorubicin. Targeting the JAK pathway may be promising in the clinical management of dogs with T cell lymphoma especially in cases resistant to traditional cytotoxic drugs.

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Comparison of bacterial DNA found in plant-based and animal-based dry canine diets

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The canine gut microbiome describes a vast and dynamic collection of symbiotic microorganisms in the gastrointestinal (GI) system and is essential for overall host health. Proportions of microbial populations can shift throughout a dog's life, as is influenced by diet, age, or disease state. The GI tract is exposed to exogenous bacterial antigens present in the diet that may stimulate innate immune responses and cause the proportions of microbial taxa to shift. The primary purpose of this study is to take a census of bacterial DNA found within plant-based and animal-based dry dog foods. A 'puppy', 'adult', and 'senior' diet from nine different canine diets is currently being investigated. Based on preliminary data, the major genera of bacterial DNA groupings from two plant-based foods (*Lactobacillus*, *Methylobacterium*, and *Tyzzere*) were mostly different than those found in six samples of animal-based foods (*Lactobacillus*, *Sphingomonas*, *Blautia*, and *Pseudoflavonifractor*). We have been compiling isolated and amplified bacterial DNA extracted from various food samples and are in the process of sequencing 16S rRNA amplicons from these samples. We anticipate to move into the second phase of our research plan as the summer progresses, to perform bioinformatic and statistical analysis on DNA sequence data. We may then compare the microbial makeup of plant-based and animal-based diet samples by identifying and organizing bacterial clusters into taxonomic groupings and operational taxonomic units (OTUs). By doing so, we will be able to more clearly describe the major potential diet-sourced bacterial antigens exposed to the canine GI tract, and in the future, understand more about how they may affect our canine companions.

Research Grant: Western University of Health Sciences (WUHS), College of Veterinary Medicine (intramural funds)

Student Support: Western University of Health Sciences, Office of Research and Biotechnology

Verification of feeding tube placement based on tracheal superimposition on thoracic radiographs

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Early enteral nutrition, or tube feeding, in small animal patients is critical in order to prevent nutritional deficiencies and also to help the patient recover from disease. Thoracic radiography is one of the simplest ways to verify feeding tube placement, yet there is little literature examining this, specifically feeding tube superimposition on the trachea. The objective of this study is to verify the presence of superimposition of the feeding tube on different parts of the trachea, and then compare the superimposition with feeding tube misplacement. Electronic medical records of dogs and cats at the University of Florida Small Animal Hospital were retrospectively examined to identify all patients who had thoracic radiographs taken for tube checks between January 2013 and May 2018. The search identified a total of 815 dogs and 306 cats. Superimposition, or lack thereof, of the feeding tube over the trachea was recorded. The results of this study are still under review and will be presented at the National Veterinary Scholars Symposium.

Research Grant: Unknown

Student Support: Joyce Novotny Scholarship from the Blue Buffalo Foundation

Age is a determinant factor of cancer metastasis in mice

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How does age contribute to cancer metastasis? The elderly population continues to increase at an unprecedented rate worldwide. Furthermore, cancer incidence increases with age, and yet the therapeutic choices for the elderly cancer patients are limited. Understanding how age affects cancer progression will help developing better treatments for the elderly. In this study we hypothesize that cancer metastasis increases with age due to age-specific factors from the tumor microenvironment. To test our hypothesis, we used a syngeneic metastasis model by inoculating intravenously murine oral cancer cells (MOC-2) into young and old mice C57BL/6 mice (3- and 20-month-old respectively). Here we show that the old mice have more tumor colonization in the lungs as compared to the young ($P < 0.05$). The tumor area was quantified from the hematoxylin and eosin-stained histological lung sections using the ImageJ analysis. We also evaluated tumor inflammation and necrosis using the same slides. There was no link between inflammation with either tumor area or age. The local tumor size correlates with necrosis area and is independent of age. These data suggest that age does not affect the local inflammatory responses, and tumor necrosis is solely driven by local tumor expansion. We next asked whether tumor vasculature and immune cell composition correlate with tumor proliferation. We will examine vascular density and integrity, and immune cell infiltrates by immunostaining for endothelial cells, cell-adhesion molecules, immune cell markers and mitotic markers. We hypothesize that the worsening of the vasculature permeability and changes in the immune cell composition contribute to the rapid tumor proliferative activity in the old mice.

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Comparison of axillary and inguinal temperature to rectal temperature in healthy guinea pigs (*Cavia porcellus*)

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Core body temperature is an essential health parameter. Temperature aberrations can indicate certain disorders, influence clinical management decisions, and serve as a prognostic indicator for patient recovery. Historically, rectal temperature measurements have been utilized in companion animals, however there is growing interest in less-invasive methods including auricular and axillary measurements to determine core body temperature. In this study, triplicate temperature measurements (axillary (AT), inguinal (IT), and rectal (RT)) were performed in 19 healthy guinea pigs (7 male, 12 female). Paired sample t-tests were utilized to evaluate differences in the mean body temperature measurements between each body site. There was no agreement in body temperature measurements between the body sites. The IT ($36.3^{\circ}\text{C} - 39.7^{\circ}\text{C}$) was significantly lower than both AT ($36.8^{\circ}\text{C} - 39.9^{\circ}\text{C}$; $t = -3.401$, $P = 0.003$) and RT ($38.2^{\circ}\text{C} - 40^{\circ}\text{C}$; $t = -5.068$, $P < 0.0001$), and AT was significantly lower than RT ($t = -3.485$, $p = 0.003$). These results indicate that AT, IT, and RT cannot be used interchangeably to represent core body temperature. However, these data provide a basis for clinicians to infer RT from AT and IT ($\text{RT} = \text{IT} + 0.83^{\circ}\text{C}$, $\text{RT} = \text{AT} + 0.44^{\circ}\text{C}$). Additional investigation is planned to assess these temperature relationships in animals suffering from disease.

Research Grant: None

Student Support: University of Illinois College of Veterinary Medicine

Antibody mediated protection against RSV after immunization with a VSV-G and F recombinant

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Respiratory Syncytial Virus (RSV) is an enveloped ssRNA virus that causes severe upper respiratory tract disease in young, immunocompromised, and geriatric patients. While this disease has a high mortality rate in these demographics, there is no licensed RSV vaccine available. Previous studies have shown that the fusion (F) glycoprotein is a potential vaccine target as it is necessary for infectivity. This study aims to explore whether or not a combination of two glycoproteins would confer protection, and whether or not this protection was B cell or T cell mediated. The fusion glycoprotein and the attachment glycoprotein (G) were utilized because they are both envelope proteins, and are the target for neutralizing antibodies and T cells. A recombinant vesicular stomatitis virus was generated which expressed both the F and the G protein, and tested in cotton rats which display increased susceptibility to RSV compared to mice. Cotton rats were inoculated with the recombinant VSV twice, and then challenged with RSV. Immunization with the VSV recombinant led to the induction of antibody and a reduction in viral titer of 1.5 log. Even groups in which CD4⁺ T cells or CD8⁺ T cells, or both CD4⁺ and CD8⁺ T cells were depleted by antibody treatment were protected against infection with RSV. These findings indicated that the RSV-specific antibodies were the main correlate of protection against challenge with RSV. In summary, our study found that VSV-G and F recombinants are protective and able to induce an antibody response.

Research Grant: None

Student Support: NIH T35 Training Grant T35OD010977

The effects of acute exposure to di(2-ethylhexyl) phthalate and diisononyl phthalate on hormone levels in mice

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Di(2-ethylhexyl) phthalate (DEHP) is a synthetic chemical used ubiquitously in the environment in a variety of plastics, medical equipment, and children's toys. Individuals can absorb DEHP via dermal contact, inhalation, and ingestion. Previous studies show that DEHP affects fertility and causes endocrine disruption in both males and females, however, most of these studies focused on males. This information led to a shift to replace DEHP with other plasticizers. One such replacement is diisononyl phthalate (DiNP). Unfortunately, little is known about the reproductive effects of DiNP. Thus, the purpose of this study was to fill the knowledge gaps of how DEHP and DiNP affect female reproduction, and to test the hypothesis that acute exposure to DEHP or DiNP has negative, long-term effects on female reproductive health. Adult female CD-1 mice were orally dosed with either vehicle control (corn oil), DEHP (20 or 200 µg/kg/day, or 20 or 200 mg/kg/day), or DiNP (20 or 100 µg/kg/day, or 20 or 200 mg/kg/day) for 10 days. Mice were euthanized three months post-dosing and blood was collected to measure sex steroid hormone levels via enzyme-linked immunosorbent assays. Treatment with 100 µg/kg DiNP significantly decreased progesterone and testosterone levels compared to controls (n=5-6; $P \leq 0.05$). DiNP also moderately decreased progesterone (20 µg/kg, $P=0.082$) and increased estradiol (200 mg/kg, $P=0.083$) levels compared to controls. DEHP exposure did not significantly affect hormone levels compared to controls at any time. In conclusion, these data show that DEHP does not alter hormone levels significantly and that DiNP, a common replacement for DEHP, alters hormone levels in mice three months after exposure has stopped.

Research Grant: NIH R56 ES025147

Student Support: Office of the Director, NIH, T35 OD011145

Antibiotic resistance in *Escherichia coli* in feedlot cattle treated for bovine respiratory disease

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Bovine respiratory disease (BRD) is the most common and costly disease to manage in the North American beef cattle industry. Treatment of BRD requires antibiotics, which may foster the propagation and spread of antibiotic resistance. The objective of this study was to determine the prevalence and profiles of antibiotic resistance of *Escherichia coli* (a gut commensal bacteria) in beef cattle receiving antibiotic treatment for BRD. For 28 days, 70 crossbred calves housed in a single pen that did not receive metaphylaxis upon feedlot arrival were monitored for clinical signs of BRD. Fecal samples were taken rectally from each calf at days 0, 14, and 28. Those meeting the case definition for BRD were treated with a macrolide, tulathromycin (Draxxin, Zoetis), and had an additional fecal sample taken that same day before antibiotic administration. Standard bacterial culturing methods were used to isolate *E. coli* from each fecal sample. The broth microdilution method was used to determine the susceptibility of each bacterial isolate to various antibiotics. Thus far, 229 *E. coli* isolates were subjected to antibiotic susceptibility testing. There was no significant change in resistance to azithromycin, another macrolide, over time nor difference between isolates from treated and untreated animals. There was an overall increase in resistance to non-macrolide antibiotics ceftriaxone, ciprofloxacin, and tetracycline among all isolates from day 0 to day 28. Isolates from treated animals were overall more susceptible to ciprofloxacin and resistant to ceftriaxone. These results indicate how tulathromycin administration to cattle with BRD can alter bovine gut bacterial antibiotic susceptibility profiles independent of antibiotic class.

Research Grant: Unknown

Student Support: Meriel Veterinary Research Scholars Program

mRNA expression of Th2 cytokines differs in duodenal mucosa of healthy and diseased dogs

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Canine inflammatory bowel disease (IBD) is a group of chronic enteropathies characterized by intestinal inflammation in response to environmental factors, luminal constituents and immune responses in susceptible hosts. A recent genome wide association study (GWAS) on German Shepherd Dogs (GSDs) with IBD revealed novel single nucleotide polymorphisms (SNPs) for T helper cell (Th) 2 cytokines IL-4, IL-5, and IL-13. The Th2 pathway is initiated by the secretion of the epithelial cytokine IL-33. SNPs of these cytokines have been previously linked to increased prevalence of human IBD, specifically ulcerative colitis (UC). The aim of this study was to use RNA in situ hybridization to investigate whether there is a loss or gain of cytokines IL-13 and IL-33 mRNA expression in mucosal biopsy samples of GSD with IBD. Experimental groups included GSD-IBD (n=10), non-GSD-IBD (n=10), protein losing enteropathy (PLE) dogs (n=10), and healthy dogs (n=8). For both cytokines, results showed that there was no difference ($P > 0.05$) in average villous histological scores between groups ($P=0.10$ for IL-13; $P=0.06$ for IL-33). Statistically significant differences were reported in average subvillous scores ($P=0.02$ for IL-13; $P < 0.001$ for IL-33), average epithelial scores ($P=0.04$ for IL-13; $P < 0.001$ for IL-33), and average lamina propria scores ($P=0.05$ for IL-13; $P=0.02$ for IL-33). Expression of IL-13 was low in all groups while expression of IL-33 was increased in healthy and non-GSD-IBD groups. These findings suggest that genetic factors affecting mucosal Th2 cytokine expression are present in GSDs with IBD and that this immunomodulatory profile might serve as a novel target pathway for design of future IBD drug therapies.

Research Grant: None

Student Support: Stipend paid for by Drs. Karin Allenspach and Albert Jergens

Formation of intestinal epithelial organoids by direct reprogramming of mouse fibroblasts

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Until recently, obtaining intestinal stem cells (ISCs) from patients necessary to create organotypic cultures was difficult due to the invasive nature of the biopsy. However, advancements in our understanding of the transcriptional and epigenetic regulation of cellular identity has enabled the reprogramming of one cell type to another. Most recently Miura and Suzuki (2017) demonstrated that mouse and human fibroblasts could be reprogrammed into induced ISCs (iISCs) by forced expression of the master transcriptional regulators Foxa3, Gata6, Cdx2, and Hnf4 α . These iISCs could be grown in 3D culture and form budding organoids (BOs) that are self-renewing and contain all the different intestinal epithelial cell lineages. Our experiment attempted to replicate the results of this study by using doxycycline-inducible lentiviruses carrying Foxa3, Gata6, Cdx2, and Hnf4 α to reprogram mouse fibroblasts into iISCs that were grown into BOs in 3D organoid culture

Research Grant: R01DK106309 and R01CA168654

Student Support: T35OD010919

MALDI-TOF mass spectrometry as a tool to identify mastitis pathogens and determine antimicrobial resistance

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Antimicrobial resistance (AMR) is a growing problem in veterinary medicine. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a rapid and inexpensive diagnostic tool which produces a characteristic spectrum of peaks based on the proteins within a sample. This study aimed to investigate the performance of MALDI-TOF MS at detecting AMR and determine the accuracy with which MALDI-TOF MS identifies *Streptococcus uberis* and *Streptococcus dysgalactiae*. A total of 87 bacterial isolates were acquired from the Mastitis Pathogen Culture Collection of the Canadian Bovine Mastitis and Milk Quality Research Network. Out of the 87 isolates, 64 were identified by MALDI-TOF MS as *S. uberis* and 23 as *S. dysgalactiae*. Antimicrobial susceptibility to penicillin and tetracycline was determined using the Sensititre microdilution system and mastitis plate format for *S. uberis* isolates. MALDI-TOF MS peak data for isolates resistant to each antibiotic were compared to those susceptible to the same antibiotic. Intensity versus mass-to-charge ratio (m/z) plots were produced. Principal component analysis (PCA) was performed on a subset of the peaks from the spectra. Whole genome sequences (WGS) were obtained from all isolates and were uploaded to the Centre for Genetic Epidemiology's KmerFinder tool to identify each species using WGS. The PCA analysis indicated that two components explained 80% of the variation in the data. For penicillin, there was a trend suggesting that some peaks were able to separate resistant and susceptible isolates. However, no trend was observed for tetracycline. All *S. uberis* and 69.56% of the *S. dysgalactiae* MALDI-TOF species IDs agreed with the KmerFinder WGS IDs.

Research Grant: AVC Internal Research Funds

Student Support: Boehringer Ingelheim Veterinary Scholars Program, AVC Veterinary Student Research Award

Multi-drug resistant infections in canine and equine patients: *Escherichia Coli* and *Klebsiella*

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Current knowledge of many aspects of the epidemiology of multi-drug resistant (MDR) bacteria in veterinary medicine is unclear. The objective of the study was to determine the prevalence of MDR bacteria (*E. Coli* and *Klebsiella*) in canine and equine patients at University of Florida teaching hospital (UFTH). A retrospective study was performed broadly on all submitted cultures of canine and equine patients at UFTH from January 2011 to May 2018. MDR was defined as an isolate resistant to ≥ 1 antimicrobial agents in ≥ 3 antimicrobial classes. Then, the overall prevalence of MDR *E. Coli* and *Klebsiella* was determined. Further exploration regarding the body system affected with MDR bacteria was examined. A total of 6,085 and 1,219 canine and equine isolates were identified during the study period, respectively. From canine isolates, 17% (1,015/6,085) were *E. Coli* and 2.2% (135/6,085) were *Klebsiella spp.*, whereas in equine isolates 7% (83/1,219) were *E. Coli* and 3.4% (42/1,219) were *Klebsiella spp.*. In canine patients, 21% (212/1,015) of *E. Coli* and 13% (18/135) of *Klebsiella spp.* isolates were classified as MDR. In equine patients, 8% (7/83) of *E. Coli* and 2.4% (1/42) of *Klebsiella spp.* isolates were classified as MDR. Most of the MDR *E. coli* and *Klebsiella spp.*, from canine patients, were isolated from the urinary system (75% (159/212) and 83% (15/18), respectively). MDR *E. Coli* and *Klebsiella spp.* are commonly isolated from canine patients. Future studies evaluating the risk factors and the impact of MDR bacteria on patient morbidity and mortality are warranted.

Research Grant: CVM Patient Safety and Quality Improvement Grant Opportunity

Student Support: Unknown

Development of a simple method of static body weight distribution in neurologically normal small breed dogs

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Acute intervertebral disc herniation (IVDH) occurs commonly in dogs, and physical rehabilitation is increasingly utilized to promote recovery. Objective outcome measures capable of tracking different aspects of functional recovery are needed to optimize rehabilitation protocols. The aim of this study was to determine static body weight (BW) distribution in neurologically normal small breed dogs predisposed to Hansen type I IVDH. Healthy dogs between 1 and 10 years of age weighing less than 20 kilograms were recruited. Static BW distribution between the thoracic and pelvic limbs was acquired using commercially available digital bathroom and kitchen scales and compared to a validated pressure-sensitive Tekscan™ animal walkway system. Reproducibility between methods was determined using a paired t-test. Twenty five dogs were enrolled including 6 Dachshunds, 4 Beagles, 4 Corgis, 1 Basset Hound, 1 French Bulldog, 1 Shih Tzu, 1 Pug and 7 chondrodystrophic mixed breeds. Mean age was 4.56 years (2.69) and mean BW was 12.05 kg (3.28). Measurements could be obtained in all dogs on the digital scales and in all but one dog on the pressure sensitive walkway. Combining values for the digital scales, mean thoracic to pelvic limb BW distribution was 63% to 37% of total BW while thoracic and pelvic limb values for the pressure sensitive walkway were 68% and 32%, respectively ($P < 0.0001$). Results demonstrated that static BW distribution in a population of healthy small breed dogs was simple to obtain using digital scales but differed from values on the pressure sensitive walkway. This study generated weight distribution data for subsequent comparison to dogs recovering severe acute IVDH.

Research Grant: None

Student Support: Boehringer Ingelheim Veterinary Scholars Program, Purdue College of Veterinary Medicine

Assessing the feasibility of canine vaccination to prevent Rocky Mountain spotted fever in Arizona

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Rocky Mountain Spotted Fever (RMSF), caused by the bacteria *Rickettsia rickettsii*, is a tick-vector-borne infectious disease found throughout the United States that affects both humans and canines. In the majority of the United States, RMSF is vectored by *Dermacentor* ticks. However, in the Arizona region, *Rhipicephalus sanguineus* is the sole vector. This leads to the question of why *R. sanguineus* can act as a vector in the Arizona region, but not elsewhere. We have previously identified two separate *R. sanguineus* lineages in Arizona—a tropical and temperate lineage. Yet, the question remains whether the difference in tick lineages matters for the transmission of RMSF. Here, we are testing two possible hypotheses: 1) *R. rickettsii* strains and bacterial loads vary among tick populations across Arizona and contribute to the RMSF outbreaks in specific regions and 2) regions with historical RMSF outbreaks will have a greater prevalence of canines exposed to *R. rickettsii*. We have analyzed 144 ticks collected from around Arizona, Mexico, and New Mexico to test whether they are carriers for *R. rickettsii*. The results of this study show a 2-fold increase in *R. rickettsii* prevalence in the tropical lineage compared to the temperate lineage (Chi-square test, $P = 0.0159$). We are currently using IFA analysis to test for IgG antibodies in canines from 13 locations across Arizona to establish a baseline seroprevalence map. This data is used to mathematically model the feasibility of a canine vaccine as a method to prevent future outbreaks of RMSF within Arizona. As many aspects of this vector-disease combination are unexplored, this study is essential for understanding and preventing canine and human disease in the southwest United States.

Research Grant: Research Grant: Midwestern University One Health Student Research Grant

Student Support: Student Support: Boehringer Ingelheim Veterinary Scholars Program

PDK4 mRNA visualization with fluorescent RNAscope in fibroblasts from doberman pinschers

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The Doberman pinscher (DP) canine breed displays a high incidence of idiopathic, nonischemic dilated cardiomyopathy (DCM) with increased mortality. A common mutation in DPs is a splice site deletion in the pyruvate dehydrogenase kinase 4 (PDK4) gene that shows a positive correlation with DCM development. PDK4, a vital mitochondrial protein, controls the switch between glycolysis and oxidative phosphorylation based upon nutrient availability. Pedigree evaluations of affected dogs suggest the disease is inherited as an autosomal dominant trait. Although this trait displays incomplete penetrance and this particular mutation does not account for the entire population of DPs that develop DCM, the correlation is significant. This study investigated PDK4 mRNA expression with fluorescent RNAscope in dermal fibroblasts from DPs that were healthy (PDK4wt/wt), heterozygous (PDK4wt/del), and homozygous (PDK4del/del) for the PDK4 mutation under conditions of high (not starved) and low (starved) nutrient availability in vitro. It is suspected that fibroblasts that are homozygous and heterozygous for the PDK4 deletion will have significantly different mRNA processing than fibroblasts without the deletion. This work will provide the mechanism of action of PDK4 mRNA processing with the mutation.

Research Grant: Unknown

Student Support: Merial Veterinary Research Scholars Program, FVMA Research Scholar

Mammary stem-like cells from mammary cancer resistant and susceptible species differ in response to UV

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Different species vary greatly in their mammary cancer incidence. Like humans, mammary tumors are among the most common neoplasms in intact female dogs and cats. Conversely, cows, mares, and sows rarely develop mammary tumors. Mammary stem cells (MaSC) reside in the adult mammary glands where they maintain and regenerate the tissue. As targets for malignant transformation, MaSCs may also be cancer cells of origin. Mammosphere-derived epithelial cells (MDEC) are primary mammary cells with stem-like properties that can be isolated from diverse species and used for comparative studies. We used MDEC to compare sensitivity to DNA damage between mammary cancer resistant and susceptible species. Previous work showed that MDEC from resistant species are more growth-sensitive to DNA damage (induced by DMBA or UV) than MDEC from susceptible species. RNA sequencing and qPCR analyses revealed that horse MDEC strongly upregulate p21 and downregulate Runx1T1 in response to DMBA or UV, whereas dog MDEC do not. p21 promotes cell cycle arrest in response to various stresses, including DNA damage. The function of Runx1T1 in the context of DNA damage is less known. Using qPCR, p21 and Runx1T1 expression was analyzed following UV exposure in MDEC from additional species that are mammary cancer susceptible (human and cat) or resistant (cow and pig). We did not observe an expression pattern of these genes that is unique to resistant or susceptible species. Upcoming experiments will be aimed at identifying the mechanism by which p21 is upregulated, the temporal pattern of p21 expression following UV exposure as well as investigating the role of p21 in the UV sensitivity of MDEC from mammary cancer-resistant species.

Research Grant: None

Student Support: NIH T35 Training Grant OD010941-09

Probiotic therapy in HIV-treated humans improves prognosis-effects on the gut microbiome and immune activation

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In human immunodeficiency virus (HIV) infections, translocation of microbial products from the gastrointestinal tract (GI) to systemic circulation is a major driver of the persistent immune activation associated with worsening prognosis. Antiretroviral (ARV) therapy significantly improves disease progression and clinical prognosis; however, it is unable to restore gut health despite effective suppression of plasma HIV viremia. Prolonged therapy remains fraught with non-AIDS-related comorbidities, especially in situations in which dysbiosis is likely to be present. This was a phase II, randomized, double-blind, two-arm study to evaluate whether a therapeutic approach aimed at recovering the symbiotic host-microorganism relationship might reduce HIV-associated chronic immune activation and improve response to therapy. Here, we investigated whether the probiotic Visibio Extra Strength (ES) reduces inflammation in HIV-infected individuals on virologically suppressive ART when compared to placebo. Serum was used to evaluate changes in colonic mucosal CD4 cells, plasma intestinal fatty acid binding protein (IFABP), and plasma CXCL10/IP-10 after 24 weeks of Visibio ES. The Visibio ES arm showed increased colonic mucosal CD4⁺ T-cell reconstitution. Increased microbial diversity, decreased damage and inflammation to intestinal mucosa (IFABP), and viral load (CXCL10/IP-10), will suggest probiotic supplementation of HIV-treated individuals improves GI tract immunity and microbiome diversity, thereby mitigating inflammatory sequelae. Importantly, this symbiotic therapy may translate into a safe and effective therapeutic for the reduction of comorbidities and improved prognosis in ART treated HIV-infected individuals.

Research Grant: NIAID/ NIH Division of Intramural Research-Project Number ZIA AI001029

Student Support: National Cancer Institute; National Institute of Allergy and Infectious Disease; NIH

Comparison of iSperm to current accepted semen analysis methods

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The goal of this study was to validate the standard operating procedures for iSperm, a portable semen analysis tool, by comparing it to currently accepted semen analysis methods. Unlike traditional methods of semen analysis, iSperm, because of its portability, can be used to perform on-farm semen analysis prior to artificial insemination or cryopreservation, and it can also be used for breeding soundness exams. Since errors can occur in the process of storing and handling semen prior to AI or assisted reproductive technologies (ART) procedures, the ability to easily evaluate the sperm just prior to insemination would be very beneficial. Performing these analyses on farms is not feasible with the commonly used methods of semen analysis. This project looked specifically at sperm motility and concentration as measured by the iSperm, and how it compared to the current gold standards in semen analysis, including a hemocytometer, and densitometer. Semen was collected from rams and analyzed fresh by iSperm at a 1:10 dilution as well as with phase contrast microscopy, hemocytometer, and densitometer. Portions of the semen were then cryopreserved in OptiXcell 2 extender. The samples will later be thawed, and a post-thaw analysis will be completed by both iSperm and computer-assisted sperm analysis. A correlation of the results by differing methods will allow for the informed use of iSperm in the future.

Research Grant: None

Student Support: USDA

Retrospective and prospective analysis of the effect of serological lead burden on fractures in wild birds

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Lead is an element naturally present in the earth's core, but its extensive utilization in hunting, mining, smelting, and fuel production has resulted in vast environmental contamination. Wild birds are at significant risk for lead poisoning because the soils, vegetation, and water that make up their habitat are contaminated. Nesting and foraging behaviors result in inhalation and direct consumption of lead. Prolonged injury recovery time and associated delay of release are of particular concern in wildlife medicine and rehabilitation. The goal of this study was to conduct analysis of historical clinic records and analysis of incoming birds in order to look for correlations between blood lead level and fractures. Whole blood samples were used to obtain information about each bird's current lead burden upon intake. Lead exposure and fracture data was available for Bald Eagle, Osprey, Northern Gannet, Common Loon, and Red-Throated Loon patients dating back to 2001. Prospective data was collected for all birds belonging to species large enough for sufficient blood sample collection that presented with fractures. Serological assessment will be considered along with radiographs, clinical details, and case outcomes in order to analyze the relationship between blood lead level and fracture prevalence, healing time, and outcome in rehabilitated wild birds.

Research Grant: NIH Grant T35OD010919; Boehringer Ingelheim Grant

Student Support: NIH-Boehringer Ingelheim Summer Research Program

Symmetric dimethylarginine (SDMA) as a marker for renal insufficiency in captive cheetahs (*Acinonyx jubatus*)

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Over 80% of cheetahs (*Acinonyx jubatus*) in captivity develop some degree of chronic renal disease, which shortens and impacts their quality of life. To mitigate the negative impacts of this insidious disease, early diagnosis is a key factor in management. Symmetric dimethylarginine (SDMA) is a naturally occurring renal biomarker specific to kidney function in cats and dogs that elevates earlier than serum creatinine and blood urea nitrogen (BUN) in diseased kidneys. SDMA is often reported on non-domestic animal reference laboratory blood work panels but has not yet been appropriately validated in cheetahs. In assessing renal function, the gold standard is to measure glomerular filtration rate (GFR) and ultimately correlate this rate with the marker to be tested. Contrast agents are often used to measure GFR, such as iohexol, which is a radiolucent intravenous marker that can be measured at various time points post-administration in order to determine the clearance rate. In this study, 12 cheetahs that presented voluntarily for blood draws were sampled to determine the clearance rate of iohexol (Time 0 [presumed 0], 120 minutes and 240 minutes) and compared with the corresponding SDMA level at Time 0. The iohexol clearance test provided GFR values that allowed for SDMA levels to be interpreted in light of appropriately assessed kidney function.

Research Grant: None

Student Support: Morris Animal Foundation

Role of gut lactobacillus in priming of neural-immune responses in hypertension

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Hypertension is a highly prevalent condition that is characterized by a dysfunctional autonomic nervous system and immune system as well as an imbalance of microbes in the gut. Evidence shows that even a moderate salt intake can cause dysfunction in the autonomic nervous system, leading to an inflammatory response in the immune system mediated by IL17 production. Moderately high salt intake additionally disrupts the normal gut microbiota by depleting the beneficial bacteria, *Lactobacillus*. We hypothesized that moderate salt intake would deplete gut *Lactobacillus* and cause an increase in IL17-positive T cells, which then infiltrate the cardioregulatory brain regions and cause neuroinflammatory molecular changes that are associated with the hypertensive phenotype. Mice were given either a moderately high salt diet or control diet, and a subset of each group was chronically administered Angiotensin II. Systolic blood pressure and bacterial fecal composition were monitored over the course of the study. The combination of a moderately high salt diet coupled with the presence of Angiotensin II resulted in a significant centrally mediated systolic blood pressure increase. We suspect the moderately high salt diet alone to lead to a decline in *Lactobacillus* levels detected in fecal samples. If this increase in systolic blood pressure succeeds a rise in IL17 concentrations in both the brain and peripheral circulatory system, we can conclude that IL17 is a critical central instigator of hypertension. We propose that elevated levels of IL17 in the brain and circulatory system accompany the hypertensive phenotype succeeding a short-term moderately high salt diet.

Research Grant: R56HL136692

Student Support: Florida Veterinary Scholars Program

Plasma extracellular vesicular miRNA as biomarker for canine osteosarcoma

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Osteosarcoma (OS) is the most common and malignant bone cancer in dogs that often lead to metastasis and poor prognosis. Currently, the main methods of diagnosing OS are either radiographs or tissue biopsy, which lack sensitivity and ability for early detection. Early detection is crucial for better outcome with treatment. Circulating extracellular vesicular (EV) associated miRNA has the potential to be a biomarker for early detection since EVs contain genetic signatures of the parent cells. We hypothesize that miRNAs are differentially expressed in EVs isolated from plasma obtained from canine OS patients. Canine patients diagnosed with OS and control dogs were enrolled under IACUC approval. Plasma was isolated from whole blood after centrifugation at 1320×g for 10 min. EVs were isolated from plasma using the exoRNeasy kit (Qiagen). Using qRT-PCR, the expression levels of 12 miRNAs were measured. Statistical analysis was performed using Mann-Whitney U test and post-hoc Benjamini-Hochberg procedure. Protein targets of the miRNAs that were differentially expressed were analyzed *in silico* using Target Scan. These *in silico* findings were then validated using OS tissues and osteoblast cell lines using qRT-PCR. A total of 12 dogs per group were enrolled. MiR-16 was found to be significantly decreased (8-fold) in canine OS plasma EVs ($P = 0.00007$) compared to controls. Target scan revealed killin (KLLN), apelin (APLN), solute carrier family 9 subfamily A, member 6 (SLC9A6), cell division cycle associated 4 (CDCA4), and tumor necrosis factor-13b (TNFSF13B) as targets for miR-16. This study suggests that miR-16 may be a potential biomarker for osteosarcoma in canines. Further validation will be needed in larger canine cohorts.

Research Grant: National Institute of Health: National Center for Research Resources

Student Support: Student Summer Research Training Program

Investigation of the occurrence of hyperketonemia in subsequent lactations in dairy cows

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Hyperketonemia (KET) is defined by elevated blood concentrations of β -hydroxybutyrate (BHB $> 1.2\text{mmol/L}$) and have been associated with disease occurrence, increased culling rates, and impaired performance in dairy cows. Management can greatly affect the levels of circulating BHB during early lactation, but recently a study showed that genetic differences are also associated with the incidence of KET. Thus, the objective of this study is to address this hypothesis by determining the likelihood of the occurrence of KET in subsequent lactations. Blood BHB was measured in 4,000 dairy cows in the first week post-partum during their previous lactation. A second blood BHB concentration will be collected in the first week post-partum at the start of their subsequent lactation. Blood samples are collected from the coccygeal vessels. Blood BHB is measured using a cow-side meter (NovaVet Meter, Nova Biomedical, MA). All statistical analysis will be performed using appropriate statistical software. The relationship between occurrence of KET in subsequent lactations will be evaluated using logistic regression. The relationship between the blood BHB concentration in two subsequent lactations will be determined using the Pearson correlation. We expect that cattle diagnosed with KET in their previous lactation to be more likely to have KET in their current lactation. A total 2,500 animals are expected to remain in the herds and starting a new lactation. Our experimental results will improve our understanding on the importance of the genetic components of KET in dairy cows. To our knowledge, this will be the first study reporting the incidence of KET in subsequent lactations.

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Student Support: The Dairy group in the Department of Veterinary Population Medicine

Microsatellite instability in canine tumors

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In certain human cancers, research has indicated that high instability in microsatellites often indicates an overall high mutation rate and a deficiency in DNA mismatch repair. Compared to cancers that are microsatellite stable, cancers that demonstrate high microsatellite instability (MSI-H) have been shown to have differences in prognosis and in susceptibility to certain treatments such as immunotherapy for checkpoint inhibitors. We aim to develop a system to test for the presence of MSI-H in dogs and hypothesize that MSI-H does exist in some canine tumors. Five mononucleotide and two tetranucleotide microsatellite markers were selected from the canine reference genome. Primers were designed to amplify the selected sequences and tags were attached to the forward primers to allow fluorescent labeling, necessary for high resolution genotyping, by a “universal primer” method. Using a full 7-plex multiplex PCR, we amplified DNA from paired samples of normal and tumor DNA from 23 Bernese Mountain dogs and 1 rottweiler diagnosed with histiocytic sarcoma. Agarose gel electrophoresis was used to verify amplification, followed by high resolution genotyping using an ABI 3730 machine (MSU Genomics Core facility) and results were analyzed for evidence of instability. We did not identify MSI-H in the histiocytic sarcomas samples, suggesting that these would not be tumors in which immunotherapy would be effective. In humans, different types of tumors have different rates of MSI. The system we developed can be further used to explore whether MSI-H exists in other types of canine tumors to provide better prognosis and treatment options.

Research Grant: None

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PCR genotyping of a novel FGF4 retrogene in Pit Bull Terriers and mixed breed dogs affected by disc disease

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Intervertebral Disc Disease (IVDD) is an incredibly devastating and painful disease that affects a wide variety of dog breeds. Chondrodystrophic dog breeds such as Dachshunds and Corgis exhibit a higher than average prevalence of IVDD. Chondrodystrophy affects proper development of leg length and prematurely calcifies the intervertebral disc. IVDD is the result of herniation of this intervertebral disc into the spinal canal often causing paralysis and ataxia. A recently discovered fibroblast growth factor 4 (FGF4) retrogene on chromosome (chr) 12 is a leading cause of chondrodystrophy and a leading contributor to a majority of IVDD cases, but not all dogs affected with IVDD have the chr 12 retrogene. While investigating for the presence of additional retrogenes, a second FGF4 retrogene was identified on chr 13 in two IVDD affected Pit Bull Terriers that were negative for the chr 12 retrogene. The purpose of this study is to determine if this FGF4 retrogene may be a contributing cause of IVDD in dogs. The first avenue of investigation looked at Pit Bull Terriers and mixed breed dogs that had corrective surgery for IVDD. Two had one copy of the novel FGF4 retrogene. Next, all dogs that had IVDD which was not explained by the FGF4 retrogene on chr 12 were tested. Finally, Pit Bull Terriers that did not have IVDD were investigated to check if it was normally present in the breed. 300 dogs in total were genotyped using a three primer PCR reaction. The novel FGF4 retrogene was only identified in the original two IVDD affected Pit Bull Terriers. Future directions include investigating dogs which have the FGF4 retrogene on chr 12 to see if, when combined, the FGF4 retrogene on chr 13 affects severity.

Research Grant: NIH Student Training in Advanced Research 2018 Fellowship (T35)

Student Support: NIH Student Training in Advanced Research 2018 Fellowship (T35)

Correlating clinical and pathological findings in inflammatory CNS diseases in dogs

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Meningoencephalitis of Unknown Etiology (MUE) refers to a group of idiopathic, non-infectious inflammatory diseases of the central nervous system (CNS) in dogs. Based on histopathological characteristics, the three subtypes granulomatous meningoencephalitis (GME), necrotizing leucoencephalitis (NLE) and necrotizing meningoencephalitis (NME) are recognized. A reliable antemortem diagnosis can often not be made due to the animal presenting with non-specific signs. In cases where MUE is suspected, a guarded prognosis is given. Our long-term goal is to develop a diagnostic test that can differentiate inflammatory CNS diseases based on the pattern of their adaptive immune responses. The objectives of this project were 1) to characterize case material by correlating clinical data and histopathology and 2) to gauge the degree and type of inflammation (B vs. T cells). Formalin-fixed and paraffin-embedded brain tissue samples from dogs with inflammatory CNS disease were retrieved from the archives of the University of Guelph and University of California - Davis and classified histologically. Out of 95 cases total, 68 (72%) were classified as GME, 10 (11%) as NLE and 17 (18%) as NME. The mean age of patients with NME was significantly lower than that of patients with GME ($P = .02$) and Pugs were overrepresented among patients with NME. The compilation and analysis of clinical data is ongoing and includes assessment of therapy and survival time. PCR-based amplification of B and T cell receptor genes suggested that both lymphocyte types are present in all 3 diseases, albeit at varying numbers. These data will serve as a basis for subsequent immune profiling by high-throughput sequencing.

Research Grant: Research Grant: OVC Pet Trust (Guelph) and Center for Companion Animal Health, University of California (Davis)

Student Support: Student Support: Boehringer Ingelheim Scholars Program

Role of catfish B cells in innate and adaptive immune responses to *E. ictaluri* live attenuated vaccines

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Enteric septicemia of catfish (ESC) is a devastating disease within the catfish farming industry, resulting in large economic losses and high mortality rates annually. The causative agent of ESC is *Edwardsiella ictaluri*, an intracellular, Gram-negative bacterium. There are previously established methods of treatment for ESC, such as medicated feed, however live attenuated vaccines (LAVs) are currently being studied as a viable form of prevention. Two LAVs of *E. ictaluri*, *EiΔevpB* and ESC-NDKL1 that showed strong protection in catfish fry and fingerlings have been recently developed in our laboratory. The aim of this study was to determine the effects of the LAVs on innate and adaptive immunity, with specific focus on antibody production, phagosome formation and bacterial killing properties of catfish B cells. We showed that IgM levels in the sera of challenged fish peaked at 14 days, followed by significant decreases at 21 days, with additional increases at 28 and 35 days post-exposure, suggesting development of protective adaptive immune responses. Furthermore, the phagosome formation was observed in IgM⁺ B cells purified by magnetic sorting and exposed to both LAVs, and wild-type *E. ictaluri* in vitro. The bacterial killing assay did not show significant decreases in bacterial numbers throughout the 48-hour incubation, suggesting that although catfish B cells were capable of phagocytosis, they lacked the bactericidal ability at 4°C. These findings support the hypothesis that catfish B cells are able to respond to *E. ictaluri* LAVs by eliciting protective innate and adaptive immunity.

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Student Support: NIH T35 Training Grant T35OD010432

Investigating the role of WWOX in DNA damage repair and radiosensitivity of canine osteosarcoma cell lines

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Osteosarcoma (OS) is the most common form of malignant bone cancer in both dogs and children. Despite aggressive treatment, up to 40% of children and > 90% of dogs die due to metastatic disease. WW domain-containing oxidoreductase (WWOX) is a tumor suppressor gene that is frequently deleted in many human cancers, including OS. WWOX has been shown to play a major role in regulating DNA damage repair responses in human cell lines. Our laboratory has been studying canine OS and found that similar to human OS tumors, loss of WWOX is a frequent event in canine OS cell lines and tumors. We hypothesize that loss of WWOX in canine OS cell lines impairs DNA damage repair and cell cycle checkpoint activation, thereby contributing to the genomic instability of OS. The canine OSA8 and Abrams OS cell lines which express low basal levels of WWOX were transduced with empty vector or canine WWOX lentiviral constructs. Overexpression was validated using qRT-PCR and Western blotting. Cell lines were treated with aphidicolin (Aph) or ionizing radiation (IR) to induce DNA replication stress. Effects of WWOX overexpression on cell survival were assessed by CyQuant Cell Proliferation and clonogenicity assays. We found WWOX expression in the Abrams OS cell line decreased colony formation following IR. No effect was found with Aph treatment for either cell line, suggesting WWOX does not play a significant role in single-stranded DNA damage repair pathways in these cell lines. Studies are underway to characterize the role of WWOX in regulating cell cycle checkpoint activation. This study will provide new data regarding the role of WWOX in canine OS cell biology with the ultimate goal of identifying novel targets for therapeutic intervention.

Research Grant: Steps for Sarcoma Foundation Pilot Award

Student Support: Rainier Scholar Program

Regulation of glutamate receptors trafficking and localization by calcium and reactive oxygen species

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Recent evidence revealed a new role for reactive oxygen species (ROS) as an important signaling molecule in healthy cells. During normal physiological states, ROS alter intracellular calcium (Ca^{2+}) levels by modifying voltage-gated Ca^{2+} channels in various cell types. In neurons, these Ca^{2+} channels are vital for the proper trafficking and localization of AMPA subtype of glutamate receptors (AMPA), which is required for normal learning and memory. Preliminary results show that increased ROS levels cause abnormal AMPAR localization. This led us to ask: do ROS regulate AMPAR trafficking and localization via the modulation of voltage-gated Ca^{2+} channels? To answer this, we utilized the genetic malleability, transparent nature and molecular conservation of AMPARs in *C. elegans*. Using fluorescently tagged AMPARs and available voltage-gated Ca^{2+} channel mutants, we created genetically modified *C. elegans* that allow us to visualize the localization of AMPARs in single neurons in intact animals in real-time. To assess if ROS modulation of AMPAR dynamics is dependent on specific Ca^{2+} channels, we treated our wild-type and Ca^{2+} channel mutants with the intracellular ROS inducer rotenone before imaging. Our results indicate that increasing ROS levels down regulate trafficking and alter synaptic AMPAR distribution via a Ca^{2+} channel-dependent mechanism. Elucidating how two ubiquitous intracellular signaling pathways such as ROS and Ca^{2+} regulate AMPAR transport requires genetic control and high spatio-temporal analysis of trafficking. Understanding this novel pathway will allow for a more comprehensive grasp of what factors influence AMPAR localization and the subsequent synaptic changes that underlie learning and memory.

Research Grant: Cooperative Research Centers and Department of Biomedical Sciences, Colorado State University

Student Support: NIH Grant T35OD015130-06

A look into how an energy-dense diet induces inflammation in the feeding centers of the brain

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Obesity is an epidemic in the United States now affecting 1 in 3 people. It is defined as having a BMI of 30kg/m² or more. Energy dense diets lead to obesity and comorbidities like diabetes, heart disease, and NAFLD. Our previous studies have shown that an E-D diet can lead to dysbiosis of the gut microbiome followed by inflammation in the gut-brain axis including the nodose ganglion, vagal afferents, and hind-brain. We hypothesized that an E-D diet would increase inflammatory cytokine levels in the blood and liver. We used 6 male and 6 female Sprague-Dawley rats being fed a regular chow diet for 10 days and then an energy dense diet for 4 weeks. Measurements of caloric intake, body weight and skin temperature were measured 3 times/week. Body composition was measured weekly using NMR technique. Blood samples were collected at first and last day of baseline and last day of E-D diet to measure systemic cytokines and insulin levels. Post-sacrifice liver samples, brain, and nodose ganglia were collected and analyzed for fat content, inflammation, and cytokine levels. Our results showed that rats fed an E-D diet had a significant increase in body weight, body fat, and insulin levels. Contrary to our hypothesis, cytokine analysis showed significant decreases in several cytokines in both male and female livers and serum. The only significantly increased cytokine was TGF- β (anti-inflammatory) in female livers. The results suggest that pro-inflammatory cytokines being produced due to an E-D diet are not traveling to the brain via the systemic circulation. Therefore, the inflammation in the feeding center of the brain shown in our previous studies is linked to neural pathways rather than systemic pathways.

Research Grant: NIH No. 1R01DC013904

Student Support: Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Quantification of rickettsial load during the acute phase of experimental bovine anaplasmosis

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Anaplasma marginale is a prototypical tick-borne pathogen, which is the primary etiologic agent of bovine anaplasmosis. This disease has been characterized in depth, with well-established acute and subsequent subclinical phases. Although ascending levels of parasitemia (percent infected erythrocytes) are well established during the incubation through acute phases of bovine anaplasmosis, there are data indicating the highest rickettsial load in peripheral blood of a related pathogen, *Ehrlichia canis*, is not always correlated with the acute phase of canine monocytic ehrlichiosis. This discrepancy could be due to differences in pathogen numbers within infected host cells. Thus, the central hypothesis of this study is that total rickettsial load in the peripheral blood is directly correlated with parasitemia during the onset of acute bovine anaplasmosis. Six calves were experimentally infected with *A. marginale*, and peripheral blood was monitored throughout transitions from incubation, acute and subclinical phases of experimental *Anaplasmosis marginale*. Parasitemias and packed cell volumes were measured with standard methods. Template was isolated with a silica-based spin-column kit (Roche, Indianapolis). Quantitative PCR (qPCR) will be used to measure rickettsial loads. A related experiment will test the working hypothesis that different anticoagulants (*i.e.*, EDTA, heparin and sodium-citrate) will not affect qPCR after silica-based template isolation. This study is expected to demonstrate that parasitemia is a direct reflection of rickettsial load. Moreover, these results are expected to determine the utility of these methods for *A. marginale* transmission studies.

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Student Support: National Institute of Food and Agriculture or USDA Grant No. 12213164

Feasibility and position optimization of a wearable smartphone ECG monitor in exercising horses

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The frequency of sudden death in horses is disproportionately larger than in humans. Studies in large groups of horses are necessary to gather meaningful data, but the lack of ‘trainer-friendly’ ECG devices is a major impediment. The specific aim of this study was to determine the feasibility and optimal position for the use of a wearable ECG monitor (Qardiocore©) in horses. Exercising electrocardiograms (ECGs) from 7 horses, at 3 different positions of the monitor, were analyzed. The association ‘diagnostic ECG’ with the variables gait, position, and their interaction was examined using mixed-effects logistic regression. The association of percentage of readable ECGs and amplitude with position was assessed using linear mixed-effects modeling. The odds of the recording being diagnostic were significantly higher for positioning over the heart ($P=0.049$; odds ratio 4.9 [95% CI, > 1 to 22.8]), and no other differences among positions were significant. The percentage of recording that was readable was largest when the device was placed over the heart followed by the level of the point of the shoulder. Model-based estimates showed that percent acceptable ECG decreased with each increase in level of exercise. Position of the device over the heart provided a significantly ($P < 0.05$) larger amplitude (0.85mV [95% CI, 0.66 to 1.04mV]) than other locations. Results indicate obtaining exercising ECGs using the wearable ECG Qardiocore© is feasible. Positioning the monitor at the level of the heart provides the best combination of more diagnostic ECGs, a higher percentage of readable ECGs, and the largest QRS amplitude. This position will be tested with a larger group of ridden horses.

Research Grant: VLCS internal funds

Student Support: NIH T35 Training Grant – OD010991

The effect of estrous stage on larynx physiology in rats

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Women report a variety of voice changes associated with their menstrual cycle including breathiness, changes in pitch, unsteady pitch and loss of high notes. These voice changes are termed dysphonia premenstruales. Erythema and edema have also been reported in women experiencing dysphonia premenstruales. This study set out to determine if female rats experience gross or microscopic changes in the larynx depending on their estrous stage. To determine estrous stage, cytology of daily vaginal smears was stained with Modified wright stain and evaluated by light microscopy. Estrous stages (proestrus, estrus, and diestrus) were determined based on vaginal cell type proportions. Once a rat reached the desired estrous stage, she was culled and her larynx was harvested. Larynges were fixed in neutral buffered formalin and embedded in paraffin according standard methods. Larynges were sectioned in the coronal plane and stained with hematoxylin and eosin (HE), Masson’s trichrome, Verhoeff-van Gieson, Alcian blue, and Movat’s pentachrome. Differential staining pattern and morphology will be reported at poster presentation. Results of this study will determine the viability of female rats as comparative animal models of dysphonia premenstruales.

Research Grant: National Institute of Deafness and Communications Disorders

Student Support: Purdue Veterinary Medicine’s 2018 Veterinary Scholars Summer Research Program

Addition of IL-10 response to hyperoxic and normoxic conditions by the Mas agonist AVE-0991

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Bronchopulmonary dysplasia is a chronic lung disease that affects mostly premature newborns. The disease results from excess oxygen associated with prolonged use of mechanical ventilation or a respirator which causes apoptosis of cells in the lungs which leads to inflammation of lung tissue. Our lab uses an hyperoxic gas chamber to replicate the use of a respirator. Prior lab experiments have shown that AVE-0991, a non peptide agonist of the angiotensin (1-7) Mas receptor, inhibits apoptosis and has anti-inflammatory effects in cells treated with hyperoxic gas. To further understand the mechanisms behind these findings, our lab will test the levels of an inflammatory-related cytokine, IL-10, in A549 cells after being treated with AVE-0991 and hyperoxic gas. We hypothesize that AVE-0991 will reverse the effects of hyperoxic gas on IL-10 release by the cells. Levels of IL10 will be tested in cells treated with AVE-0991 and cells without AVE-0991 that were exposed in hyperoxic and normoxic gas for respective timepoints. A549 cells were cultured and when at the right confluency, were treated with AVE-0991 and exposed in hyperoxic and normoxic gas chambers. ELISA kits will then be used to test for the levels of IL-10 cytokines in the cells. We predict that cells treated with AVE-0991 will have higher levels of IL-10 than the cells not treated with AVE-0991. Understanding the relationship between IL-10 with AVE-0991 and hyperoxic gas will help us to better understand the mechanisms behind apoptosis and inflammation associated with excess oxygen and may lead to new ways to treat and prevent this disease.

Research Grant: None

Student Support: NIH R25HL103156

Developmental competence of ovine and caprine vitrified oocytes using a closed system

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The current study investigated the developmental competence of cryopreserved (via vitrification) ovine and caprine in vitro matured oocytes that were subjected to in vitro fertilization (IVF) procedures. We hypothesized that developmental competence of non-vitrified and vitrified ovine and caprine oocytes as assessed by oocyte maturation, embryo cleavage and blastocyst rates would not differ significantly. Ovine and caprine ovaries were collected from a local abattoir. After 24 hours of in vitro maturation (Day -1), the ovine and caprine oocytes were split into 3 groups: (control group) oocytes underwent conventional IVF; (denuded group) oocytes were denuded and oocytes with an extruded polar body (MII) underwent IVF; (vitrification group) oocytes were denuded and checked for a polar body as previously described, vitrified, warmed and then subjected to IVF. Maturation, cleavage, and blastocyst rates were recorded on Days 0, 2, and 7, respectively. Results included: ovine maturation rate: 48.9%, cleavage rate: 40%, 30%, and 0% and blastocyst rate: 24%, 0%, and 0% for the control, denuded, and vitrified groups, respectively; caprine maturation rate: 49.5%, cleavage rate: 0%, 0%, and 2.85% and blastocyst rate: 0%, 0%, and 0% for the control, denuded, and vitrified groups, respectively. These results are not in agreement with cleavage and blastocyst rates previously obtained from our lab during the spring and fall. It is possible that hot environmental temperatures during the summer months may have had detrimental effects on oocyte developmental competence, therefore, drastically reducing the oocyte maturation, embryo cleavage and blastocyst rates.

Research Grant: None

Student Support: Boehringer Ingelheim

Examination of the *Mannheimia haemolytica* genotypes in dairy cattle isolated at the WVDL from 2015 to 2018

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Mannheimia haemolytica is a bacterial pathogen that is normally found within cattle upper respiratory tracts; however, certain strains can migrate to the lungs causing bovine respiratory disease in cattle upon stress, transport or viral infection. Previous research using isolates predominately from beef cattle has demonstrated that *M. haemolytica* genotype 1 is non-pathogenic and is not antimicrobial resistance and genotype 2 pathogenic and multidrug resistant, particularly to macrolides. Therefore, it is predicted that dairy cattle *M. haemolytica* isolates from the upper respiratory tract will be genotype 1 and isolates from the lungs will be genotype 2 and multidrug resistant. Using MALDI-TOF mass spectroscopy, references strains of genotype 1 and 2 *M. haemolytica* spectra were imported from Loy et al. (date). These spectra were used as a library to assign genotypes for *M. haemolytica* isolated between July 2015 and July 2017 to the Wisconsin Veterinary Diagnostic Laboratory (WVDL). We used that information along with traditional minimum inhibitory concentration (MIC) data and pathoclinical findings that were performed as routine testing to determine, if for dairy cattle, genotype 1 is indeed non-pathogenic and susceptible and genotype 2 is multidrug resistant and virulent. Preliminary data demonstrate shows more variation in genotypes isolated from dairy cattle than beef. It is important to understand the genotype and antibiotic resistance distribution in dairy cattle, as this data may be used to determine if antibiotic susceptibility testing will be performed on genotype 1 isolates at the WVDL. Clinicians can use this information to limit antibiotic usage to only those *M. haemolytica* isolates that are genotype 2.

Research Grant: None

Student Support: Dept of Pathological Biosciences School of Veterinary Medicine, University of Wisconsin-Madison

Optimizing conditions for non-viral mRNA transfection of bone marrow cells using mineral-coated microparticles

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Growth factors such as bone morphogenetic protein-2 (BMP-2) are key players in bone and cartilage regeneration and repair. However, to be therapeutic, clinical use of BMP-2 requires very high doses which is commonly associated with adverse side effects such as ectopic bone growth. Non-viral transfection of bone marrow cells with mRNA lipoplexes offers an attractive way to enhance localized growth factor expression at the site of an osteochondral defect, thereby improving healing and reducing complications while also avoiding the safety concerns associated with viral gene delivery. Our study utilized mineral-coated microparticles (MCMs) that were designed to function as a reservoir system for non-viral gene carriers by binding delivered mRNA, limiting off-target effects through localization, and maintaining adequate transfection efficiency. Using reporter genes, we tested transfection efficiency and cytotoxicity in cell cultures by varying mRNA concentration, MCM and reagent ratios, and incubation times. Our results indicate that mRNA is superior to plasmid DNA in terms of short term transfection efficiency, and that MCMs have shown efficacy in improving delivery of mRNA to transfect several cell types of interest to bone healing. Based on these findings, MCM-mediated mRNA lipoplex delivery represents a promising strategy for overexpressing therapeutic genes in orthopedic applications, and future studies will evaluate whether MCM-mediated BMP-2 mRNA delivery can be used to promote bone and cartilage healing *in vivo*.

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Student Support: NIH T35 Training Grant 5T35OD011078-08 (PI: Bjorling)

The effect of irradiated human squamous cell carcinoma cells on microglia activation and survival

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Previous studies in mouse models have demonstrated neuroinflammation following radiation therapy (RT) targeting distant (i.e. non-CNS) tissue. The mechanism leading to this inflammation is unknown. The hypothesis is that inflammatory mediators produced by irradiated tissue lead to neuroinflammation and contribute to the cognitive deficits seen in cancer patients prescribed non-brain RT. The purpose of this study was to identify and measure cytokines produced by human squamous cell carcinoma cells (TR146) after irradiation and determine their effect on human microglia (HMC3). Growth curves and radiation dose-response were determined to define the ideal culture media and target radiation doses. To simulate the effect of peripheral tumor treatment on microglia, media from irradiated TR146 cells (0, 3, 6, 9, and 12 Gy) was transferred to microglia at 1, 6, and 24 hours (h) post-treatment. To determine microglial viability, MTS assay was performed 48h following serum transfer. To determine changes in their activation state, microglia were stained for Iba1 expression 48h after serum transfer. TR146 cells had decreased dose-related viability and clonogenic survival after radiation. Viability of microglial cells following media transfer from irradiated TR146 cells did not demonstrate a clear dose-related decrease in viability. Future work will include measurement of cytokine production from media collected over time from irradiated TR146 cells and co-culture of microglial cells with irradiated TR146. Results will inform the underlying mechanisms contributing to non-brain radiation related cognitive dysfunction and permit strategic intervention to mitigate radiation side effects.

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Student Support: National Institutes Of Health, Award Number T35OD011118

Sedation protocols in juvenile Blue Poison Dart Frogs (*Dendrobates tinctorius azureus*)

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Blue poison dart frogs (*Dendrobates tinctorius azureus*) are maintained by several zoological institutions and are commonly kept by amphibian pet owners. Sedation or light anesthesia is often necessary to enhance the quality and safety of general physical examinations, routine diagnostics, and therapeutic procedures. Currently, there is a lack of published, systemically-derived injectable sedation data in amphibians, including poison dart frogs. Clinicians must extrapolate information regarding drug dosages and combinations from other unrelated species which leads to a lack of desired clinical drug efficacy and potentially harmful side effects including death. The purpose of this study was to develop and evaluate partially reversible and safe sedation protocols in blue poison dart frogs that would achieve a moderate to deep level of sedation suitable for performing a variety of clinical procedures. A blinded, complete crossover study was performed in which two sedation protocols (ketamine-dexmedetomidine-midazolam versus alfaxalone-dexmedetomidine-midazolam) were administered subcutaneously in juvenile blue poison dart frogs (n = 10). Heart rate, pulmonic respiratory rate, various reflexes (including Von Frey filament testing) and behavioral parameters were monitored after sedation injection. Antagonists for dexmedetomidine and midazolam (atipamezole and flumazenil, respectively) were administered 50 minutes after the initial injection of sedation. Preliminary results demonstrated that both sedation protocols provided safe and effective sedation with rapid reversibility.

Research Grant: University of Wisconsin-Madison School of Veterinary Medicine Companion Animal Fund

Student Support: University of Wisconsin-Madison School of Veterinary Medicine

Determining cryptic speciation in two- and three-toed sloths using molecular phylogenetics

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Brown-throated three-toed sloths (*Bradypus variegatus*) and Hoffmann's two-toed sloths (*Choloepus hoffmanni*) are classified as Least Concern by the International Union for the Conservation of Nature (IUCN) due to historical understanding that these species are widely distributed in Central and South America. However, two distinct populations of *C. hoffmanni* are recognized and both species are being threatened due to habitat destruction and fragmentation, illegal wildlife trade, and in the case of *C. hoffmanni*, consumption by local populations. Given that sloth populations have been isolated over time, there is evidence that these species may comprise distinct evolutionary units that would warrant independent classification and protection. Moreover, recent genetic studies have provided support for cryptic speciation. The aim of this study is to construct the first molecular phylogenetic tree to encompass two- and three-toed sloths across their range in Central and South America using DNA samples collected from wild populations and museums as well as saliva samples from sloths housed in North American zoos. DNA and mitochondrial (16S) and nuclear (ENAM) genes will be amplified, and sequenced. Using the sequences, a Bayesian phylogenetic tree will be built and population genetic statistics will be calculated. I hypothesize that two- and three-toed sloths will actually comprise multiple cryptic species that have each accrued local genetic variations based on isolation by distance. Data from this study will be used, if necessary, to change the IUCN status of two- and three-toed sloths and to propose appropriate conservation measures for potential new cryptic species.

Research Grant: Systematics Research Fund of the Linnean Society and the Systematics Association

Student Support: School of Veterinary Medicine, University of Wisconsin-Madison

Characterizing larval epidemiology of the meningeal worm (*Parelaphostrongylus tenuis*)

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Meningeal worm (*Parelaphostrongylus tenuis*) is a parasite causing fatal neurologic disease in accidental ruminant hosts. Its prevalence in a declining Minnesota moose (*Alces alces*) population was found to be 20-35% in adult mortalities, indicating a need to further understand its epidemiology. Larvae are shed by the definitive host, white-tailed deer (*Odocoileus virginianus*), with a gastropod intermediate host. The current methodology to estimate shedding rates uses manual counts of extracted larvae per gram of feces, using a modified Baermann technique. This method may be susceptible to high user error and is time intensive. Our research seeks to compare the current methods with metagenomic and molecular tools for the detection and quantification of larvae, and to assess genotypic variability. White-tailed deer fecal pellets were collected in Grand Portage, MN, during peak larval shedding in winter and early spring. Larvae were manually counted, with 5-10 individuals extracted per deer sample (n=57). Fifty-nine percent of fecal samples were found positive, with spatially heterogeneous shedding rates ranging from 0.3 to 291 larvae/gram. Fecal samples will be subjected to a newly developed droplet digital polymerase chain reaction to compare quantification of larval shedding rates with manual methods. Individual extracted larvae will also be genotyped by restriction site-associated DNA sequencing to evaluate within-host genetic variability, for use in comparing inter-host relatedness and spread across the landscape. These tools will be used to more reliably and efficiently investigate the epidemiology of meningeal worm, to better understand transmission risks and routes of introduction.

Research Grant: College of Veterinary Medicine Population Systems Signature Grant, Van Sloun Foundation, University of Minnesota Genomics Center GenoPitch

Student Support: Morris Animal Foundation Veterinary Summer Scholars Award (D18ZO-604)

The role of Tc17 cells in enhancing neutrophil recruitment to the Chlamydia-infected genital tract

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Chlamydia trachomatis is the leading cause of sexually transmitted bacterial infection worldwide and leads to serious pathological sequelae in the female upper genital tract. Our laboratory has shown that antigen (Ag)-specific CD8 T cells are required for pathogenesis in the genital tract, whereas others have demonstrated a role of neutrophils using the mouse model of *C. muridarum* infection. We have recently also found that Ag-specific CD8 T cells modulate neutrophil infiltration into Chlamydia-infected genital tracts, suggesting that these cell types act in a serial pathogenesis cascade. IL-17 is a known chemoattractant for neutrophils, and CD8 T cells (Tc17 type) are capable of producing this cytokine. Therefore, we hypothesize that Chlamydia-specific CD8 T cells enhance neutrophil recruitment into infected genital tract via IL-17 production. In order to address this, we will use the OT-1 transgenic mouse (OT-1) wherein all CD8 T cells are restricted to recognition of ovalbumin peptide and therefore unable to recognize and respond to chlamydial antigens. These mice will be replete with purified splenic CD8⁺ T cells from wild type mice or IL-17 knockout CD8 T cells, and subsequently infected intravaginally with *C. muridarum*. The frequency of various inflammatory cell types including all leukocytes (CD45⁺), neutrophils (Ly-6G⁺), macrophages (F4/80⁺), dendritic cells (CD11c⁺), and CD4⁺ and CD8⁺ T cells in the genital tract will be analyzed by flow cytometry on day 10 following infection. We expect to find that adoptive transfer of WT, not IL-17^{-/-}, CD8⁺ T cells into OT-1 mice will enhance infiltration of various inflammatory cells, including neutrophils, into the Chlamydia-infected genital tract.

Research Grant: National Institutes of Health grant 2R15AI101920 and Midwestern University Faculty Startup Grant

Student Support: Midwestern University and Boehringer Ingelheim Summer Research Fellowship

Effects on equine neonates of exogenous progestogens given to late gestation mares

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Mares with high-risk pregnancies (e.g., placentitis) are often given exogenous progestogens (progesterone, altrenogest) to maintain pregnancy. Although the use of these steroids in some mares is justified, for many of them it lacks justification. Progestogens can cross the placental barrier and potentially affect the health of the fetus and newborn foal. Our research group recently showed that endogenous progestogens such as progesterone are associated with disease severity in critically ill foals. High progesterone was associated with maladjustment syndrome. Therefore, it is reasonable to assume that progestogen administration to pregnant mares can lead to perinatal diseases. Maladjustment syndrome (“dummy” foal syndrome) and sepsis are the main causes of death in newborn foals. We hypothesize that the exogenous progestogens given to pregnant mares contribute to the dysregulation of the hypothalamus-pituitary-adrenal axis (HPAA) and to steroid imbalances, contributing to foal disorders and mortality. We will measure steroid hormones in healthy and sick foals born to mares treated and untreated with progestogens. Results will be associated with treatment, disease severity, and mortality. Progress: We have collected blood samples and clinical information from ~200 foals. Hormone measurement is ongoing and results are forthcoming.

Research Grant: OSU Equine Research Funds

Student Support: Epperson Summer Research Scholar

IgE concentrations in bronchoalveolar lavage fluid from horses with severe pasture-associated equine asthma

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Equine asthma is the most common respiratory disease of horses. Horses with severe asthma demonstrate respiratory distress at rest. Stall-associated severe equine asthma predominates in horses housed in continental climates and has been clearly associated with exposure to moldy hay. However, the etiology of a pasture-associated form of severe equine asthma (equine pasture asthma, EPA) affecting horses during conditions of high heat and humidity is unclear. We identified Basic Helix Loop Helix 40 (BHLHE40) as differentially expressed in the lung transcriptome of EPA horses. BHLHE40 is a TH2 signature gene in human asthma that is central to IgE production. This is curious because eosinophilic airway infiltrates typify Th2 responses. However, horses with EPA demonstrate neutrophilic airway inflammatory infiltrates. In human asthma, neutrophilic airway infiltrates with strong antigen-specific IgE responses are diagnostic of asthma with fungal sensitization. Accordingly, we hypothesize that increases in BHLHE40 in the lung transcriptome of EPA horses will be reflected in increased IgE in their bronchoalveolar lavage fluid (BALF). To examine this hypothesis, total IgE was quantified in BALF of EPA (N=8) and non-diseased control horses (N=11) using an equine IgE-specific ELISA. Horses were sampled during timing of seasonal asthma exacerbation and remission in the diseased horses. Significant differences in total IgE concentrations were not identified in comparisons between diseased and control horses, nor in comparisons within groups by season. Efforts to quantify antigen-specific IgE are warranted in order to determine the significance of increased BHLHE40 in BALF samples of horses with pasture-associated asthma.

Research Grant: USDA-NIFA Agriculture and Food Research Initiative (AFRI) Animal Health Program competitive grant no. 2015-67016-23172

Student Support: Mississippi State University College of Veterinary Medicine

Pre-operative dermatological findings for dogs undergoing total hip replacement: a retrospective study

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Total hip replacement (THR) is a surgical procedure recommended to alleviate pain and functional deficits associated with the coxofemoral joint. Standard of care at the Veterinary Medical Teaching Hospital (VMTH) for THR patients requires a pre-operative dermatological evaluation to help mitigate post-operative surgical site infection (SSI)--the incidence of post-operative SSIs in patients included in the study was 0%. The medical records of 111 dogs that received a THR at VMTH over a five year period were reviewed. Of the 111 dogs, 17 received THR surgeries bilaterally resulting in 128 total cases included in the study. Seventy one (71) cases defined by passing a dermatological evaluation prior to THR were assigned as Group A. The remaining 47 cases did not pass a single, or multiple, dermatological evaluation(s) prior to eventual THR (Group B). Dogs were examined for skin lesions by VMTH dermatologists prior to anticipated surgery. The incidence of failure to receive THR for a dermatological reason (lesional skin) at first presentation in this study was 39.1%. The average time to THR surgery for cases in Group B was 119.9 days. There were 77 total failure events in the study, 14 of which occurred at the time of surgical clipping. Coat length, age of the patient, or season at presentation did not have a significant effect on the incidence of failure when considered independently. Most dogs in Group B had lesions attributable to superficial pyoderma, due to suspected underlying allergic skin disease. The data of this ongoing descriptive study will hopefully be utilized to better inform dog-owners and general practitioners of potential risk factors necessitating postponement of THR.

Research Grant: None

Student Support: Boehringer Ingelheim VSP, Texas A&M College of Veterinary Medicine & Biomedical Sciences

The role of gut microbiome in susceptibility to diarrhea associated with enteric pathogens in shelter kittens

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Shelter animals are especially at risk for gastrointestinal (GI) infections due to environmental factors and stress. There is a need for research on the prevalence of GI disease and the association between disease, enteric pathogens, and microbiome composition in cats. Previous research in humans, cats, and dogs has demonstrated that the microbiome composition has a large impact on resisting pathogen colonization. We hypothesize that the gut microbiome differs in shelter kittens with and without diarrhea, and specific differences in microbes present can be linked to the presence of and susceptibility to selected pathogens. Fecal samples were collected from kittens upon entry into a local animal shelter. Samples were collected from seven kittens, but two were excluded due to poor sample quality. Fecal DNA extraction and PCR amplification of the V4 region of 16S was done prior to sequencing by Illumina Miseq at the MSU Genomics Core. The sequence results will be analyzed on the pipeline Mothur. Our findings are expected to show differences between the microbiomes of kittens depending on the presence of diarrhea and specific enteric pathogens. Detection of enteric pathogens was done utilizing MacConkey agar culture plates for isolation of Gram-negative enteric bacteria, PCR detection of bacterial genes in fecal DNA, and fecal flotation for identification of intestinal parasites. Fecal DNA from two kittens contained DNA specific for *Campylobacter spp.* One sample was sufficient for fecal flotation and was negative for intestinal parasites. This study is important because it could lead to a more specific probiotic regimen, which could ameliorate GI disease, limiting shelter costs and improving kitten health.

Research Grant: None

Student Support: NIH Grant T35OD016477 to Michigan State University

Exploiting biological priors to enhance cruciate ligament rupture candidate locus discovery in dogs by GWAS

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Canine cruciate ligament rupture (CR) is a devastating complex disease with genetic and environmental components. It is life-changing for dogs as stifle arthritis inevitably occurs and risk of a second rupture is very high. While there are many risk factors that predispose dogs to developing CR, other than breed, few have consistently been associated with CR. Estimates in commonly affected breeds suggest that CR has moderate heritability (~0.25-0.45). Currently, limited work has been done to investigate the genetic basis of CR. Thus, the specific aim of this project is to identify differentially expressed genes associated with canine CR. Cranial cruciate ligament (CL) and synovium from four CR affected and four controls were collected and matched across variables known to influence CR. RNA sequencing generated a library of strand-specific paired-end 125bp reads. After correction for multiple testing, 374 differentially expressed genes were identified between CR case and control samples in synovium tissue. Analysis with DAVID identified C-type lectin and MHC Class II pathways, which play important roles in immune response, as significantly enriched in synovium tissue. There were 186 differentially expressed genes in CL but no significantly enriched pathways. We will also use an enhanced Bayesian mixture model analysis (BayesRC) for discovery of CR candidate loci. We expect this work to advance understanding of the genetic architecture of the disease and could identify therapeutic targets for disease-modifying medical treatment, screening of at-risk dogs, and tailored clinical care of the individual. These findings may also translate to understanding similar ligament and tendon ruptures in horses and humans.

Research Grant: Companion animal grant funding and Melita Grunow Family Professorship

Student Support: Morris Animal Foundation

Purification of murine pancreatic lipase - Initial findings

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The purification of pancreatic lipase has been reported for many species. Tightly bound acidic phospholipids make purification of pancreatic lipase difficult. The objective of this study was to purify and partially characterize murine classical pancreatic lipase (mPL). Pancreata were collected from mice sacrificed for unrelated research studies. Lipids were removed by excising gross fat and by extraction with organic solvents. The delipidated pancreatic tissue was further purified by anion-exchange chromatography using a Tris-buffer containing phenylmethanesulfonyl fluoride (PMSF) and benzamide as protease inhibitors. Anion-exchange chromatography mPL was further purified by size exclusion and cation-exchange chromatography. Then, mPL was partially characterized by SDS-Page and N-terminal amino acid sequencing. To date mouse pancreata have been delipidated and extracted and lipase activity was maintained. Anion-exchange chromatography produced variable results and the chromatographic method had to be adjusted. This study is a prerequisite for developing an immunoassay for the measurement of murine pancreatic lipase immunoreactivity in mouse serum for the study of mice with experimental exocrine pancreatic disease.

Research Grant: Gastrointestinal Laboratory at Texas A&M University intramural funds

Student Support: Gastrointestinal Laboratory at Texas A&M University

Colon-targeted delivery of a novel treatment for *Tritrichomonas foetus* infection in cats

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Tritrichomonas foetus, a prevalent protozoal pathogen of the colon, is a cause of chronic large bowel diarrhea in cats worldwide. Currently, the only treatment is a drug called ronidazole, which is unable to clear the infection in almost 40% of cases and can cause neurotoxicity. A novel treatment was demonstrated to be more effective *in vitro* at lower doses than ronidazole. However, when given orally to *T. foetus* infected cats, the drug failed to clear the infection due to absorption into circulation before reaching the colon. We hypothesize that a colon-targeted tablet could be developed and would improve efficacy by enabling delivery specifically to the site of infection. Guar gum is a plant-derived β -1-4-linked polysaccharide that cannot be metabolized by mammalian enzymes but is susceptible to degradation by colonic bacteria. Guar gum-encased drug tablets were created with a hydraulic press. They were tested for their ability to withstand simulated gastric and small intestinal environments yet dissolve in the simulated colon. After selecting the best coating, we compounded the core drug with barium tracer prior to guar gum coating and tracked the tablet *in vivo* via radiography following oral administration to healthy client-owned cats. High-performance liquid chromatography (HPLC) assays of plasma and fecal samples were used to assess targeted dissolution of the tablet in the colon. These studies will enable resumption of clinical trials of the new drug for treatment of cats with ronidazole-resistant *T. foetus* infection, with potential applications for colon-targeting of a variety of drugs to the feline colon.

Research Grant: Winn Feline Foundation

Student Support: Boehringer Ingelheim Veterinary Scholars Program, Fund for Discovery

Assessment of AAVR contribution to AAV8-luciferase *in vivo* transduction post retro-orbital delivery

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Adeno-associated virus (AAV) is a viral vector of broad tropism that can be engineered to deliver DNA in gene therapeutic technologies. However, its mechanisms for targeted tissue delivery remain poorly understood, hampering its therapeutic use. A transmembrane protein receptor KIAA0319L (AAVR) has been identified as an essential host factor for transduction of human and mice cells, but it is still unclear how AAVR contributes to tissue tropism *in vivo*. This study aims to assess the correlation of AAV transduction *in vivo* to differences in AAVR expression in tissues. We first determined expression of AAVR in mice tissues by immunohistochemistry. We then infected adult WT mice by retro-orbital injection of an AAV8 expressing a luciferase transgene and assessed luciferase activity in tissues. Additionally, mice that constitutively express AAVR under a strong promoter (CAG), Knock-In, were also infected. At 1 and 2 weeks post-infection (p.i.), functional transduction was assessed *in vivo* by bioluminescence to examine expression levels and kinetics. On day 16 p.i., animals were euthanized and organs harvested to assess luciferase and AAVR expression. *In vivo* imaging did not show significant differences in expression between WT and KI mice. Organ luciferase activity normalized to weight showed high expression in liver and heart of WT mice, while only moderate expression was observed in KI mice liver and lungs. Results show that in some tissues, the levels of AAVR may be critical, and its overexpression changes *in vivo* AAV tropism. Identifying a sensitive antibody for recognition of AAVR to compare expression levels in tissues remain to be determined for a more accurate characterization of AAVR expression in a murine model.

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Student Support: NIH/ORIP T35 Training Grant OD010989

Triple negative breast cancers are highly sensitive to ferroptosis

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Breast cancer is a leading cause of death for women in the developed world, and is widely divided into two categories: triple negative or receptor positive breast cancer. Triple negative breast cancers (TNBCs) do not express the estrogen, progesterone, or HER2 receptors, and are generally more aggressive than their receptor-positive counterparts. For receptor positive cancers, drugs can target the expressed receptors to inhibit cell proliferation. This is not an option for TNBCs, leaving them vulnerable only to more aggressive therapies such as standard chemotherapeutics, radiation, or surgery. Ferroptosis is a recently uncovered form of programmed cell death in which iron(III) ions oxidize cell membrane lipids, disrupting the cell's redox balance, leading to oxidative stress and cell death. We recently began exploring the sensitivity of assorted breast cancers to ferroptotic stressors. It was initially found that TNBCs were more sensitive to direct iron treatment, and accumulate reactive oxygen species more readily than receptor positive cells. We also examined the sensitivity of breast cancers to erastin, a small molecule that induces ferroptosis by blocking system Xc, a key regulator of cell redox balance. While erastin was found to have an average IC_{50} of 2 μ M for breast cancer cells regardless of receptor status, the maximal effect of the drug was much greater for TNBCs. By comparison, significant numbers of receptor positive cells were able to survive erastin treatment even at high concentrations of the drug. This effect was exaggerated by adding a small amount of iron(III) nitrate to the cell culture. These findings suggest that inducing ferroptosis may be an attractive target for treating TNBCs.

Research Grant: National Institutes of Health

Student Support: Veterinary Investigators Program at Cornell University College of Veterinary Medicine

Immune response to high frequency irreversible electroporation treatment of canine hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) incidence rates and mortality rates have been steadily increasing in humans over the past two decades. Liver transplantation is the only curative treatment; but most patients do not qualify. HCC in dogs is the most common form of hepatobiliary neoplasia and surgical excision is also the preferred treatment. The liver is an immunologically rich organ that provides a tolerant immune environment. This tolerogenic environment presents a hinderance for treatment of HCC. Reversing the tolerogenic environment of the liver may provide potential for treatment of hepatic neoplasia. A proof of concept clinical trial of canine HCC patients treated with high frequency irreversible electroporation (H-FIRE) demonstrated local recruitment of CD3+/CD4-/CD8- T-cells in the zone between ablated tumor and normal liver. Our project hypothesizes that H-FIRE treatment induces an immunostimulatory microenvironment in the treated HCC, compared to pre-treatment tumor biopsies. Pre- and post-treatment samples were obtained from three dogs diagnosed with HCC undergoing the H-FIRE study. Eight normal liver samples from healthy dogs were selected as normal controls. Gene expression was analyzed using RT-qPCR. A custom panel analyzing 89 potential genes associated with inflammation and cancer was constructed to detect changes in gene expression in pre- versus post-treatment H-FIRE HCC samples, normalized to the normal liver group. We expect to identify gene expression signatures associated with immune stimulation in the post-treatment group which will provide further insight on the tentative immunostimulatory effect of H-FIRE ablative therapy in HCC and highlight the value of dogs as a spontaneous cancer model.

Research Grant: Veterinary Memorial Fund, Grayton Friedlander Memorial Fund

Student Support: Virginia-Maryland College of Veterinary Medicine

Evaluation of canine cognitive function and exclusion criteria in a phase II rapamycin clinical trial

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The goal of this ongoing project is to perform a randomized, placebo-controlled, double-blind phase II clinical trial of rapamycin (a specific inhibitor of the mechanistic target of rapamycin complex I (mTORC1), a central regulator of cell growth) in healthy middle-aged dogs to determine whether low-dose rapamycin can (1) significantly improve age-related cardiac function, (2) positively impact age-associated cognitive decline, and (3) increase activity. Dogs are randomized to receive either rapamycin orally at 0.025 mg/kg or a placebo three times per week for the first six months of the one-year study. Comprehensive clinical monitoring is performed at 3-month intervals. Two components of this trial are the subjects of this abstract. Each canine nominee must satisfy three levels of inclusion criteria: an initial demographic survey, a medical record screening, and an enrollment appointment with a physical exam, comprehensive lab work and echocardiography. Excluded candidates are recorded, and the phase, origin (dog versus owner) and reason for the exclusion of each will be analyzed as the first component. In addition to clinical monitoring, three cognitive task experiments are used to evaluate whether or not a dog's performance in working memory, inhibitory control, and perseveration error change over the course of a year, and if any such change is associated with rapamycin. Evaluation of the development, administration and performance outcomes of these cognitive tasks is the second component. At the time of abstract submission, 10 dogs have been excluded and 7 dogs have been enrolled. All dogs excluded or enrolled by July 20, 2018 will be analyzed for presentation at the 2018 National Veterinary Scholars Symposium.

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Student Support: Texas A&M College of Veterinary Medicine & Biomedical Sciences

Comparing the significance of animal shelter parasite control programs on heartworm infection in LA mosquitos

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Previous studies (McKay et al., 2013) have demonstrated a dramatic increase in the percentage of mosquitoes infected with *Dirofilaria immitis* (DI), the causative agent of canine heartworm (HW) disease, when collected near a (HW+ microfilaremic) dog versus those collected in a general HW endemic region (73.7% versus 7.3%, respectively). However, little research has been done at an individual animal shelter level, specifically comparing if the way each shelter manages their HW+ dogs can make a difference in DI-infected mosquito rates in the surrounding areas. Microfilariae (Mf) are the blood circulating stage in HW+ dogs and are responsible for infecting mosquitoes and maintaining the HW transmission cycle. Currently, there is a macrocyclic lactone HW preventive product with a FDA-approved label claim for the treatment/elimination of the Mf life stage but unfortunately, these microfilaricidal products can be expensive and not every shelter can access them. Therefore, the overall purpose of this project is to investigate the relationship between an animal shelter's parasite control program and their surrounding mosquito populations to determine if there is a statistical variance in DI levels. Using geospatial technology, 3 shelters were selected with similar environmental conditions (St. Martinville, Iberville, and Tangipahoa Parish), and each shelter's parasite control protocol was reviewed. Mosquitoes have been collected and identified to genus, and we have begun DNA extraction and PCR amplification for DI-specific genomic material from the samples. We hypothesize that shelters that currently use microfilaricidal drugs on HW+ resident animals will have lower rates of DI infection in their neighboring mosquito populations.

Research Grant: None

Student Support: Boehringer Ingelheim

Effect of acupuncture on recovery from general anesthesia in shelter cats

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Acupuncture point GV-26, at the nose, has been shown to shorten anesthesia recovery in humans and animals. Shortening the time to recover from general anesthesia is beneficial to avoid several possible complications such as cardiac arrest, apnea, hypoxia, hypothermia, and monitoring difficulties. Although atipamezole can be used to reverse cats from dexmedetomidine, the drug is expensive and many shelters have limited resources. The aim of this study is to investigate the effect of acupoints GV-26 and Wei-jian stimulation on recovery from general anesthesia in shelter cats that also received a low dose of atipamezole. In this study, 30 cats of any breed, sex, or age from regular shelter population undergoing ovariohysterectomy or orchiectomy surgery were randomly assigned to one of three groups. Cats were premedicated with a single IM injection composed of dexmedetomidine (20 mcg/kg) and ketamine (5 mg/kg) with butorphanol (0.5 mg/kg). Isoflurane was administered to cats during surgery. Acupuncture was started after surgery. Group A (n=10) received dry needle acupuncture at GV-26 and Wei-jian. Group B (n=10) received electroacupuncture at GV-26 and Wei-jian. Cats in both groups also received a low dose of atipamezole IM (5 mcg/kg). Group C (n=10) received no acupuncture and atipamezole IM (10 mcg/kg). Respiratory rates, heart rates, and temperatures were recorded before and after the treatments. Time to palpebral reflex, time to swallow reflex, time to return of voluntary motor function, and time to lifting head were recorded as well. We plan to reach our 30 cat goal by mid-July, and at that point, the data will be assessed for statistical significance and the results will be evaluated.

Research Grant: None

Student Support: Boehringer Ingelheim Research Scholars Program

Low pH endocytic entry of pseudorabies virus into multiple cell lines

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The alphaherpesvirus pseudorabies virus (PRV) is the causative agent of pseudorabies, a disease of great economic and welfare importance in swine and dead-end hosts such as cats and dogs. Like the prototypical alphaherpesvirus herpes simplex virus 1 (HSV-1), PRV establishes primary infection at epithelial surfaces, followed by lifelong latency in peripheral nervous system neurons and periodic reactivations at the initial site of infection. PRV infects multiple species and cell lines, especially under experimental conditions. Herpesviruses utilize multiple entry pathways in a cell-specific manner, which may contribute to broad tropism. We demonstrate the low pH endocytosis pathway is an important route of entry for PRV into multiple laboratory model cell lines. PRV entry into PK-15, Vero, and SK-N-SH cells was inhibited by ammonium chloride, a weak base that accumulates in endosomes and raises the normally-acidic pH. Entry was also inhibited when endosomal acidification was blocked by the carboxylic ionophore monensin. Inhibition occurred in an agent concentration-dependent manner and at non-cytotoxic levels of agent. Time-of-addition experiments indicated that lysosomotropic agents exerted their effects at an early stage of infection, likely virus entry. Inactivation of virions by acid pre-treatment is a hallmark of viruses that utilize a low pH-activated entry pathway. Pre-treatment of PRV virions with a mildly acidic pH of 5.0 to 6.0 inhibited viral entry into PK-15 and Vero cells, consistent with a low pH entry activity of PRV.

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Student Support: Boehringer Ingelheim

WSU Student Summer Research Fellowship

WSU Research Scholars Program

Herpes Simplex Virus 1 (HSV-1)-mediated oncolytic virotherapy for treating ovarian cancer

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Ovarian cancer has the fifth highest mortality rate among women and accounts for the most deaths compared to other female reproductive cancers. To date, there are limited therapeutic options for advanced disease. Oncolytic herpes simplex viruses (oHSVs) have shown promise in cancer therapy and have received FDA approval in treating melanoma. Our aim was to determine the efficacy of an attenuated oHSV vector, constructed to express the sodium iodide symporter (NIS) gene (VC2-NIS) for monitoring infection and spread in tumors. To assess VC2-NIS infection capacity, a panel of mouse and human ovarian cancer cell lines were tested. Four cell lines (2008, SKOV-3-luc, IG-10-pluc, and HM-3-pluc) showed high expression of NIS compared to uninfected cells or cells infected with a control vector (VC2), by quantification of radioactive ^{99m}Tc uptake. To confirm NIS expression, cells were analyzed by flow cytometry using an anti-NIS antibody; high expression levels of NIS were detected in cells infected with VC2-NIS. These results were correlated with high levels of ^{99m}Tc uptake detected in cells by SPECT imaging. An assessment of VC2-NIS infection in IG-10-pluc cells showed a graded dose response with a 50% effective dose value (ED₅₀) of 0.27 ifu/cell. A cell-killing assay was conducted using luciferase activity as a biomarker. SKOV-3-luc, IG-10-pluc, and HM-3-pluc cell lines were harvested at 0, 6, 12, 24, 48, and 96 hours after infection with 0.5 ifu/cell VC2 or VC2-NIS and lysates were measured. In all cell lines, luciferase activity increased until 24 hours and then subsequently decreased, indicating cell cytotoxicity. Overall, these findings support the further development of VC2-NIS for treating patients with ovarian cancer.

Research Grant: National Institute of Health T35 Grant

Student Support: Louisiana State University School of Veterinary Medicine

Development of a miniature pig model of Juvenile Osteochondrosis Dissecans (JOCD)

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Juvenile osteochondrosis dissecans is a developmental orthopedic disease that leads to intra-articular formation of (osteo) cartilaginous flaps or fragments in juvenile human subjects. It is characterized by the development of zones of focal necrosis in the bone subjacent to articular cartilage. Untreated lesions may eventually progress to early osteoarthritis. A miniature pig model that mimics the pathogenesis and clinical features of JOCD would provide an invaluable resource for the development of novel diagnostic and treatment methods for JOCD. In the reported study, induction of JOCD was attempted by surgically interrupting the vascular supply to the epiphyseal growth cartilage of the femoral condyle. It is hypothesized that the resulting ischemia will trigger focal necrosis of the epiphyseal cartilage leading to the failure of endochondral ossification, thus recapitulating the pathogenesis of JOCD. The progression of the induced lesions is being monitored via serial MRI imaging and, upon euthanasia, the femora will be harvested for histological evaluation. To date, the animal cohort has undergone JOCD induction surgery, as well as 2-week and 4-week MRIs. While there appears to be some localized lesion progression under MRI, definitive results cannot be determined yet. Completion of the pending imaging procedures and final histological analysis is necessary to determine the success of miniature pig JOCD model creation.

Research Grant: University of Minnesota, Department of Orthopedic Surgery, preclinical research grant

Student Support: National Institutes Of Health, Award Number T35OD011118

Examining routes of prion entry into the gut of white-tailed deer, *Odocoileus virginianus*

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Chronic wasting disease (CWD) is a fatal, contagious, neurodegenerative prion disease of cervids and is documented in 25 states in the United States. Transmission of CWD is poorly understood but has been linked to exposure to infectious material, including bodily fluids, feces, contaminated water, soil, or food. Studies in cervids demonstrate oral exposure to infectious material can result in CWD infection. Prions are thought to enter the gastrointestinal tract, undergo transcytosis via microfold cells (M-cells), and subsequently move from the blood to the brain. M-cells are gut epithelial cells specialized for the transport of molecules from the gut lumen to the host immune system. M-cells can be found in the follicle associated epithelium overlying Peyer's patches in the small intestine and in the villi throughout the GI tract. Research has shown links between oral prion disease susceptibility and M-cell abundance. In this study, our goal was to optimize a stain that allowed us to identify M-cells in white-tailed deer, *Odocoileus virginianus*. We obtained small intestinal and rectal samples from 30 CWD-negative deer harvested from urban and suburban Cleveland Metroparks in 2018. We then tested M-cell targeting lectin [i.e., *Ulex europaeus* agglutinin (UEA-1)] or antibodies -for visualization by immunofluorescence. Optimizing this technique will allow us to compare M-cell abundance in deer with and without CWD or from different environments - with the ultimate goal of understanding how M-cells or other cells in the gut epithelium influence CWD susceptibility. As M-cell abundance is influenced by the gut microbiota, ongoing work also includes characterizing the gut microbiota of urban, suburban, and CWD-positive deer.

Research Grant: Ohio State University Department of Veterinary Preventative Medicine and the Infectious Diseases Institute

Student Support: Cliff M. Monahan Summer Research Scholar

Potentiation of macrophage response to *M. tuberculosis* via vitamin A-dependent nuclear receptor activation

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The development of active tuberculosis, the leading cause of death due to infectious disease, is often the result of known risk factors, either intrinsic or extrinsic. Recently, vitamin A deficiency has been implicated as such, however the mechanisms underlying this risk are yet to be elucidated. The aim of this study is to better understand the role of vitamin A in macrophage immune function, through investigating nuclear receptors (PPAR γ , RAR, and RXR) and enzymes (RALDH1A1 and RALDH1A3) associated with vitamin A's metabolic pathway. Administration of agonists of these targets should lead to their activation, which we hypothesize will improve the innate response to infection and consequently antimicrobial capacity. Bone marrow-derived macrophages obtained from guinea pigs were used as an in vitro cell model of inflammatory macrophages preferentially infected by *M. tuberculosis* in vivo. Through quantification of protein expression by western blot and gene expression analysis using qRT-PCR, nuclear receptor targets and immune parameters were assessed in context of administration of retinoic acid, the bioactive form of vitamin A, and a known PPAR γ agonist - rosiglitazone, or combination of both in uninfected macrophages and those infected with *Mtb*. Bacterial growth in the infected cells of these same treatment groups was measured through quantification of colony forming units (CFUs), and was compared to an MTT viability dye to assess impact on antimicrobial activity. Initial results show reduced expression of pro-inflammatory cytokines and improved cellular viability in all treatment groups, suggesting increased macrophage immune response through vitamin-A dependent pathways.

Research Grant: NIH NIAID U19AI111224

Student Support: NIH Grant T35OD015130-06

Improving the IVF efficacy of porcine embryos through the alteration of IVF protocol and medium selection

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In Vitro Fertilization, or IVF, has become an important technology in animal agriculture, particularly in the dairy and beef industries. Although it has played less of a role in the swine industry, it is a necessary technology for introducing direct genetic modifications into animals, which requires high quality, in vitro-derived oocytes for its success. Specialized media are needed to allow the immature oocytes to complete meiosis and be fertilized in vitro, and for the resulting zygotes to develop to blastocysts and provide offspring. Recent developments have led to increases in the efficiency of producing in vitro-derived piglets. In particular, this laboratory has described a maturation medium containing 3 cytokines (FGF2, LIF, and IGF-1) called FLI medium, which doubled the efficiency of nuclear maturation of oocytes in cumulus-oocyte complexes (COC's) and their development to blastocysts in vitro. The objective of our study is to compare the efficacy of producing blastocysts after maturing the COC in FLI medium and control medium. At the end of the 44h in maturation medium, half the oocytes in each group are denuded of their cumulus cells and subjected to IVF (conventional method) while the remainder are allowed to retain these cells and undergo IVF (modified method). This is the first study to investigate combinations of these variables during IVF protocol with FLI media. Values of $P \leq 0.05$ will be considered to support the conclusion that differences between cleavage and blastocyst percentages of FLI vs non-FLI oocytes are significant. Optimization of IVF methods in the presence of cumulus cells could improve cleavage and blastocyst percentages, reduce polyspermy and enhance efficiency in swine IVF techniques.

Research Grant: IDEXX-BioResearch

Student Support: None

The germination and sporulation of *Bacillus anthracis* in topsoil

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Bacillus anthracis is a rapidly fatal, spore-forming zoonotic bacterial pathogen. Infection results from exposure to the environmentally persistent spores residing in soil. Spores are the infectious form of this pathogen. The common belief is that spores germinate and proliferate in the host's body and then the vegetative forms sporulate in the carcass post mortem when growth conditions become unfavorable. Whether *B. anthracis* spores are capable of germination, proliferation, and subsequent sporulation in soil is a topic of debate. This study incorporates two experiments to A) determine if *B. anthracis* spores can germinate in topsoil and B) determine if vegetative forms can sporulate in topsoil and if the presence of blood increases the sporulation efficiency. We hypothesize that spores are capable of germination and sporulation in topsoil environments and that blood has a positive effect on sporulation efficiency. For experiment A, spores were inoculated into soil samples, incubated, and viable bacteria were recovered from the soil. Each recovered sample was halved and one half was heat treated to kill vegetative bacteria and thus permit the identification of spores. Heat treated and non-heat-treated halves of the samples were diluted and plated to enumerate the number of spores and total viable forms (vegetative cells and spores), respectively. For experiment B, vegetative bacteria were inoculated into soil samples with varying amounts of blood. Samples were collected over time and enumerated. The results would indicate whether germination, proliferation, and sporulation can occur in soils and may contribute to soil contamination levels.

Research Grant: USDA National Institute of Food and Agriculture, Animal Health project 1014418

Student Support: Endowment established by IDEXX-BioResearch

Assessment of *Haemonchus contortus* burden in a captive pronghorn population at a zoo

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Haemonchus contortus is the most economically significant parasite in small ruminants due to the severity of parasitism and the emergence of anthelmintics resistance. Haemonchosis is also of great concern in the management of exotic ruminant collections and pronghorn (*Antilocapra americana*) can be especially susceptible to heavy parasitism. However, most prevention practices are derived from the husbandry of sheep and often times these practices are not feasible or have not been validated in captive non-domestic ruminants. The study objectives included the following: (i) to characterize pronghorn intestinal nematode parasite-burden, (ii) to identify *H. contortus* using lectin staining and DNA methods, and (iii) to evaluate the impact of management on fecal ova shedding over a summer period in the pronghorn. The study also evaluated the fecal egg counts of bison housed within the same enclosure as the pronghorn to determine if *Haemonchus* species are shared between hoof stock within an exhibit and to assess any potential transmission of parasites between species. The study results will be used to optimize fecal sampling and detection of gastrointestinal nematodes ova.

Research Grant: OSU CVM Ecosystem Health Unit

Student Support: Boehringer Ingelheim Veterinary Scholar

A survey of veterinarian and owner attitudes, beliefs and perceptions regarding pain in dogs

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Pain scales have been validated to standardize pain assessments in veterinary hospitals but typically do not extend to include small animal owners, arguably the most vital resource of observational information of patient well-being after hospital discharge. In the reported study, the level of agreement between veterinarians and owners regarding attitudes, beliefs and perception of pain in dogs undergoing medical and surgical procedures requiring anesthesia was analyzed. It is hypothesized that factors such as evaluator background, patient familiarity, and observational environment, affect the level of agreement between the subjects. Surveys were developed to assess each of the constructs at the patient level and applied to stakeholders by a single trained investigator using Qualtrics. In order to construct a complete picture of these constructs, the theory of planned behavior was employed to establish a subjects' baseline bias including: veterinary knowledge and formative experiences. Data was collected and consolidated into the statistical program R. In total, 53 dog owners and 90 veterinarians completed the survey; one owner declined to complete the survey. The most frequent reasons for anesthesia were surgery (n=20), dental procedures (16) and diagnostic imaging (15). Comprehensive statistical analysis is ongoing thus precluding reporting of final results and conclusions. cursory assessment suggests possible strong similarities and differences between stakeholders for some of the constructs. These findings could be further developed with research and education to optimize perioperative pain care for patients in the hospital and at home.

Research Grant: University of Minnesota, College of Veterinary Medicine

Student Support: National Institutes Of Health, Award Number T35OD011118

Evaluating systemic inflammation as a contributor to the development of canine idiopathic epilepsy

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The majority of canine epilepsy cases are considered idiopathic; however, there is increasing evidence to suggest that inflammatory conditions and the cytokines derived from systemic inflammation may contribute to the vulnerability of the brain to seizures. We hypothesize that chronic inflammatory disease can contribute to the development of epilepsy in dogs. We propose that epileptic dogs have a high incidence of concurrent skin and/or GI inflammatory conditions, and that these changes in epileptic dogs are reflected by an increase in inflammatory cytokines in blood plasma. Medical record search identified candidate dogs diagnosed with idiopathic epilepsy with and without signs of inflammatory disease. 197 surveys were sent to dog owners to collect information on medical, environmental, and dietary history. Responses from the 94 received surveys (47% response rate) were used to send a follow-up survey. The responses (70/94, 74% response rate) were used to categorize dogs as epileptic/inflammatory (E+/I+), epileptic (E+), or control (C) based on their reported incidence of inflammatory signs. Plasma samples were collected from 35 epileptic dogs (E+/I+ and E+) and 19 healthy control (C). Cytokines will be measured by either ELISA (IL-1 β) or the Milliplex MAP Canine Cytokine/Chemokine Magnetic Bead Panel (TNF- α , IL-6, IFN- γ , IL-2, and IL-10). We expect that E+/I+ dogs will display the most elevated cytokine profile, but that E+ dogs will still be elevated when compared to the normal dogs. A better understanding of the pathogenesis of epilepsy will provide opportunities for the development of improved and novel therapies.

Research Grant: NCSU CVM Nutrition Program, Molly's Seizure Studies Fund

Student Support: Morris Animal Foundation Veterinary Scholars Program

ECGes: A mobile application to help with ECG interpretation in dogs and cats

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It is estimated that 30% of dogs and cats seen at a general practice have an underlying arrhythmia. However, detecting arrhythmias via electrocardiogram traces may require extensive training beyond what is provided in veterinary school. Therefore, there is an urgent need for a portable application to help veterinarians and veterinary students to correctly interpret ECGs. This project represents the development of a mobile app to help with the identification of arrhythmias and conduction disturbances in dogs and cats. Our hypothesis is that by using our mobile app, users will correctly identify arrhythmias more frequently than with no aid. Development of the app was divided into four phases: validation, content generation, alpha testing, and beta testing. We began by validating a stepwise decision matrix that guides the user to the most likely arrhythmia based off of various questions. Numerous ECG traces were tested, and the algorithm was modified as needed. After validation, app developers at WesternU built the scaffolding of the app from the decision matrix. During the second phase, a synopsis for each of the arrhythmias was generated and programmed based on cardiology textbooks and primary literature. While in the third phase, we tested a series of ECG traces to see if the app would give us the correct interpretation. The usability and layout of the app was also evaluated. The app is currently undergoing beta testing. Users were asked to interpret five ECG traces without aid, and then to reinterpret the same traces using the developed app. We expect that this study will positively impact the clinical management of veterinary patients by providing an easy-to-use tool for the precise identification of arrhythmias.

Research Grant: Boehringer Ingelheim

Student Support: Western University of Health Sciences, College of Veterinary Medicine, Office for Research

Eosinophil-mediated clearance is blocked by a *Bordetella* blood-responsive regulon

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Bordetella spp. cause respiratory diseases in humans and animals. *B. pertussis* the causative agent of whooping cough in humans and is restricted to this host, while *B. bronchiseptica* is able to cause respiratory illness in many mammals, including mice. This allows us to study the host/pathogen interaction in the natural infection setting in the robust murine model. A particular feature of *Bordetella* spp. infections is their capability to persist by down-regulating innate and adaptive response. Recent work from our lab lead to the discovery of one *Bordetella* spp. gene (*bsr*) that is involved in recognizing host immune signals and altering bacterial gene expression to adapt accordingly. We found that *bsr* interferes with host glycosylation by modifying host glycoterrain. These altered glycans in the secreted mucins lead to a more robust CD11b+ cell recruitment. Based on the glycan studied (SiglecF) and the CD11b+ cells numbers, we hypothesized that eosinophils may be involved in clearance of the infection. Our results revealed an increase of eosinophil recruitment in lungs, and a characteristic peak in the numbers of eosinophils in the spleen on day 7 coinciding with the peak of infection. To test the role of eosinophils in *Bordetella* spp. infections, eosinophil-deficient mice were intranasally challenged with 50 ml of PBS containing 5×10^5 bacteria, and the results revealed that eosinophils mediate *Bordetella* spp. clearance. Overall, our results demonstrate that although eosinophils were known for their role in allergies and parasite infections, they are necessary for the clearance of bacterial infections, possibly by mediating interactions with the adaptive response, opening new possibilities for treatments.

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Student Support: NIH T35 Training Grant 5T35OD010433-12

The prevalence of exercise-induced pulmonary hemorrhage in high goal polo horses

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Exercise induced pulmonary hemorrhage (EIPH) is a common condition in high speed exercising horses that affects the respiratory system and in some cases horse performance. EIPH has been studied extensively and new strides have been made to better understand this condition however, few studies have focused on high goal polo horses. The primary aim of this study is to assess the prevalence of EIPH in high goal polo horses and to evaluate the possible association between the presence of Tracheal Mucus Accumulation (TMA) and EIPH. In 2017 and 2018, a single video endoscopy evaluation was performed on 109 polo horses within 120 minutes of competing. Two different cohorts of polo horses were evaluated: a start season cohort (2018) comprised of 66 horses, and an end season (2017) cohort comprised of 43 horses. Endoscopic evaluations were independently reviewed and graded by two equine specialists. Preliminary results indicated that 15% of all horses had a level of EIPH. Specifically, 30% of the end season cohort manifested EIPH, while only 5% of the start season cohort had some evidence of EIPH. Additionally, 38% of horses with EIPH had TMA and 25% of the horses negative for EIPH had some degree of TMA. The prevalence of EIPH appears to be lower among high goal polo horses in comparison with thoroughbred horses. Results suggests other factors such as training methods and judicious use of the horse in the polo sport may account for the low prevalence of EIPH. Differences between both cohorts could be explained by various factors such as repetitive training and discrepancies between weather conditions through the season of both years. TMA may be associated with the prevalence of EIPH in high goal polo horses.

Research Grant: Ross University School of Veterinary Medicine

Student Support: Merial Veterinary Research Scholars Program

Antimicrobial resistance risk factors in pig slaughterhouse workers, meat products, and environmental samples

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The incidence of antimicrobial resistance (AMR) is a growing concern worldwide, not only in the human population but also in the animal population. Some of the risk factors leading to increased AMR include increased or inappropriate use of antimicrobials, and transmission via contaminated food, and the environment. A major multinational European project, Ecology from Farm to Fork Of microbial drug Resistance and Transmission (EFFORT) is seeking to understand the development of AMR within the food chain. The aim of this particular study within the EFFORT project is to determine any associations between AMR and the slaughterhouse process as well as to evaluate occupational risk factors for employees. A Dutch swine slaughterhouse was visited June 2015 and July 2016. Samples collected for testing were 503 human feces (with questionnaire), 60 swine manure, 259 swine carcass, 355 employee gloves, 33 air and 198 end meat products. Two specific AMR genes, namely tetW and ermB, were quantified along with 16S for normalization. Significant risk factors for AMR gene abundance in human samples included gender, nationality, and year but not job location. Significant associations were found between the slaughterhouse process and the presence of the AMR genes on gloves, carcass and in air, however all meat samples were below the level of detection. In conclusion, the slaughterhouse process has been a significant factor for carcass and environmental samples but not for humans. Additional research is planned to continue to identify and quantify additional risk factors and search for solutions to AMR.

Research Grant: EU FP7-KBBE-2013-7

Student Support: Boehringer Ingelheim Veterinary Research Scholars Program

Intra-articular AAV-mediated overexpression of interleukin-10, for the prevention of equine osteoarthritis

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Equine athletes are prone to osteoarthritis (OA) often caused by joint injury and cartilage damage after a traumatic event. Following joint trauma, inflammatory and catabolic cytokines are upregulated leading to the degradation of the extra-cellular matrix (ECM). If severe enough, posttraumatic inflammation can cause joint degeneration and posttraumatic osteoarthritis (PTOA), a progressive and debilitating disease. PTOA currently has no effective treatment and there is no disease-modifying OA drug to halt or reverse progression. Interleukin-10 (IL-10), a cytokine with potent anti-inflammatory effects, plays a potential role in cartilage disorders. The main objective of this study was to investigate intraarticular expression of equine IL-10 following direct injection of an AAV5-IL-10 vector into the middle carpal joint. Following injection, serial joint circumference measurements and synovial fluid analysis was performed to evaluate the inflammatory response to vector injection. Preliminary results demonstrate a decrease in joint circumference and total nucleated cell count in AAV5-IL-10 joints compared to saline injected joints. Synovial fluid total protein was not significantly different between the groups. Future directions include quantification of IL-10 in the synovial fluid and serum of horses using multiplex ELISA. We expect there will be significantly increased amounts of IL-10 over the 84-day study period in equine joints treated with the AAV5-IL-10 vector.

Research Grant: Boehringer Ingelheim

Student Support: NIH grant T35OD010919

Carbon dioxide surgical laser versus scalpel excision of soft tissue sarcomas and mastocytomas in dogs

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Lasers have been used by veterinarians for a variety of surgical procedures, but little has been published on laser use in veterinary oncology. This study examines the use of a carbon dioxide laser for excision of soft tissue sarcomas and mastocytomas in dogs, comparing the laser to traditional surgical excision. The University of Missouri Veterinary Health Center's computerized medical record system was searched to find all cases of soft tissue sarcomas and mastocytomas that were surgically excised from dogs from December 2004 to May 2018. For each tumor type (soft tissue sarcoma, n=58; mastocytoma, n=91), the following data was tabulated: intraoperative blood loss (ml), surgical time (minutes), anesthesia time (minutes), tumor size (cm), presence of contaminated margins on histopathology, and occurrence of wound complications. The results for each of the above parameters for soft tissue sarcomas were 5 (0-150), 89 +/- 41.8, 155 +/- 59.1, 3 (0.5-18), n=8, and n=1 for laser excision (n=17), compared to 10 (0-50), 75 +/- 51.1, 124 +/- 60.7, 4.8 (0.5-25), n=15, and n=6 for traditional excision (n=41). The results for each of the above parameters for mastocytomas were 5 (0-40), 105 +/- 51.1, 161 +/- 68.2, 2 (0.5-9), n=4, and n=2 for laser excision (n=17), compared to 5 (0-200), 77 +/- 46.5, 125 +/- 56.5, 2 (0.3-18), n=20, and n=6 for traditional excision (n=74). It appears that for both tumor types there was little difference in intraoperative blood loss. Laser excision took longer to perform, but resulted in fewer contaminated margins and fewer surgical wound complications. Sound conclusions cannot be made without statistical analyses and evaluation of other data collected but not mentioned here.

Research Grant: None

Student Support: University of Missouri College of Veterinary Medicine

Whole-genome sequencing to identify AMR determinants in *Salmonella* obtained from the dairy worker environment

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The presence of zoonotic pathogens in dairy herds, such as *Salmonella enterica*, present a public health challenge to consumers and dairy workers alike. The CDC estimates an incidence of 1.2 million cases of salmonellosis per year, with around 450 deaths. Concerningly, the rise of antimicrobial resistance (AMR) has the potential to put us into what former CDC Director Tom Frieden called a “post antibiotic era.” Analyzing the public health risks of AMR spread may be one of the primary steps towards its prevention. Identifying the unique DNA markers (such as integrons or single nucleotide polymorphisms) that may confer resistance genes to *Salmonella spp.* could aid in the early detection, prevention, or treatment of infections among human and animal populations. The objective of this research is to utilize Whole-Genome Sequencing to identify AMR determinants obtained from *Salmonella spp.* in the dairy worker environment. In 2015, *Salmonella* isolates were collected from the dairy worker environment, including personal protective equipment such as gloves/cloth bibs/shoes, and surfaces such as floors/walls/etc. As literature varies, both TET (tetrathionate with iodine) and RV (Rappaport Vasiliadis) were used to maximize culture yields. After PCR analysis, 41 samples were determined to be *Salmonella enterica* Serotype Dublin. These 41 samples were utilized for the current research. They were cultured in BHI broth for DNA extraction using Qiagen DNEasy protocol. Once complete, extractions will be sent to Dr. Scaria's lab at South Dakota State University for Whole-Genome Sequencing. Raw data files will be returned to CSU and assembled De Novo using Geneious or other computer software, to identify common patterns related to AMR.

Research Grant: CSU High Plains Intermountain Center for Agricultural Health & Safety

Student Support: USDA Animal Health & Disease Program (NI17AHDRXXXXG018)

Assessing medical comorbidity burden in a rhesus macaque model of Autism Spectrum Disorder

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Autism Spectrum Disorder (ASD) is a neurodevelopmental condition characterized by social deficits and repetitive behaviors. Individuals with ASD also exhibit at higher rates than the general population a variety of well documented medical comorbidities. However, despite its high prevalence (1 in 59 children) and significant cost (\$268 billion annually), ASD remains poorly understood. This is due, in part, to the difficulty of studying disease biology directly in patients and the fact that rodent models lack face validity to the human condition. We have addressed this barrier to progress by developing a novel monkey model of ASD, in which naturally low-social (LS) vs. high-social (HS) monkeys spend less time interacting with conspecifics and exhibit greater social cognition deficits. To further validate our model, here we tested whether LS vs HS monkeys exhibited an excess burden of the medical comorbidities documented in people with ASD. The medical records of N=164 male rhesus monkeys, previously classified as LS or HS, were reviewed and scored (blinded to social group) for bouts of diarrhea, inflammation, and rare conditions, such as cardiac issues, traumas, and/or death. The chart review period for each monkey spanned birth to an end date specified by the monkey's current status (i.e. shipped, deceased, present, or enrolled in a different project at time of record review). Identification of comorbidities associated with social classification, like what is seen in humans, would provide further evidence of the value of our naturally-occurring rhesus monkey model for advancing our understanding of the complex features observed in people with ASD.

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Student Support: NIH/ORIP T35 OD010989

Multi-parameter analysis of skin lesions in MRL/lpr mice model of chronic cutaneous lupus erythematosus

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Chronic cutaneous lupus erythematosus (CCLE) is a photosensitive autoimmune disease characterized by a strong type I interferon-associated inflammation. The lupus mouse model MRL/lpr is the most intensively studied CCLE model, however, global patterns of gene expression and inflammatory pathways leading to local skin damage are unknown. The objectives of this study were to characterize chronic skin lesional transcriptome of 10 MRL/lpr mice; lesional and non-lesional skin biopsies were obtained 3 to 8 weeks after the onset. We extracted mRNA from skin biopsies and the transcriptome was assessed using RNA-seq. Gene expression of lesional skin was compared to that of non-lesional skin specimens (1.5-fold change, 0.05 adjusted *P*-values). Histopathological findings revealed a mild lymphocyte-rich interface dermatitis and folliculitis with zones of basal keratinocytes vacuolation. Comparing the lesional mRNA expression to non-lesional skin, we identified a large set of 3,042 genes significantly up-regulated. The Th1 signal (STAT1, OASL, MX1, IFN γ) and T-cell trafficking chemokine, CXCL10, were among the upregulated genes. The differentially expressed genes (DEGs) within the MRL/lpr lesional pathology signature reflect an activated immune and inflammatory system and belong to dys-regulated KEGG pathways such as Jak/Stat signaling pathway, T-cell receptor-, chemokine-cell receptor-signaling pathway and cytokine-cytokine receptor interaction. Functional annotation with human CCLE revealed mechanistic pathway and processes commonality (type I interferon pathways, apoptotic/survival pathways). In conclusion, the MRL/lpr skin lesions show robust immune and inflammatory responses that resemble human CCLE transcriptome results.

Research Grant: Self-funded

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Expanding our knowledge of fat: Is stretch-activated Piezo1 found in perivascular adipose tissue?

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Adipose tissues including brown and white are known for their functions of energy storage, structure support and thermoregulation. Over the past 10 years adipose tissues have been revealed to be dynamic endocrine tissue, in particular, perivascular adipose tissue (PVAT) the fat directly outside the vascular wall. A standing question is how changes in vascular pressure are transmitted to PVAT. Piezo1 is a non-selective stretch-activated ion channel expressed in the endothelial cells of developing blood vessels. We hypothesize that the Piezo1 channels are present in the PVAT and actively modify vascular tone. Immunohistochemistry was used to identify the presence of Piezo1 in PVAT of rat thoracic aorta, superior mesenteric artery (SMA), mesenteric artery, retroperitoneal adipose tissue (RPfat) as non-PVAT adipose and kidney (positive control). Sections of tissues were incubated with and without primary antibody against Piezo1 overnight. FITC-labeled secondary antibody was used to visualize Piezo1. Slides were imaged with an inverted microscope and nuclei were counterstained with DAPI. Piezo1 was found to be located in the endothelium of the aorta and SMA, smooth muscle of mesenteric artery and SMA, in PVAT of aorta and SMA, and was also found in the RPfat (n=5 different rats). Future directions include a contractility study with Yoda (Piezo1 channel activator) in the vessels with or without PVAT to determine if Piezo1 channels in the fat affect the contractility of the vessel. These results open the door to future studies of the Piezo1 activity in channels of the PVAT of aorta, SMA and mesenteric artery.

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Student Support: NIH grant R25HL103156

Asthma - not just an airway disease

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Asthma is a common chronic disease of the lungs that is characterized by airway inflammation and narrowing, together with abnormal bronchoconstriction in response to specific allergens. Current treatments focus on mitigating and/or preventing bronchoconstriction of the conducting airways. However, chronic allergic asthma can result in airway remodeling, decreased lung compliance and an overall decrease in pulmonary function, which may be related to decreased production of pulmonary surfactant. Given that the Alveolar Type II (ATII) pneumocyte is responsible for the production of surfactant, we hypothesized that chronic asthma results in abnormal ATII cell function and metabolism. To test this, we used the well-established DRA (dust mite/ragweed/*Aspergillus fumigatus*) asthma model. 8-12 week old, female BALB/c mice were sensitized by i.p. injection of DRA + Alum on Days 0 and 5, then challenged with intranasal DRA on Days 12, 13 and 14. Control mice received PBS at all timepoints. On Day 15 measurement of airway responses to increasing doses of the muscarinic agonist methacholine using the Scireq flexiVent FX system showed increased airway hyperresponsiveness in the DRA group. Bronchoalveolar lavage fluid was collected for total and differential cell counts. Eosinophils in the DRA group were increased. Finally, lungs were removed and ATII cells were isolated. To profile gene expression in the ATII cell for the DRA and PBS control mice, we will use the Qiagen Mouse Allergy & Asthma RT2 Profiler PCR Array, which looks at 84 genes known to be associated with asthma. Increased understanding of the role of ATII cells in the pathogenesis of asthma may lead to identification of novel therapeutic targets for this condition.

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Student Support: NIH T35 Training Grant T35OD010977

A comparative study of hepatic abscesses in antibiotic-free cattle and conventional cattle

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Production feed rations predispose cattle to rumenitis and subsequently hepatic abscesses. The aims of this study are twofold: 1) to correlate severity of hepatic abscesses to carcass quality in antibiotic-free cattle and conventional cattle and 2) to compare the prevalence and severity of hepatic abscesses in antibiotic-free cattle to that of conventional cattle. At time of slaughter, livers were graded on a scale of 0 to 3, 0 meaning no abscesses present, 1 meaning mild abscessation, 2 meaning moderate abscessation, and 3 meaning severe abscessation. Carcasses were graded according to the USDA quality grading scale. Liver scores were correlated to carcass scores. A univariate regression was used to determine differences in abscess prevalence and severity between types of cattle and between producers. The study is ongoing, and preliminary results will be presented.

Research Grant: None

Student Support: Boehringer Ingelheim Veterinary Scholars Program

CRISPR/Cas9 phage and antibiotic combination therapy against *Staphylococcus aureus* biofilm

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Infections with Methicillin-resistant *S. aureus* often cause biofilm-associated infections such as diabetic foot ulcers and osteomyelitis, which are refractory to current vancomycin treatment. Biofilm is a sessile microbial community in which bacterial cells are closely associated with polysaccharides, proteins, and other nutrients, which forms physical barriers against host immune response and penetration of antibiotics. As a result, bacteria in biofilm are 1000 times resistant to antibiotics than their free-living counterparts. Since bacteriophages produce enzymes that disintegrate biofilm structure that will aid penetration of small molecular weight antibiotic, such as fosfomycin, we hypothesized that combination therapy using fosfomycin and bacteriophage could be better therapeutic approach against biofilm-associated *S. aureus* infection. The treatment of vancomycin alone partially reduced the biofilm structure (~50%) but has no effect on viable cell counts. The treatment of fosfomycin alone showed better reduction of biofilm (~75%) and viable cell counts (~2 log scale). The treatment of phage alone completely removed biofilm (~95-99%), but still remained viable cell counts (4 log scale). The combination of phage and vancomycin therapy did not improve the effect, but the combination with phage and fosfomycin significantly reduced the viable cell count more than 6 log scale. These results demonstrate that the combination of phage and fosfomycin showed greatest effect against *S. aureus* biofilm. Currently, we are integrating CRISPR/Cas9 system into the phage genome to improve the effect of phage therapy.

Research Grant: NIH T35 summer research program

Student Support: Unknown

Molecular characterization of *Salmonella* Typhimurium isolated from pigs, humans, and the environment

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Salmonella Typhimurium is the number one cause of bacterial food-borne illnesses in humans in the United States. The majority of these cases can be traced back to animals, soil and retail food products. The aim of this study was to use a one-health approach to characterize isolates of *S. Typhimurium* from humans (n=100) and compare it temporally and spatially related isolates from swine (n=22) and the environment (n=22) and determine the phenotypic and genotypic similarity/diversity among them. We utilized broth microdilution to determine the minimum inhibitory concentration (MIC) of these samples against a panel of fourteen different antibiotics representing nine classes. We genotypically characterized the human *S. Typhimurium* samples (n=22) using whole genome sequencing and PCR to identify antimicrobial resistance (AMR) genes. The MIC results indicated that isolates from humans (n=31), swine (n=22) and the environment (n=22) displayed frequent resistance to sulfisoxazole, ampicillin and tetracycline. Interestingly, the swine (n=11) and environmental samples (n=10) displayed more resistance to chloramphenicol than the human samples (n=7) despite its ban in food animals. We identified fourteen AMR genes and the presence of class I integrons. The samples containing integrons had the most genotypic and phenotypic similarity of all the Typhimurium isolates. Tetracycline resistance and β -lactamase producing genes were noticeably more prevalent in swine and environmental samples and these two sources appear to have the most similarity among them as a whole. Determining these relationships will be a great tool for professionals attempting to mitigate the threat AMR *salmonella* poses.

Research Grant: NIH FDA u18

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Influenza induces robust vascular regeneration mediated by both canonical and non-canonical progenitor cells

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Acute respiratory distress syndrome instigated by influenza is a common cause of mortality in infected individuals, producing as many as 500,000 deaths annually. Despite increasingly thorough models of epithelial regeneration in the lung, the cell types and mechanisms responsible for endothelial repair after injury are almost entirely unknown. Our studies aim to reveal the progenitor cell type(s) and mechanisms responsible for pulmonary endothelial repair post-influenza. Flow cytometry, in situ immunofluorescence, and endothelial-specific lineage tracing was performed, at various time points post-infection, to elucidate the means by which endothelium is repaired. Our results showed that mice challenged with H1N1 influenza demonstrated robust endothelial proliferation throughout the course of injury repair. EdU analysis demonstrated that as much as 23% of the endothelium was newly generated 27 days post-flu. Strikingly, endothelial proliferation was at least as robust as epithelial, highlighting the unappreciated importance of vascular repair. Additionally, immunofluorescence analysis showed co-localization of micro-thrombi and proliferating endothelium indicating a possible mechanism of angiogenesis. Lastly, using VECAD-CreERT2/*Isl*-RFP mice to lineage trace pre-existing endothelium, we observed strong evidence of a non-endothelial source of endothelial restoration (vasculogenesis) that may work in conjunction with resident endothelial stem cells (angiogenesis) to mediate regeneration. Overall, these results provide new biological insights into the importance and progression of vascular endothelial regeneration, thus unveiling mechanisms that might be exploited for therapeutic interventions.

Research Grant: University of Pennsylvania, School of Veterinary Medicine, Biomedical Sciences Startup Funds

Student Support: Meriel Veterinary Research Scholars Program, NIH Grant T35OD010919, Boehringer Ingelheim Grant

Differential activation of hepatocyte TF procoagulant activity by apoptotic and necrotic cell death

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Tissue factor (TF) is the transmembrane receptor for coagulation factor VII/VIIa and the primary activator of the coagulation cascade. The TF:VIIa complex expressed by liver parenchymal cells (i.e., hepatocytes) lacks procoagulant activity under normal conditions. However, when the liver is injured, the TF:FVIIa complex initiates intrahepatic coagulation. The mechanisms required to activate TF:VIIa procoagulant function are unknown. We tested the hypothesis that hepatocyte TF:FVIIa procoagulant activity is increased by hepatocyte injury and functionally connected to the mode of cell death. Necrosis of primary mouse hepatocytes was induced by the toxicant acetaminophen (0-5 mM) and Fas-dependent apoptosis induced using Jo2 antibody (0.5 mg/mL). APAP treatment increased outer membrane permeability consistent with necrotic cell death, indicated by release of alanine aminotransferase (ALT). A slight, but significant (~1.7 fold) increase in hepatocyte TF procoagulant activity occurred 7 hours after APAP treatment, but did not increase concomitant to near complete cell death observed after overnight treatment. In contrast, induction of apoptosis with Jo2 caused a dramatic increase in TF activity prior to loss of outer membrane integrity, and this continued to increase over time. The results indicate that loss of outer membrane permeability, as in necrotic cell death, is insufficient to elicit large increases in TF procoagulant activity. By comparison, apoptotic cell death elicits rapid and robust increases in TF procoagulant activity. Overall, these results suggest that apoptotic cell death, as opposed to necrosis, is a more procoagulant form of hepatocyte cell death.

Research Grant: R01 DK105099

Student Support: R25HL103156

Determining the function and regulation of genes selectively induced by acidic pH in the presence of glycerol

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Tuberculosis is an infectious bacterial disease that is caused by the bacterium *Mycobacterium tuberculosis* (Mtb). Mtb arrests its growth at pH 5.7 in the presence of glycerol as the sole carbon source but continues its growth on other carbon sources such as pyruvate. The transcriptional profile of Mtb grown in the presence of glycerol or pyruvate at acidic or neutral pH indicated that select genes, *prpC* and *prpD*, were upregulated by glycerol at acidic pH, but not pyruvate. *prpCD* are known to be induced during times of propionate toxicity as part of the methylcitrate cycle. Other genes *Rv0188*, *Rv0841*, *Rv2557*, and *Rv2558* displayed similar expression patterns as *prpCD*, but have no known function. We hypothesize that *Rv0188*, *Rv0841*, *Rv2557*, and *Rv2558* are involved in propionate toxicity response in the presence of glycerol at pH 5.7. An alternative pathway, the methylmalonyl-CoA pathway can relieve *prpCD* when vitamin B₁₂ is introduced. Thus, the expression of *Rv0188*, *Rv0841*, *Rv2557*, and *Rv2558* will be determined using quantitative real time polymerase chain reaction (qRT-PCR) under the conditions of Glycerol at pH 7.0 and 5.7 in conjunction with or without Vitamin B₁₂. The results from qRT-PCR will be analyzed using the $\Delta\Delta C_t$ method. We hypothesize that these genes, *Rv0188*, *Rv0841*, *Rv2557*, and *Rv2558* will not be induced in the presence of Vitamin B₁₂ suggesting their role in propionate toxicity. Understanding the role these genes play in the metabolic processes of *Mycobacterium tuberculosis* is important as it helps us better understand mechanisms of bacterial pathogenesis and discover new ways to treat the disease.

Research Grant: NIH-NIAID R01AI116605

Student Support: NIH grant R25HL103156

Synovial response to bone marrow mononuclear cells as an equine joint therapy

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Osteoarthritis is characterized by macrophage-driven synovial inflammation. When normal regulatory macrophage (M2) functions are overwhelmed, an inflammatory (M1) macrophage phenotype is elicited. Bone marrow mononuclear cells (BMNC), a rich source of macrophages, control inflammation and produce essential molecules for cartilage metabolism, making them an intriguing target for joint therapy. The *objective* of this study was to define the synovial response to BMNC injection in normal and inflamed equine joints. We *hypothesized* that there would be greater M2 polarization and less histological evidence of inflammation in BMNC-treated joints compared to saline-treated controls. Following induction of a synovitis model, horses (n=7) were treated with intra-articular BMNC or PBS. Horses were euthanatized after 6 days and synovial membrane biopsies were harvested for histology. H&E-stained sections were graded and immunohistochemistry was performed for macrophage markers CD206 (M2), CD86 (M1), and CD14 (pan), and interleukin-10. Inflammation scores for all evaluated parameters were lower in BMNC-treated joints compared to untreated (PBS): cell infiltration (2.1 vs 3.0), vascularity (1.7 vs 2.6), intimal hyperplasia (0.7 vs 1.4), subintimal edema (2.0 vs 2.6), and overall (6.6 vs 9.6). Staining patterns for CD86 were comparable between groups; however, staining in untreated joints was more intense. CD86 was also more markedly expressed towards the base of the synovial villi. Results for additional markers and response to BMNC in normal joints are pending. The decreased cell infiltration and vascularity in BMNC-injected joints suggest that treatment with macrophage-rich BMNC has the potential to regulate synovial inflammation.

Research Grant: Grayson-Jockey Club Research Foundation

Student Support: Virginia-Maryland College of Veterinary Medicine

Serum vitamin D levels in Hispaniolan Amazon parrots (*Amazona ventralis*) housed indoors over time

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Vitamin D (Vit D) is a hormone that can be orally ingested or synthesized in the body when skin is exposed to ultraviolet radiation (UV), typically from sunlight. In captivity, birds with no sunlight exposure may experience Vit D deficiencies that may contribute to a hypocalcemic condition, even when provided Vit D supplemented diets. An initial pilot study indicated there were significant differences in Vit D serum levels between the indoor-housed birds and historically outdoor-housed birds used in this study. The goal of this study was to determine if Vit D, ionized calcium, and ionized magnesium levels were maintained in the historically outdoor-housed Hispaniolan Amazon parrots, subsequently deprived of natural UV light exposure, over time. To accomplish this, the authors compared vitamin D levels in the same two groups of birds, with no access to natural or artificial UV light exposure for either group of birds, since the initial pilot study approximately 18 months ago. The first group of birds (N=9) have been housed indoors for approximately 20 years with no exposure to UV light from any source. The second group of birds (N=8) have been housed under the same conditions as the first group for approximately 18 months. The blood samples were sent to Michigan State University: Diagnostic Center for Population and Animal Health for testing. There were no significant differences in the Vit D levels of the first group over time. However, the second group experienced a significant decrease in vitamin D levels over time, comparable to the level of the first group, as well as a decrease in ionized calcium. These findings support the authors' hypothesis that serum vitamin D levels decrease in absence of UV light.

Research Grant: Research Support: Paula and Milton Shepard Professorship

Student Support: Student Support: Dr. Kenneth F. Burns Lectureship and Clinical Clerkship Foundation

Effects of leptin on equine lamellar keratinocytes: potential role in endocrinopathic laminitis

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Equine metabolic syndrome (EMS)-associated laminitis (EMSAL) is currently the most common cause of equine laminitis, a devastating orthopedic condition of equids. Serum [leptin] is commonly increased in horses with EMS, as many are overweight; however, its role in EMSAL has not been well characterized to date. Leptin exerts mitogenic effects on keratinocytes and can regulate the pro-inflammatory response of human epidermal keratinocytes. In addition, the euglycemic hyperinsulinemic clamp (EHC) model can acutely increase circulating [leptin] in healthy adult humans. The aim of this study is to investigate the roles of leptin and leptin receptor (lepR) in EMSAL. Healthy Standardbred horses (n=16) were randomly assigned to receive either 1) an EHC for 48h (or until signs of lameness developed; EHC, n=8) or 2) a saline infusion for 48h (CON, n=8). Real-time PCR analysis of lamellar tissue revealed significant downregulation of leptin and lepR gene expression in response to the EHC ($P=0.0002$ and 0.0158 , respectively). Lamellar immunohistochemistry (IHC) showed diffuse expression of lepR within keratinocytes of the primary and secondary epidermal lamellae in both groups. These results suggest that lamellar keratinocytes appear capable of responding to circulating leptin, as well as that leptin does not appear to be locally produced within the lamellae. Measurement of serum leptin concentrations revealed that 5/8 of the EHC horses had acutely increased leptin levels at 6h in response to the model. Further clarification of the role of leptin in EMSAL is warranted given these results and the prevalence of hyperleptinemia in EMS patients.

Research Grant: USDA Grant (NIFA AFRI 2014-06397)

Student Support: Rainier Scholars Program

Impaired expression of AP Endonuclease-1 impairs fetal development and leads to embryonic loss

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Background: Apurinic/apyrimidinic endonuclease-1 (APE-1) is induced in response to the accumulation of reactive oxygen species (ROS) and plays multiple roles in response to oxidative stress including: regulation of redox-sensitive genes; inhibition of ROS accumulation; inhibition of apoptosis and; repair of abasic sites in oxidative DNA damage. While a complete APE-1 knockout is embryonic lethal, a novel hypomorphic (HM) mouse model was developed in which APE-1 expression is inhibited ~80%. The goal of this project was to test the hypothesis that reduced expression of APE-1 in HM embryos initiates resorption during organogenesis.

Methods: MRI and serial necropsies indicated that fetal resorption is observable by gestation day 12.5. Necropsies were performed at critical time points and samples were collected to assess DNA, protein, and histology.

Results: Breeding of heterozygous mice yields ~50% of the HM offspring compared to what would be predicted by Mendelian genetics. A mating of a HM male with a heterozygous female showed a higher resorption:fetus ratio than heterozygous pairings (3:5 vs ~1:8), suggesting the HM genotype is prone to resorption. In multiple necropsies, resorption sites were observed, as well as smaller fetuses compared to littermates, fitting the phenotype of neonatal HM mice. PCR of DNA samples is in progress to genotype the healthy and resorbed fetuses. Protein expression by western blot and immunofluorescent will be compared across different genotypes and gestation periods to associate APE-1 expression in embryonic loss. Histology of fetal HM mice will be analyzed at various times to determine if the lack of APE-1 is contributing to pathology in organs or tissues.

Research Grant: Chiba UCSD Immunology Initiative and the Center for Mucosal Immunity, Allergy and Vaccine Development

Student Support: NIH funded program for Students Training in Advanced Research (STAR)

Excisional wound healing in diabetic and nondiabetic mice treated with amnion-derived matrix-Pilot Study

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Human amniotic tissues enhance wound healing through anti-inflammatory, antimicrobial, and tissue regeneration properties. Porcine-derived amniotic tissues may be a source for noninvasive, larger and less expensive wound treatment products. The objective of this pilot study was to evaluate wound healing of full-thickness excisional skin wounds treated with a novel porcine amnion-derived acellular matrix (PAAM) in diabetic and nondiabetic mice. Our hypothesis was that full-thickness excisional skin wounds treated with PAAM would heal quicker and with less scarring than untreated wounds in diabetic and nondiabetic mice. Two excisional wounds were created on the cranial dorsal midline of 10 diabetic (Group D) and 10 nondiabetic (Group ND) mice. One wound was treated with PAAM and one was an untreated control. Wound surface area (SA) was measured biweekly and wounds were harvested weekly from 2 mice per group for histologic evaluation. Mean initial wound SA was larger on Day 0 for treated than untreated wounds in group D, while treated and untreated wounds in group ND were similar in size. In group D, treated wounds were larger than untreated wound, but both were healed by day 21. In group ND, treated wounds were smaller than for untreated wounds, but both were healed by day 17. No inflammation was grossly present in treated and control wounds. In conclusion, treated and untreated wounds showed a similar rate of healing in both diabetic and nondiabetic mice, with treated wounds possibly healing faster. No adverse effects were noted. Disparity in wound size in diabetic mice may be the result of refining the wounding technique. Additional larger studies to examine wound healing properties when treated with PAAM are warranted.

Research Grant: Triad Life Sciences, Inc.

Student Support: NIH NCRR T35 Grant

Investigating the respiratory virome of backyard poultry and need for owner education about poultry pathogens

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Since the Minnesota outbreaks of Highly Pathogenic Avian Influenza (HPAI) in 2015, the Minnesota Veterinary Diagnostic Laboratory (MVDL) has seen an increase in the number of phone calls, emails, and cases received from small flock owners (SFOs) regarding respiratory disease in their flocks. Many SFOs are concerned that any respiratory sign is due to HPAI, when in most cases infectious laryngotracheitis virus (ILT), infectious bronchitis virus (IBV), Newcastle disease virus (NDV) or *Mycoplasma gallisepticum* (MG) have been identified alone or as a mixed infection. SFOs need to know about common pathogens that may affect their birds and how to control them. Through the use of an online survey format, a twenty-question survey was sent to two well-visited Minnesota Facebook groups and to SFOs whose flocks were field tested. The survey assessed SFO background, general recognition of poultry diseases, use of medications and vaccines, and sources of information regarding disease. Approximately 168 SFOs responded to the survey. Many (73%) SFOs had kept birds for less than five years and expressed a lack of knowledge of poultry diseases. In addition, SFOs voiced there was a shortage of educational resources available. The field project involved visits to individual farms/households to collect tracheal swabs from 3-6 birds in each flock. Swabs were collected from poultry flocks in Minnesota counties that were historically either positive or negative for HPAI in 2015 and pools of these samples were analyzed by Next-Generation Sequencing (NGS) to determine the respiratory virome. This information will be useful in improving small flock health and will help to identify potential risks to both small flocks and commercial poultry.

Research Grant: Poultry Respiratory Disease Coordinated Agricultural Project 2017-2018

Student Support: USDA National Needs Fellowship for Enhancing Animal Production (Grant 11978724)

Cytokine expression after porcine islet xenotransplantation in immunosuppressed and tolerant nonhuman primates

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Pancreatic islet cell transplantation has demonstrated value as a therapeutic modality in Type 1 diabetes that can restore insulin independence and lower risk of secondary complications. Although highly effective, scarce human donor pancreata has limited its widespread use. One potential solution is the use of porcine islets, which produce a near-identical insulin. Porcine islet xenografts undergo rejection via a primarily T-cell-mediated process in nonhuman primate (NHP) models. Xenograft survival depends on mitigating this response, via either long-term immunosuppression, or ideally, inducing a state of immune tolerance to the graft. Immune tolerance is enforced by immunosuppressive cytokines mediating regulatory T cell function. Conversely, tolerance can be broken by pro-inflammatory cytokines signaling effector T cell expansion, survival, and memory. Characterizing cytokine profiles in xenotransplanted NHPs exposed to either conventional T-cell directed immunosuppression or tolerogenic protocols has the potential to uncover the molecular basis of regulatory mechanisms central in fostering tolerance and prolonging graft survival. We studied the systemic cytokine response to islet xenotransplantation in 17 diabetic cynomolgus macaques that received either immunosuppression or a tolerogenic protocol. Cytokines were measured with a multiplex assay from serum samples collected at serial time points during follow-up, and profiles were stratified by graft functional outcome for comparison. In the future, we plan to study how cytokine expression varies by compartment, to evaluate accuracy of cytokine profiles in predicting the fate of transplanted islets, and to exploit tolerogenic mechanisms with targeted cell therapies.

Research Grant: National Institutes of Health Grant U01AI120130

Student Support: Boehringer Ingelheim Veterinary Scholars Grant

The effect of transdermal flunixin meglumine on resolution of otitis media in pre-weaned Holstein heifers

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Aiming to reduce the age at first calving (AFC), dairy farmers work to expedite the development of heifers into productive members of the lactating herd. Disease processes that affect pre-weaned calves decrease growth rates and effectively delay reproductive maturity, making them detrimental to both the individual calf and the profitability of the farm. Otitis media (OM), infection of the inner ear, is most commonly caused by *Mycoplasma bovis*. Clinical signs of OM arise due to inflammation and include inappetence, recumbency, ipsilateral ear droop, head tilt, and ptosis. At a 4,100 cow dairy in central New York, OM has a 47.8% morbidity. We performed a prospective cohort study to determine the impact of OM on average daily gain (ADG) in 165 pre-weaned Holstein heifers and nested a randomized trial to assess the effect of pain mitigation on calves with OM. The calves were weighed 5 days a week until 14 days of age, then 2 days a week up to 35 days of age. Concurrently, calves were diagnosed with OM by farm personnel and treated with either a placebo or transdermal flunixin meglumine (TD; non-steroidal anti-inflammatory, 3.3 mg/kg). The mean age at diagnosis was 23 days. There was a trend for OM calves to have a lower ADG than healthy calves until 23 days of age (1.66 lb/day versus 1.77 lb/day, $P = 0.1588$). Administering TD resulted in an increase in ADG from 1.46 lb/day pre-OM to 2.21 lb/day in the first week post-OM diagnosis ($P = 0.002$). Our ongoing research aims to determine if application of TD reduces the number of antimicrobial treatments given to OM calves.

Research Grant: None

Student Support: Ruth L. Kirschstein National Research Service Award 5T35OD 10941-09

Surveillance and genomic characterization of AMR *E. coli* and from an ovine colony on Navajo Nation

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Antimicrobial resistance has emerged as a threat to global health in recent decades. There is need to quantify the prevalence of antimicrobial resistant (AMR) bacteria in various contexts so that methods of reducing risk contributing to its dissemination may be identified. Few studies offer insight into resistant populations in food production sheep. Sheep living on Navajo Nation typically grow up in a pastoral setting, and enteric AMR bacteria dissemination amongst ovine with this context has not been reported. Antimicrobial use is infrequent in the flock we tested at Navajo Technical University due to low risk of infection; but, breeding rams are leased to various shepherds and have unknown exposure to sheep and environments potentially harboring AMR bacteria during breeding season. To investigate, we gathered fecal and environmental samples from this flock and its housing unit during non-breeding season to establish a baseline prevalence of enteric *Salmonella* spp., extended spectrum beta-lactamase gene bla_{CTX-M} , and AmpC gene bla_{CMY} . We predicted that antimicrobial resistance patterns found in sheep isolates will be the same as resistance patterns from environmental isolates taken from housing surfaces. Selective media using antimicrobial drugs cefoxitin and cefepime were used to isolate colonies. We discovered that 1.79% of samples showed phenotypic resistance indicating bla_{CMY} , and 0% of samples showed phenotypic resistance indicating bla_{CTX-M} . We are continuing to characterize environmental samples and confirm genotypes within the lab. Data gathered could further elucidate how food animal production methods affect dissemination of AMR bacterial populations.

Research Grant: NIH T35 Research Grant

Student Support: Wittum Lab

Genomics of methicillin-resistant *Staphylococcus pseudintermedius*

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The gram-positive bacterium, *Staphylococcus pseudintermedius*, is a major cause of canine skin and soft tissue infections (SSTIs), pyoderma, ear infections, otitis externa and urinary tract infections (UTIs). Because methicillin and multidrug-resistant *S. pseudintermedius* cases are becoming more frequent, it is important to understand the evolution and mechanism of acquired antibiotic resistance. This project utilizes whole-genome sequencing to identify genetic changes associated with antibacterial resistance. *S. pseudintermedius* genome sequences from public databases will be analyzed using a pipeline established in our laboratory. The multilocus sequence type (MLST), phenotype profile, antibiotic resistant genes, and virulence genes of each strain will be identified. We expect that an isolate exhibiting elevated minimum inhibitory concentration (MIC) against an antibiotic will have a corresponding mutation or gene associated with phenotypic resistance. The strains with high MICs to multiple antibiotics are expected to bear a greater number of resistance mutations. In addition to known mutations, we expect to discover some novel mutations that may be associated with high MIC and multidrug resistance. Information from this study will provide insight for better management and control of the pathogen, and future development of treatment options.

Research Grant: College of Veterinary Medicine

Student Support: Office of the Director, NIH, T35 OD011145

Anti-inflammatory cytokine profile of synovium constructs

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Osteoarthritis (OA) is an irreversible degenerative joint disease affecting 30 million adults across the United States. Regenerative therapy approaches, such as injection of autologous mesenchymal stem cells (MSCs) into OA joints has shown transient efficacy. Surgical transplantation of a viable synovium extracellular matrix (sECM) seeded with synovium MSCs (sMSCs), termed synovium constructs (sConstructs) has been shown to stimulate chondrogenesis in vivo in rat stifle lesions. This novel approach may offer a longer term and broader delivery of chondrogenic proteins. Research in our laboratory has shown that sConstructs in culture produce a mild level of the pro-inflammatory cytokines (IL-1, IL-6 and TNF- α), whereas sConstructs co-cultured with peripheral blood mononuclear cells (PBMCs) have a dampened inflammatory response. sConstructs were produced from decellularized sECM seeded with sMSC, and co-cultured with PBMCs. Analysis of TGF- β , IL-1ra and IL-10 was performed on the co-culture media by ELISA. The anti-inflammatory cytokines TGF- β ($P < 0.03$) and IL-1ra ($P < 0.013$) were increased (4- and 3-fold increase, respectively) in media from sConstruct-PBMC compared to sConstruct alone. IL-10 was below the level of detection in both the sConstruct-PBMC and sConstruct alone. In the presence of sConstructs, immune cells such as PBMCs, can improve the environment by reducing the inflammatory and increasing the anti-inflammatory cytokine profile. This modulatory effect of immune cells may be a mechanism for the benefits on cartilage identified with sConstruct in vivo and thereby support the efficacy of sConstruct as a novel treatment option for OA.

Research Grant: Equine Research Grants Program, College of Veterinary Medicine, The Ohio State University

Student Support: Boehringer Ingelheim Research Scholars Program

Establishing healthy baseline blood chemistry values in native missouri bat species

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With the advent of handheld analyzers such as the Abaxis VetScan i-STAT 1, requiring only 95 μ L of whole blood or less for measuring chemical blood parameters such as electrolytes, researchers can now measure these blood parameters using non-terminal blood collection methods. In bats, blood chemistry values have only recently begun being investigated and have largely focused on hibernating bats and have solely focused on comparing bats affected and unaffected by White-nose Fungus (*Pseudogymnoascus destructans*). Previous studies have not looked at healthy bats in the summer when they are actively foraging. The purpose of this study is to determine variability of blood chemical parameters and determine the number of bats required to establish healthy baseline blood chemistry parameters in wild-caught and captive bats. We will use a VetScan i-STAT 1 Handheld Analyzer using CG4+ and CHEM8+ cartridges to determine the blood chemistry parameters in native Missouri bats. We will analyze the blood from a minimum of 10 members of each of the following species: Big Brown Bats (*Eptesicus fuscus*), Little Brown Bats (*Myotis lucifugus*), and Red Bats (*Lasiurus borealis*).

Research Grant: Missouri Department of Conservation and USDA US Forest Service

Student Support: None

Effects of a kisspeptin analog on serum concentrations of LH, FSH, and testosterone in prepubertal bulls

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Kisspeptin (KP) is a neuropeptide integral in regulating puberty and GnRH release. Compound-6 (C6), a KP analog, may have greater clinical applications than KP. To determine the acute and subacute effects of KP and C6 on serum concentrations of LH and FSH, prepubertal bull calves were assigned to one of three treatment groups [Saline (n = 4), KP (n = 4; 20 nmoles), or C6 (n = 4; 20 nmoles). Treatments were administered IM daily for four consecutive days. Blood samples were collected every 15 minutes for six hours immediately following treatment administration on Day 1 (acute) and Day 4 (subacute). Serum concentrations of LH and FSH were determined by radioimmunoassay. For each period, effects of treatment, time, and interactions on LH and FSH concentrations and pulse parameters were analyzed using procedures for repeated measures with JMP Software (SAS Inst. Inc., Cary, NC). During the acute period, LH concentrations were greatest following administration of C6 ($P < 0.0001$). During the subacute period LH concentrations tended to be greatest following administration of C6 ($P = 0.051$). Number of LH pulses were least following administration of C6 ($P = 0.02$). The LH nadirs were greatest following administration of C6 ($P = 0.04$). Mean FSH concentrations were greatest during the acute period ($P < 0.0001$). During the subacute period FSH concentrations ($P = 0.0254$) and number of FSH pulses ($P = 0.0168$) were least following administration of C6. In conclusion, acute and subacute C6 increased LH concentrations and pulse parameters and subacute C6 decreased FSH concentrations and pulse parameters. Despite suppression of gonadotropins with subacute C6, KP analogs may have application to affect the onset of puberty in livestock.

Research Grant: Select Sires

Student Support: UTCVM Center of Excellence; AVMA/AVMF 2nd Opportunity Summer Research Scholarship

Travelers' diarrhea: *Providencia alcalifaciens* can infect humans and calves, but what about mice?

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Providencia alcalifaciens (Pa) is a cause of travelers' diarrhea in people but the mechanism of diarrhea remains unknown. Pa has two Type 3 Secretion Systems (T3SS-1a and -1b) that genetically resemble the T3SS-1 of *Salmonella*, which is essential for invasion of epithelial cells and the development of diarrhea. We previously used the calf ligated ileal loop model to demonstrate that the Pa T3SS-1a is required for intestinal colonization. The purpose of our study is to develop a mouse model of Pa infection and to establish whether the T3SS-1a and T3SS-1b are needed for intestinal infection. Since mice have colonization resistance to numerous enteric pathogens, we hypothesize that alteration of the microbiota is needed for development of a robust Pa infection. Mice were treated with streptomycin or water prior to infection with the WT organism ($\sim 10^8$ CFU) and body weights and fecal shedding were monitored. Mice with disturbed microbiota were infected with either the WT or Pa mutants ($\sim 10^8$ CFU) that have gene deletions for each T3SS. We found a significant increase in Pa colonization in mice treated with streptomycin for the first 4 days of infection, but then no difference in colonization. We found no significant differences in the degree of colonization for either TTSS mutant but a reduced intestinal persistence by day 7 post-infection for the TTSS-1b mutant and by day 10 for the TTSS-1a mutant. This experiment will provide a better understanding of the role of Pa pathogenesis in the intestine of hosts of varying species.

Research Grant: CVM Practice Plan

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Nutritional practices, knowledge, attitudes and perceptions of clients at a small animal teaching hospital

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Nutrition and exercise are critical components of an animal's overall health status. However, despite their importance, these topics frequently go undiscussed during veterinary visits. As such, it would be beneficial to fill this knowledge gap on what pet owners are feeding, why, and how they are informing these choices. Objectives were to: 1) determine teaching hospital small animal clientele practices, knowledge, attitudes and perceptions regarding nutrition and exercise, and 2) evaluate their recall on veterinary recommendations related to these. Clients visiting the Atlantic Veterinary College Teaching Hospital were invited to complete a questionnaire, and descriptive statistics were used to evaluate the data. A total of 143 clients were invited to participate, and 101 surveys were completed (71% response rate). In a typical week, pets ($n=112$) were fed canned food (32%), dry food (88%), processed treats (57%), pet chews (24%), dietary supplements (13%), cooked food (29%), raw food (14%), raw fruits or vegetables (41%) and rawhides or bones (15%). Participants ($n=101$) stated that they trusted their veterinarian's recommendations regarding their pet's nutrition as follows: strongly agree (74%), somewhat agree (23%) and neutral (3%). However, clients ($n=98$) reported that they had interest in discussing exercise with their veterinarian as: strongly agreed (27%), somewhat agreed (36%), neutral (27%), somewhat disagreed (3%), strongly disagreed (6%), and no interest (2%). Our work helps better understand pet owner's practices and attitudes, and builds on other work highlighting a need for improved communication with clientele.

Research Grant: Atlantic Veterinary College

Student Support: Atlantic Veterinary College Veterinary Student Research Award

Effects of developmental programming with EDCs on hypothalamic monoamines and neuroendocrine functions

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Exposure to endocrine disrupting chemicals (EDCs) such as diethylhexyl phthalate (DEHP) and bisphenol-A (BPA) during pregnancy is commonly encountered these days. Many of these EDCs are produced in high volume in the US and are widely used in the manufacture of many plastic products that we use on a daily basis. Exposure is through oral, dermal or inhalation routes and these EDCs can easily cross the placental barrier and reach the offspring. Thus, the fetuses are at risk of being exposed to EDCs during the "critical development window." This exposure can indeed program the developing fetus and predispose it for dysfunctions and diseases during adulthood. The purpose of the present study is to investigate developmental programming potential of DEHP and BPA in rats. Pregnant female Sprague-Dawley rats were exposed to the vehicle or 5 µg/kg BW of BPA or 7.5 mg/kg BW of DEHP or the combination of BPA and DEHP from gestation day 6 through day 21. The male and female offspring were monitored for changes in growth pattern and feeding from weaning through adulthood. When the offspring were around 4 months of age, they were sacrificed and the brains were removed, frozen, sectioned and discrete hypothalamic nuclei including the paraventricular nucleus (PVN) were microdissected. Monoamine concentrations in the PVN will be measured using HPLC-EC and correlated with changes in central and neuroendocrine functions such as feed intake and the hypothalamo-pituitary-adrenal axis activity as the PVN is known to be critically involved in regulating these functions. It is expected that changes in brain monoamines in the PVN might be involved mediating EDC programming-induced changes in central and neuroendocrine functions.

Research Grant: UGA Foundation

Student Support: Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Correlates of mucosal immune control of feline enteric coronavirus replication

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Feline enteric coronavirus (FECV) is a ubiquitous virus that causes minimal or no clinical signs in cats. Mutation of FECV can lead to the emergence of feline infectious peritonitis virus that causes a deadly effusive and/or granulomatous disease. Previous research has shown that cats infected with FECV can clear the virus or become intermittent or persistent virus shedders. It is thought that rapid waning of the humoral immune response predisposes cats to reinfection. To date, there has not been a comprehensive assessment of the mucosal immune response associated with FECV infection and clearance. A closed cat colony with circulating FECV infection was studied longitudinally to assess mucosal immune correlates of protection. Blood and fecal samples were collected monthly and colonic biopsies were obtained at an arbitrary time 0. Virologic assessment included PCR detection of virus in feces and colonic tissue. Immunological assessment included FECV-specific serum IgG and fecal IgA. Lamina propria lymphocytes from colon biopsies were phenotyped using flow cytometry and were assessed for FECV-specific IgA and IFN-g by ELISPOT. Expression of IL17 and FoxP3 was assessed by qRT-PCR. Although histopathology of colonic biopsies from cats shedding virus was unremarkable, an inflammatory state was indicated by total IgA producing cells, IFN-g production and increased IL17:FoxP3. FECV-specific IgA was also associated with viral replication. Whether mucosal IgA and serum IgG represent correlates of protection will be assessed by functional assays. This information will be used to design an orally-delivered mucosal vaccine to prevent FECV replication and thereby mutation to FIPV.

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Student Support: Ms. Morgan was supported by Boehringer Ingelheim Fellowship

Breed association of natural killer (NK) cell number and function in dogs

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Unlike T-cells, natural killer (NK) cells do not require antigen specificity or priming to kill tumor targets and are not implicated in graft-vs-host disease or cytokine release syndrome. However, evaluating novel NK cell-based therapies is difficult due to an inability to expand mouse NK cells for doing so. In contrast, canine NK cells can be expanded and canines spontaneously develop several cancers homologous to humans. However, it is difficult to predict which canine breeds will have higher NK cell numbers and be better donors for testing NK cell therapies in canine cancer patients. The purpose of this study is to determine canine inter-donor variability in number of NK cells in four dog breeds (Greyhound, Labrador Retriever, Golden Retriever, and Staffordshire Terrier) and produce enough adoptively transferred NK cells to conduct a clinical trial in dogs with cancer. It has been shown that NK cell numbers vary substantially between donors. Flow cytometry will be used to determine number of NK cells (identified by NKp46 antibody) after red blood cell lysis of heparinized blood from 10 dogs each of the four specified breeds from The Ohio State University Veterinary Medical Center Canine Blood Bank donors and healthy individuals; NK cell expansion will be conducted using a feeder-cell system developed by the Lee Laboratory at Nationwide Children's Hospital for human NK cells. We expect inter-donor variability in number of NK cells (approximately 2-3% of lymphocytes) to be partially breed-associated. We also expect absolute NK cell numbers to be lower in Greyhounds since they are known to have lower lymphocyte counts. These results will facilitate the selection of ideal canine NK cell donors for cancer immunotherapy.

Research Grant: None

Student Support: Rohovsky Summer Research Scholar

Evaluation of the BRAF mutation in canine gliomas

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Malignant gliomas are among the most common primary tumors of the central nervous system in humans and dogs with a poor prognosis for both species. Conventional diagnostics include magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) analysis, and a biopsy of tumor tissue. The latter is essential to confirm diagnosis since advanced imaging does not enable differentiation between neoplasia and inflammatory disease; however, brain biopsy is invasive, challenging and associated with considerable morbidity. A number of mutations have been documented in human brain malignancies, although the mutational landscape is relatively unknown in the dog. The BRAF gene is an oncogene that causes cells to proliferate uncontrollably when mutated, contributing to neoplastic transformation. BRAF is known to be mutated in some canine cancers, such as urothelial carcinomas. Recently, an ultrasensitive digital droplet PCR (ddPCR) test has been developed by the Breen Lab that enables the detection of BRAF mutations within urine samples from dogs with urothelial carcinoma. We hypothesized that BRAF would be mutated in a subset of canine gliomas and that these mutations would be detectable within cell-free tumor DNA within CSF. We analyzed DNA isolated from 20 histologically confirmed gliomas using ddPCR, and found that three were positive for the BRAF mutation. The next steps include isolation of tumor and cell-free DNA from dogs with matched tissue and CSF samples and screening those for the mutation. If successful, such an assay would expand the available diagnostics, allowing for a less-invasive "liquid biopsy" confirmation of glioma.

Research Grant: NC State Cancer Genomics Fund

Student Support: NC State CVM Boehringer Ingelheim Veterinary Scholars Program

Establishing hematologic and biochemistry reference values in common leopard geckos (*Eublepharis macularius*)

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Hematologic and biochemistry reference values are an incredibly powerful tool in the arsenal of the clinical veterinarian, and yet for many exotic species there is very limited published information available on the subject. Leopard geckos, a well-represented reptile in the pet trade, lack an established reference range for these common diagnostic tests, making clinical diagnosis and treatment more challenging. While there are publications regarding this subject in other species of gecko, one must remember that the common leopard gecko is its own species and must not be thought of as a “breed” of gecko. The purpose of this study is to establish both hematologic and plasma biochemistry reference values in the common leopard gecko (*Eublepharis macularius*), allowing for better diagnostic evaluation by clinicians. Results from manual blood counts and automated plasma biochemistries from 50 adult leopard geckos, both male and female, were reviewed. These results were obtained from three separate, unrelated studies. All geckos from were housed and maintained using proper husbandry technique and were considered clinically healthy at the time of blood collection. Ranges established from these populations may be used as a basis for comparison in clinical cases.

Research Grant: None

Student Support: Boehringer-Ingelheim Veterinary Research Scholars Program

Isolation of *Staphylococcus pseudintermedius* bacteriophage

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Staphylococcus pseudintermedius is an opportunistic pathogen of dogs and is identified as an emerging zoonotic pathogen of humans. Methicillin resistant *S. pseudintermedius* (MRSP) is increasingly recognized in canine infections. MRSP is resistant to all β -lactam drugs, thus reducing treatment options. Research into alternative therapies is warranted. The goal of this study was to isolate lytic phage that could possibly be used for therapy in clinical infections and identify lysogenic phage that could be used as molecular tools for study of *S. pseudintermedius* pathogenesis. Phage therapy has shown promising potential for treating methicillin resistant *Staphylococcus aureus* (MRSA) infections and may have promise for treating MRSP infections as well. Potential lysogenic phages were identified from genomic sequences of *S. pseudintermedius*. The bacteria were observed for spontaneous release of phage into tryptic soy broth, extracted and tested against other *S. pseudintermedius* isolates to identify candidates to use for transduction. To isolate lytic bacteriophage, residual bandage material from dogs with skin and soft tissue infections was collected and immersed in TSB and incubated at room temperature. The broth was extracted and sterilized, enriched overnight, and spotted on lawns of *S. pseudintermedius*. Phages that formed plaques on the bacterial lawns were isolated for further characterization. Resultant bacteriophage will be further purified, imaged, sequenced, and tested against a bank of 65 human *S. pseudintermedius* isolates.

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Student Support: NIH (NIH 5T35OD010991-14) and the VMSRTP. SVL was supported by a TAMU Diversity Fellowship

Detection of the invasive fish parasite *B. acheilognathi* using loop-mediated isothermal amplification (LAMP)

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The Asian fish tapeworm (*Bothriocephalus acheilognathi*, syn. *Schyzocotyle*) is an invasive generalist parasite of freshwater fish that is known to detrimentally affect native fish populations, endangered fish species and freshwater ecosystems. *Ba* has been shown to be spread through the transport of infected fish, particularly bait fish and copepod intermediate hosts. Despite its harmful potential, there is not an efficient or accurate way to detect *Ba* in water or fish samples before transport. The aim of this project was to develop and implement a precise and specific isothermal assay to detect the parasite from an environmental sample (i.e. bait water) to prevent the spread of *Ba* and therefore infection of at-risk fish populations. Six loop-mediated isothermal amplification (LAMP) primers were designed for a conserved 18S rRNA region of *Ba*. Detection of genetic material was quantified with a FAM fluorescent label, performed on the Agdia AmpliFire instrument. Our LAMP assay successfully amplifies and consequently detects extracted *Ba* DNA; thus far its exact specificity when compared to native cestodes and other potential material in bait water is still being examined. The sensitivity of the assay is yet to be completely determined; evaluating the minimum quantity of *Ba* DNA required for detection among the multitude of other genetic material in water samples is ongoing. However, early assay trials have positively detected samples with purified *Ba* DNA concentration as dilute as 1×10^{-1} copies/ μ l. Future application of our assay would be a low-cost test that could rapidly and accurately detect invasive parasite DNA from environmental samples on site to mitigate the spread of the Asian fish tapeworm.

Research Grant: USGS Ecosystems Mission Area

Student Support: Boehringer Ingelheim Veterinary Scholars Program Grant (PI: Bjorling)

Intestinal dysbiosis in dogs with atopic dermatitis

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Canine atopic dermatitis is a common pruritic inflammatory skin condition associated with environmental allergens, and genetic and immune factors. Dogs have a diverse intestinal microbiota that is thought to play a role in the development of allergies. The aim of this study was to test if dogs with atopic dermatitis have an altered microbiota. Fecal samples from 20 dogs with atopic dermatitis and 12 healthy dogs were analyzed. Quantitative PCR was used to determine the abundance of bacterial groups in each sample, then the dysbiosis index was calculated from these values. Flow cytometry analysis was used to determine the percentage of IgE-coated fecal microbiota. Mann-Whitney tests were used to compare the dysbiosis index, abundance of bacterial groups, and the percentage of IgE-coated fecal microbiota between healthy dogs and dogs with atopy. Dogs with atopic dermatitis had a significantly increased dysbiosis index ($P = 0.0068$). Dogs with atopy had significantly lower total fecal bacteria ($P = 0.0009$), *Faecalibacterium* ($P = 0.0056$), and *Fusobacterium* ($P = 0.0056$) than healthy dogs. They also had significantly higher *Blautia* ($P = 0.0116$). There was no significant difference in the abundance of *Turicibacter*, *Streptococcus*, *E. coli*, or *Clostridium hiranonis*. There was no significant difference in the percentage of IgE-coated fecal microbiota between healthy dogs and dogs with atopy. In conclusion, there was a difference in the composition of the microbiota between healthy dogs and dogs with atopic dermatitis. These findings warrant further studies to determine whether modulation of the microbiome would be beneficial to dogs with atopy.

Research Grant: None

Student Support: Boehringer Ingelheim VSP, Texas A&M College of Veterinary Medicine & Biomedical Sciences

Using multiplex assays to screen for tick-borne pathogens in animals and ticks of Grenada, West Indies

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Tick-borne diseases are a major concern in Grenada, West Indies, particularly due to the tropical climate. Because of the isolation of Grenada, variability in the populations of tick-borne pathogens is suspected, but not well studied. Traditional single plex PCR assays are the standard tests for tick-borne diseases but multiplex PCR assays have proven to be more efficient, cost effective, and can detect up to 50 different pathogens with a smaller sample size. The goal of the study was to screen for tick-borne pathogens in DNA collected from 20 dogs, 23 cattle, 17 small ruminants and eight ticks using both a 22-plex commercial kit (MultiFLEX Tick-borne Panel, Luminex) and a 7-plex in-house assay. Agreement between the two assays for detection of *Anaplasma* and *Ehrlichia* positives was determined. The 22-plex assay was designed to detect *Anaplasma*, *Borrelia*, *Ehrlichia*, *Rickettsia*, *Coxiella*, *Bartonella*, *Babesia*, Powassan virus, and Tick-Borne Encephalitis virus. The 7-plex assay was customized to detect *Anaplasma* and *Ehrlichia* sp., some known to be in Grenada. The 22-plex assay detected *Anaplasma* sp. in cattle, goat, and ticks. *Ehrlichia* sp. DNA was only detected in the tick samples. In contrast, the in-house 7-plex assay defined *Anaplasma* and *Ehrlichia* species specific DNA in multiple ticks, dogs and bovine samples. Two coinfecting dogs were identified, one with *A. platys* and *E. canis* the other with *E. canis* and *E. chaffeensis*. The overall agreement between the two assays for detection of *Anaplasma* and *Ehrlichia* sp. was 85% and 70%, respectively. Additionally, the kit assay identified pathogens not previously described in Grenada. Future sequencing studies are planned to confirm the presence of these other pathogens.

Research Grant: Windward Islands Research and Education Foundation One Health Grant Research Initiative 01-26-8

Student Support: BI Veterinary Research Scholars Program, KSU Center of Excellence for Vector-Borne Diseases

Canine endometrial and ovarian histology throughout the estrous cycle

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Reproductive diseases in the domestic dog and their wild counterparts such as wolves, African wild dogs, and fennec foxes, have a significant economic and conservation impact. Gross and histologic examination of the reproductive tract are basic tools for understanding and resolving these diseases. Despite this, little basic research on the varying microscopic morphology of the reproductive tract throughout the estrous cycle has been done. We hypothesize that ovarian structures and uterine histomorphology accurately predict the stage of the estrous cycle in the dog. To demonstrate this, 60 sets of full thickness uterine and ovarian samples were analyzed from bitches in various stages of the estrous cycle. Parameters measured include differentiation of the stratum spongiosum and basalis, endometrial gland density, surface epithelium height, and follicular density. T tests were utilized in statistical analysis. We are hopeful these findings will have predictive potential with respect to canine reproductive and overall health, impacting the work of theriogenologists and conservationists alike.

Research Grant: None

Student Support: NIH Grant T35OD016477 to Michigan State University

Radiographic quantification of tibial long axis shift using the anatomical-mechanical axis angle

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Tibial long axis shift (TLAS) is defined as a change in the mechanical tibial axis (MTA; a line connecting the centers of the stifle and tarsus) after surgery for cranial cruciate ligament disease (CCLD). TLAS may benefit patients with proximal tibial angulation (PTAng), but may worsen outcomes for patients with straight tibiae. Currently described methods for measuring TLAS, using the distal tibial axis/proximal tibial axis angle (DPA) and the anatomical-mechanical axis angle (AMA), are infrequently used because they are cumbersome and require digital image overlay. The purposes of this study were to A) compare two simplified methods for measuring PTAng to the two established methods, B) describe normal values associated with the novel methods, and C) evaluate the use of all four methods for quantifying TLAS. We hypothesized that there would be a strong correlation between the four measurement methods and that all methods would allow quantification of TLAS. Digital imaging software was used to measure 185 medio-lateral radiographs of Labrador Retriever tibiae who underwent CCLD-surgery at CSU. Pre- and postoperative radiographs were measured in triplicate by a single observer. Mean preoperative values were 6.33° for DPA, 2.25° for AMA, 2.40° for novel method A, and 2.58° for novel method B. Mean postoperative values were 6.31° for DPA, 1.83° for AMA, 1.95° for novel method A, and 2.20° for novel method B. Preliminary results indicate strong correlation between the four methods for pre-operative measurements. DPA could not quantify TLAS in 102/185 patients because surgical implants obscured landmarks. AMA and both novel methods allowed for quantification of TLAS in all patients.

Research Grant: Young Investigator Grant Program

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Peroxisome proliferator-activated receptors pathway utilized to control lipid metabolism in Dengue infection

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Dengue viruses annually infect an estimated 390 million people with 96 million becoming symptomatic and 500,000 people developing severe dengue disease requiring hospitalization. Despite this immense global burden, prevention still relies upon vector control due to effective vaccines remaining in experimental stages. Contemporary flavivirus research has shown that the dengue virus life cycle is associated with host cell lipids, with cellular membranes aiding in viral replication and particle generation as well as host lipid metabolism being altered to create an environment that favors viral replication. Our novel idea is that the peroxisome proliferator-activated receptor pathway is implicated in this viral alteration of host lipids through its involvement in energy homeostasis and triglyceride, glucose, and fatty acid metabolism. To address this hypothesis, we are using siRNA mediated knockdown in Huh7 cells of genes involved in the initiation of the pathway and in fatty acid transport then measuring viral output with plaque assays. We will also analyze the impact of the siRNA gene knockdown using a luciferase reporter assay to measure genome replication. If the plaque assays performed using the supernatant from siRNA transfected cells produce less plaque forming units that would indicate a decrease in viral particle release. Similarly, if the luciferase assay produces diminished luciferase transcription that would indicate a decrease in viral RNA replication. These results would suggest that genes within the peroxisome proliferator-activated receptor pathway are important for the dengue virus life cycle, generating novel targets for the development of antivirals.

Research Grant: Unknown

Student Support: NIH Grant T35OD015130-06

Test method validation for detection of vitamin D (25OHD) in the black rhinoceros (*Diceros bicornis* spp.)

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Subclinical vitamin D (25OHD) deficiency may have serious implications for immune and reproductive health function. Subclinical vitamin D deficiency may exacerbate disease in critically endangered species, such as the black rhinoceros (*Diceros bicornis*). Assays exist to determine circulating 25OHD from animal serum samples, but these tests are not readily available without submission to reference laboratories, or purchase of expensive laboratory equipment (such as liquid chromatography or mass spectrometry), which minimizes access to testing in the range countries of wild animals. Enzyme immunoassays (EIAs) developed for human serum do not give valid results for most animal species, due to differences in protein and lipid matrices. In order to use these EIAs, it is necessary to extract 25OHD away from the proteins and lipids prior to using the assay. The objective of this study was to validate a human-specific enzyme immunoassay (EIA) for 25OHD for use in *Diceros* species maintained in AZA zoos within North America.

Research Grant: 2018 VCS Research Incentive Grant

Student Support: Merial

Effects of routine hoof trimming on hindhoof force distribution biomechanics at midstance in horses

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Proper hoof function is essential for soundness and performance in horses. It has been reported that the predominant cause of all equine lamenesses are pathologies within the foot. Though hoof health has been a primary concern for farriers, owners, and veterinarians, few empirical studies have investigated the scientific basis of hoof trimming, proper hoof balance, and their combined effect on hoof biomechanics. Additionally, most studies performed to date have focused on the forehoof (FH) while the hindhoof (HH) has received less attention. The aim of this study was to examine force (F), contact pressure (CP), and contact area (CA) at the medial and lateral aspects of the HH to evaluate the impact of routine hoof trimming on HH biomechanics. Nine clinically sound Arabian horses were walked across a calibrated pressure plate before and after routine hoof trimming and the F, CP, and CA at the medial and lateral aspects of the HH were recorded at midstance before and after trimming. The differences between these regions were investigated using a paired t-test. P -values < 0.05 were considered significant. Among measured variables, force increased by 25% ($P=0.007$) and contact pressure increased by 13% ($P=0.032$) at the medial aspect of the HH after routine hoof trimming. Our findings illustrate the impact of routine hoof trimming on specific regions of the equine HH, providing benchmark data for further studies on equine HH biomechanics. Analyzing data from hindlimb hoof-surface interaction in sound horses will allow for a better understanding of imbalances and pathologies that commonly cause hindlimb lameness in horses.

Research Grant: Arabian Horse Foundation, Western University of Health Sciences

Student Support: Boehringer Ingelheim Veterinary Research Scholars Program

RNA-Seq identifies mutually exclusive driver insertions in PI3K-hyperactive mammary carcinomas

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College of Veterinary Medicine, University of Minnesota (Pope); Masonic Cancer Center, Minneapolis, MN (Pope, Sokolowski, Kurata, Shu, Sarver, Temiz, Hudson, Slipek, Largaespada); Genentech Inc, San Francisco, CA (Yuan, Seshagiri); Department of Pediatrics, University of Minnesota, Minneapolis, MN (Largaespada)

The most common carcinomas in women, and one of the leading causes of cancer-related death, are invasive mammary tumors. Creating in vivo models that accurately recapitulate mammary tumorigenesis is difficult due to high genetic heterogeneity within and between tumors. Activating mutations in the catalytic subunit of PI3K are one of the most common genetic alterations in human breast carcinomas. We conducted a forward genetic screen using a Cre-inducible Sleeping Beauty (SB) transposon system in the context of PI3K hyperactivation to discover genetic events that cooperate with an oncogenic PI3K mutation. The T2/Onc3 transposon activates endogenous proto-oncogenes or inactivates tumor suppressor genes by insertional mutagenesis. In conjunction with a R26-LSL-SB11 transposase and tissue-specific Cre, the transposon mobilizes and integrates into the genome. Mice with the PI3K mutation and SB had accelerated tumorigenesis compared to controls. RNA sequencing showed molecular subtypes with correlating T2/Onc3-induced insertion mutations, including estrogen receptor (ER) positive and negative subtypes. RNA and DNA sequencing defined common insertion sites of the SB transposon, allowing for detection of frequently mutated genes. We analyzed 244 tumors and identified 20 tumor suppressor genes and 35 oncogenes as common insertion sites. Notably, some common RNA insertion sites (R-CIS) were mutually exclusive, specifically insertions in *Jup*, *Hras*, *Pten*, and *Notch1*. Additionally, *Pten* insertions were associated with ER⁺ tumors. This model provides a source of genetically heterogeneous mammary tumors with the same initiating mutation (PI3K) useful for identifying cooperating pathways and drivers of specific tumor phenotypes.

Research Grant: American Cancer Society Research Professor Award (to D.A.L.), Genentech/Roche Inc

Student Support: The Boehringer Ingelheim Veterinary Scholars Program

Evaluating accuracy of AliveCor Vet ECG device for measuring heart rate of dogs with atrial fibrillation

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The most commonly employed treatment option for dogs with atrial fibrillation is heart rate control. This requires accurate measurement of heart rate since therapy is based on a target heart rate. Any method of heart rate assessment must take into account the variability of heart rate throughout the day. Currently a 24-hour ambulatory continuous electrocardiographic monitoring (Holter) is considered to be the gold standard for heart rate assessment. However, Holter monitoring is associated with high cost and is not always practically feasible in a primary care setting. The objective of this study is to determine whether heart rate measurements made at home using the AliveCor ECG device provides an accurate measurement of resting heart rate in dogs with atrial fibrillation as compared to a 24-hour Holter monitor. Holter monitors were placed on four dogs that met the inclusion criteria. Owners were instructed to obtain multiple 5 minute mean heart rate measurements using the AliveCor Vet application at home when the dogs were at rest. The mean difference between the 24-hour Holter and AliveCor ECG obtained heart rates was 12.4 beats per minute. AliveCor ECG provided a mean heart rate within 18.3 percentage of Holter obtained heart rate in the study dogs. This pilot study showed that AliveCor ECG is a feasible method to obtain accurate heart rate in dogs with atrial fibrillation with acceptable deviation from mean. Further studies with larger sample size is required to evaluate its clinical use.

Research Grant: None

Student Support: Boehringer Ingelheim Veterinary Scholars Grant to Michigan State University

Expansion of biliary tree stem cells through culture in conditions for intestinal stem cell expansion

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Stem cells within the biliary tree have been identified as a source of progenitor cells for the liver, pancreas and gallbladder. Because biliary tree stem cells (BTSC) are highly pluripotent; they could be of invaluable use in regenerative medicine. Their usefulness in regenerative medicine is dependant the ability to isolate, culture, and expand these cells in vitro. BTSC have been isolated and cultured to form organoids. However a process for expanding these organoids in vitro has not yet been developed. BTSC are developmentally similar to the stem cells found within the small intestine. We hypothesized that they might have similar growth requirements. We attempted to expand BTSC in culture, using media conditions known be successful in expanding intestinal organoids. BTSC organoids were cultured in wells containing either media alone, media with matrigel, or media with matrigel and growth factors. Preliminary observations were of increased size and morphology changes for organoids cultured in media with matrigel and growth factors. PCR analysis will follow to determine the differentiation status of the organoid cells.

Research Grant: Unknown

Student Support: Boehringer Ingelheim Veterinary Scholars Research Program

Discovery of inhibitors against the *Cryptosporidium parvum* lactate dehydrogenase (CpLDH)

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Cryptosporidium parvum is a gastrointestinal apicomplexan that is a common infectious parasite in companion animals, livestock, and humans. *C. parvum* frequently causes water-borne outbreaks around the world. Moderate to severe diarrhea is most common, but *C. parvum* can be deadly for immunocompromised individuals since the one FDA approved drug is only for immunocompetent individuals. *C. parvum* lacks the Krebs cycle and cytochrome-based respiration, so glycolysis is relied on to produce ATP. Lactate dehydrogenase (LDH) is a key enzyme required to regenerate NADH and to remove the end product in the glycolytic pathway. LDH inhibitors have been found to inhibit *Cryptosporidium* lactate dehydrogenase (CpLDH) enzyme activity and inhibit parasite growth in vitro. This suggests that CpLDH is a potential, effective target for developing treatments for cryptosporidiosis. The aim of this project is to validate the inhibition of selected compounds on CpLDH enzyme activity. The long-term goal is to identify the most efficacious compounds with anti-CpLDH enzyme activity to evaluate their selectivity on host and parasite LDH and evaluate their anti-cryptosporidial efficacies. We tested over 100 preselected compounds with 50% inhibition at 10 μ M from the TimTec ActiProbe-10K library of 10,000 compounds. We identified 18 compounds with CpLDH inhibition of 50% or greater at a concentration of 2 μ M. Inhibitory kinetics provided further information on the potency of their inhibitory effects. These highly inhibitory compounds can be used as structural templates to build analogs with greater inhibitor efficacy, for structure-activity analysis (SAR) and for lead optimization.

Research Grant: NIH Western Regional Center for Biodefense and Emerging Infectious Disease (WRCE, U54 AI057156)

Student Support: NIH 5T35OD010991-14

Comparison of injectable and transdermal flunixin meglumine effects on pregnancy after embryo transfer in cows

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Embryo transfer (ET) is an important tool for increasing the reproductive potential of valuable beef cows. Pregnancy rates following embryo transfer can be improved using flunixin meglumine to decrease levels of PGF2a, thereby maintaining adequate uterine environment and hormone levels to support embryo development in the recipient cow. The objective was to compare the effects of injectable and transdermal flunixin meglumine on pregnancy in embryo transfer recipient beef cows. Angus-cross beef cows (n=726) designated in estrus after synchronization were used as embryo recipients for frozen-thawed embryos at 7 d after estrus. At the time of ET, cows received injectable flunixin meglumine IM (n = 273; INJFM), transdermal flunixin meglumine (n = 271; TDFM) or no treatment (n=182; CON). Pregnancy rates were determined by ultrasonography 35 d after ET. The ET pregnancy % was not influenced by dams age, embryo stage, embryo grade, treatment by temperament score, treatment by ET difficulty score, embryo stage by ET difficulty score, and embryo grade by ET difficulty score interactions ($P > 0.1$). Controlling for temperament ($P < 0.05$); ET difficulty score ($P < 0.05$); and dams BCS ($P < 0.05$), the treatments affected the ET pregnancy % ($P < 0.05$). The ET pregnancy % for cows in INJFM treatment group (63.7%) was significantly greater than CON group (52.2%), an 11 % increase in ET pregnancy. The ET pregnancy % for cows in TDFM treatment group (58.7%) was not different from cows in CON or INJFM groups ($P > 0.1$, $P > 0.1$ respectively). Considering the expense and effect on ET pregnancy %, it is advisable to implement injectable over transdermal flunixin meglumine treatment following ET.

Research Grant: Merck Animal Health, USA

Student Support: Boehringer Ingelheim Veterinary Scholars Program, WSU College of Veterinary Medicine

3D imaging of lineage tracing in the mouse prostate

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Information on the spatial organization of whole organs or embryos, including at the cellular level, is essential for many areas of developmental biology and cancer research. A number of techniques, such as CLARITY, 3DISCO and PACT, have been developed to allow three-dimensional (3D) imaging of molecularly labeled structures within large intact tissues. The purpose of this study was to evaluate the applicability of 3D imaging to visualize basal, luminal, and neuroendocrine cells of the prostate after immunostaining and cell lineage tracing with tdTomato reporter. To this end, we harvested prostates, immunostained them with markers for basal (KRT5+), luminal (KRT8+), and neuroendocrine (SYP+) cells, and tested tissue clearing protocols followed by imaging with confocal and light sheet microscopy. Furthermore, we applied the optimized clearing techniques to tracing KRT5+ cell lineage. Our results showed that optimized PACT protocol based on an immersion in a denaturing agent (6% SDS in PBS) and in a sorbitol-based solution (sRIMS) made the samples transparent enough to image 2 mm-thick prostate samples. Along with appropriate staining with dyes and fluorescent antibodies emitting at high wavelengths, this technique allowed visualization of individual cells in prostate samples. We also observed that KRT5+ cells appear to be mostly located in the proximal part of the prostatic ducts, the known place of prostatic stem cells. In sum, the optimized PACT technique is well suitable for acquiring spatial information about prostate cells, and will be an important tool for cell fate analysis of prostate lineages, including rare neuroendocrine cells.

Research Grant: NIH/NCI grant (R01CA197160) to Alexander Yu. Nikitin, and NYSTEM postdoctoral fellowship (C30293GG) to Mallikarjun Bidarimath.

Student Support: Boehringer Ingelheim Veterinary Scholars Program (BIVSP)

Purification of mouse embryonic stem cell derived cardiomyocytes (mESC-CMs) by metabolic selection

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Cardiovascular disease is the number one cause of death in the United States. Conventional treatments, such as drug therapy, heart surgery, or transplantation, are not ideal approaches due to their side effects and potential complications. Recently, stem cells have become the focus of regenerative medicine because of their ability to self-renew and differentiate into multiple cell lines, including cardiomyocytes (CMs). Thus, CMs derived from embryonic stem cells (ESC-CMs) hold great potential to repair damaged heart tissue by direct transplantation; however, the differentiated cardiac cell population may contain non-cardiac cells, such as fibroblasts. It is known that most somatic cells produce ATP via glycolysis, whereas CMs can effectively use lactate to generate energy. The main objective of this project is to examine the purification efficacy of mouse ESC-CMs (mESC-CMs) using the lactate-supplemented method. Briefly, the mESC-CMs were exposed to glucose-free medium, supplemented with 4 mM L-lactic acid on Day 14 after initial differentiation, while a control group received regular cardiac differentiation medium that contained glucose. On Day 20 all cells were detached and stained with cardiac α -actinin-2 antibody and Hoescht 33342. The mean data of % of α -actinin-2 positive cells were compared between the two groups. The lactate method resulted in $99 \pm 1.17\%$ purity of CMs compared to $56 \pm 9.36\%$ ($P < 0.001$) in the control group. CMs in both groups exhibited continuous beating under 37°C , indicating the functional properties of cardiac muscle. This indicates that purifying the mESC-CMs with the lactate-supplemented method significantly increases their purity; however, more replicates are needed to increase the sample size.

Research Grant: NIH-1R15HL140528-01

Student Support: T35OD011887 NIH Office of Director

Utilizing microsatellites to track genetic diversity in *Cytauxzoon felis*

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Cytauxzoonosis is a tick-borne, hemoprotozoal disease of domestic cats whose causative agent is the apicomplexan, protozoan parasite, *Cytauxzoon felis*. Previously, this disease has been associated with extremely high fatality rates, however, more recent reports have been emerging of domestic cats surviving the infection with and without treatment. This variability in clinical outcomes leads us to question if distinct genotypes of this parasite can be linked to clinical outcome. In this pilot study, we focused on utilizing microsatellite loci (highly variable, short tandem DNA repeat regions) as a tool to assess genetic diversity in *C. felis* populations across the southeastern United States. Initially, we found 36,460 microsatellites within the *C. felis* genome. After sorting to identify those with the longest repeat regions, we created primer sets for 30 microsatellite loci spread out across unique *C. felis* contigs. We observed positive amplification of *C. felis* positive DNA with 29 of the 30 primer sets. Next, to validate polymorphic microsatellite loci, we utilized a standard genotype assay and tested our primer sets against 12 *C. felis* positive samples from diverse geographical locations in Georgia, Arkansas, Missouri, and Florida. We identified 16 of the 30 microsatellite loci as polymorphic, and further tested these against 24 *C. felis* positive samples evenly distributed across Georgia and Arkansas. By establishing these polymorphic microsatellite loci within the *C. felis* genome, we aim to explore the population genetics parameters of *C. felis*, establish distinct genotypes within the population, and assess if specific genotypes can be linked with clinical outcomes.

Research Grant: None

Student Support: UGA Swanson Family Feline Research Fund: Summer Research Stipend

Improvement of bioink accommodating stem cells for bio-fabrication of cartilage

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With the increasing demand for organs and tissues for transplantation, alternative methods to synthesize living tissues have been attempted. A promising solution is biofabrication, which combines 3D bioprinting and cell (stem cell) technology. Gelatin, chitosan, cellulose and alginate are commonly used for formulation of bioinks for bioprinting, but although printable, these materials do not emulate a native tissue environment. Further optimization is needed to improve cell survival, growth and differentiation. The extracellular matrix (ECM) is a natural component that provides an environment for cells in the native tissues, we hypothesize that adding dECM to the formulation of bioink can improve cell survival, proliferation and differentiation. The following two Aims are proposed: Aim 1 is to characterize decellularized ECM (dECM) derived from different tissues with different methods; Aim 2 is to improve formulation of alginate /cellulose-based bioink with dECM for cartilage 3D bioprinting. In Aim 1, dECM was obtained from skin and cartilage through two methods, one being chemical and the other being a physical-enzymatic method. The dECM obtained were then assessed for levels of decellulization and content of major dECM components such as collagen (COL) and glycosaminoglycans (GAG). The results indicated that the physical-enzymatic method is more effective at decellularization. Results for composition of the dECM are yet to be interpreted. In Aim 2, dECM was mixed with alginate /cellulose at different concentrations to formulate bioinks for printing cartilage constructs. We are currently testing survival, proliferation and chondrogenic differentiation of tissue-derived stem cells loaded onto the constructs.

Research Grant: National Institute of Health

Student Support: NIH T35, LSU SVM, and Louisiana Board of Regents

Pilot project to determine benefit of BVD testing along with Iowa Green tag preconditioning program

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Persistently infected calves are the primary reservoir of Bovine Viral Diarrhea Virus (BVDV) and are a source of horizontal and vertical transmission. It has been reported in previous studies that 17% to 50% of persistently infected calves reach breeding age, ultimately posing a threat to feedyards (Larson et al., 2004). In Iowa, there is a well-established preconditioning program called the Iowa Green tag program where calves are vaccinated, dewormed, and castrated. Currently, testing for BVDV is not included. In this study, the goal was to determine if BVDV testing, in addition to the preconditioning program, returned a sufficient premium to justify the expense of testing. Calves that were enrolled in the study underwent normal preconditioning processing, and an ear notch was obtained for BVDV testing. Data was collected from feeder cattle auctions in various locations across Iowa during September 2017, November 2017, and January 2018. The analysis in this paper used a partial budget to compare the expected returns of BVDV tested calves along with selling calves under the current requirements of the Iowa Green tag program. Additionally, a hedonic model was used to determine the value of feeder cattle characteristics and market-related factors including lot size, breed, sex, weight, and condition to see if there was a significant effect of BVDV testing on the price obtained for the calves. The results showed that as price declined at a nonlinear rate, weight increased. The advantage of BVDV tested Green tag calves was maximized in the middle of the weight range (300 to 900 pounds). Overall, the study demonstrated that, within certain parameters, it is advantageous for producers to test their calves for BVDV.

Research Grant: Research Grant: Iowa Veterinary Medical Association

Student Support: Student Support: Unknown

The effect of cannabidiol on seizure activity in uncontrolled canine epilepsy

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Epilepsy is one of the most common neurologic condition in dogs. Of those affected, 20-30% of dogs suffer from uncontrolled seizures while receiving standard therapy. There is a need for a medication that can control epileptic seizures with minimal adverse side effects. Cannabidiol (CBD) has been used medicinally in humans for years and, as a result, awareness of CBD as a possible treatment for epilepsy in pets has increased. However, the pharmacokinetics, side effects, and therapeutic potential of CBD in dogs have not been previously studied. The results of a pharmacokinetic study revealed that exposure in dogs is dose-proportional and that oral CBD-infused oil provides the best pharmacokinetic profile. An initial safety study demonstrated that CBD is well tolerated in dogs with the most significant side effect being diarrhea. A preliminary clinical trial was then performed to study the effect of oral CBD-infused oil on seizure activity in dogs with uncontrolled epilepsy. Results from this study indicate that there was a significant difference in seizure reduction in dogs taking CBD-infused oil compared to dogs taking placebo oil. Dogs in the treatment group also experienced an increase in alkaline phosphatase (ALP) levels while dogs in the placebo group did not experience a significant change in ALP levels. Minor fluctuations in phenobarbital and potassium bromide serum levels were noted in dogs in both groups, but there were no significant changes in either group. These results provide the first evidence that CBD could be used to minimize uncontrolled seizures in dogs. Further randomized, double-blinded studies must be performed to fully investigate the potential of CBD as a treatment for uncontrolled epilepsy.

Research Grant: AKC Canine Health Foundation Grant No. 02323

Student Support: AKC Canine Health Foundation Grant No. 02533

Transfection of bovine kidney cells with mRNA for the human cathelicidin, LL-37

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Bovine respiratory disease (BRD) is the most common cause of morbidity and mortality in feedlot cattle in the U.S. Methods to improve host immunity could help cattle resist BRD. This study investigated the feasibility of mRNA therapy to induce expression of an immunotherapeutic, the human cathelicidin LL-37, for eventual use to treat or prevent BRD. The objectives were: 1) to transfect Madin Darby bovine kidney (MDBK) cells with exogenous mRNA encoding the reporter protein luciferase; 2) to measure expression of LL-37 in MDBK cells transfected with LL-37 mRNA and 3) to determine whether supernatants or cell lysates from MDBK cells transfected with LL-37 mRNA for expressed antiviral effects against bovine herpesvirus-1 (BHV-1) or bovine respiratory syncytial virus (BRSV). MDBK cells were transfected with mRNA for luciferase in Viomer Red (VR) or Lipofectamine 3K (L3K) transfection reagent. Results from the bioluminescence assay confirmed luciferase expression. 46.62 relative light units (rlu) were measured for VR transfected cells vs 0.01 rlu in VR transfected control cells. MDBK cells were transfected with LL-37 mRNA using L3K or VR; expression was assessed using immunocytochemistry (ICC) but was not confirmed. Supernatants and cell lysates from MDBK cells transfected with LL-37 mRNA for 6 or 24 hours were assessed for antiviral effect against BHV-1 and BRSV using TCID50 assays to quantify virus in treated vs control cultures. Supernatants and lysates from LL-37 transfected cells did not have significant antiviral effects against BHV-1 or BRSV. Tests are ongoing to determine whether modified culture conditions lead to protein expression of LL-37 and induction of antiviral effect by transfected MDBK cells.

Research Grant: None

Student Support: NIH T35 Training Grant T35OD010432

Testing the detection of DNA barcoded microcrystals as a novel mosquito marking method

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Mosquitoes are important vectors of disease related pathogens. To inform the prevention and response of disease outbreaks involving transmission by mosquitoes, it is important to understand their dispersal behavior. One current method for understanding mosquito dispersal is mark-release-recapture (MRR). Mosquitoes trapped in the field are labeled with a fluorescent dye, released, and recaptured at varying distances from the release site. This current technique, however, often has low recapture rates, sometimes less than 1%. This necessitates the release of extraordinarily large numbers of marked mosquitoes to obtain robust and meaningful data. Therefore, the need for a more powerful method of measuring mosquito dispersal behavior is evident. We are working with a group of biochemical engineers to validate the use of cross-linked microcrystals packed with unique DNA barcodes. We hypothesize that these microcrystals can be used as marking technology for mosquitoes through voluntary ingestion or topical self-application at field bait stations. Ultimately, mosquitoes will be trapped and screened for DNA barcodes to determine at which station(s) crystals were acquired. The objective of this pilot study is to optimize detection methods for using microcrystals as genetic markers for mosquitoes. The specific aims of this project are: 1) modify mosquito DNA extraction protocols to maximize retrieval of DNA from within the microcrystals, and 2) optimize molecular detection of specific DNA barcodes from marked mosquitoes. Using this novel technology, information regarding disease transmission and its relation to the ecological behavior of mosquitoes will be expanded, furthering the potential for prevention and control measures.

Research Grant: This work was supported by the USDA National Institute of Food and Agriculture, Animal Health and Disease project 2017-36100-06008 and Colorado State University.

Student Support: USDA Animal Health & Disease Program NI17AHDRXXXXG018

Determining the effect of obesity on stem cell growth, proliferation, and the incidence of breast cancer

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Obesity is a growing epidemic with 39.8% of individuals in the United States currently affected. Obesity is associated with an increased risk for a number of diseases, including postmenopausal breast cancer. Obese women have increased severity and mortality of breast cancer post-diagnosis, but the link between the two is unclear. Breast cancer risk may be determined in part by the number of mammary epithelial stem/progenitor cells that serve as targets for transformation. Our lab has previously shown in both mice and humans that obesity directly correlates with increased epithelial stem/progenitor activity. We hypothesize that obesity increases epithelial stem/progenitor cells which will enhance mammary tumor formation. To test this hypothesis, we isolated mammary epithelial cells from *trp 53*^{-/-} mice and transplanted the cells into the mammary glands of 3-week-old wild type recipient mice. Mice were placed on either a High Fat Diet (HFD) or a Control Diet (CD) until 8 weeks of age or until tumor formation to evaluate changes in the mammary gland environment. After 8 weeks, we collected the mammary glands from HFD and CD mice and isolated the epithelial cells. Epithelial cells were grown in culture in colony-forming and mammosphere assays to quantify stem/progenitor activity. We observed that HFD mice had an increase in both size and number of mammospheres that formed compared to CD mice. HFD mice also had an increase in size and number of colonies growing in colony-forming assays. These results suggest that HFD mice have increased numbers of mammary stem cells present compared to CD mice. Future studies will examine how increased stem cells impact mammary tumor incidence.

Research Grant: This work was supported by Susan G. Komen CCR153332611 (LA) and NIH T32OD010423 (TC)

Student Support: This work was also supported by NIH T35OD01107 (HR)

Anti-ELTD1 monoclonal antibody therapy improves survival times and tumor volumes in mice with glioblastoma

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Oklahoma Medical Research Foundation (Zalles, Ziegler, Smith, Saunders and Towner)

High-grade gliomas, like glioblastoma, are the most common primary brain tumor in adult humans and are also a common primary brain tumor in dogs. Prognosis is poor, with a mean survival time of 15 months for humans, despite surgical resection of the tumor, radiation and treatment with the anticancer drug, temozolomide. Because glioblastoma is a very vascular tumor, treatments that target angiogenesis are a promising therapeutic pathway. The goal of this study was to evaluate the efficacy of monoclonal antibody (MAb) therapy against ELTD1 (epidermal growth factor, latrophilin, and 7 transmembrane domain-containing protein 1 on chromosome 1), a G-protein-coupled receptor and glioblastoma biomarker with upregulated expression in endothelial cells. We hypothesized that treatment with anti-ELTD1 MAb would disrupt angiogenesis and result in a significant increase in survival time and decrease in tumor volume for treated animals. For this project, G55 human glioblastoma cells were orthotopically implanted in the right cerebral hemisphere of nude, athymic mice, and tumor volumes were monitored with MRI. Treated mice lived an average of 6 days longer than untreated mice ($P < .05$) and average treated tumor volume was 31% of the average untreated tumor volume ($P < .05$). In conclusion, results suggest that anti-ELTD1 monoclonal antibody therapy has a significant anti-tumor effect and shows potential as a therapeutic agent in the treatment of glioblastoma for humans and canines.

Research Grant: Oklahoma Medical Research Foundation

Student Support: Oklahoma State University CVHS SRTP

Ticking all the boxes: bureaucracy, research, and reality in reports of tick-borne diseases

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To address the increasing public-health concern about tick-borne diseases it is necessary to know where people are exposed to ticks. This study addresses the efficiency of the disease-reporting process and comparisons between five states: Illinois, Iowa, Michigan, Minnesota, and Wisconsin. Data were collected from discussions with public-health officials and by assessing each state's reporting documents. Currently, each state uses a different methodology to report tick-borne diseases, with particular differences in how geographic exposure location and travel data are collected. One important variation between states was the number of follow-up reports to gather additional information about the illness from either the patient or healthcare provider. This value also varied by disease. Another discrepancy between states was who does the follow-up, varying from public health officials to a team of student employees. Additionally, some states follow up cases with the provider and others follow up with the patient. This process results in varied detail regarding information where the tick may have been acquired. Data for Wisconsin from 2007 to 2016 revealed that at least half of the reported cases of Lyme disease every year had no information about the location the tick was encountered. Overall, these disparate results reveal a need for improved surveillance methods of tick-borne diseases in the Midwest. Increased funding and manpower, a universal form for each disease, and an equal emphasis on all tick-borne diseases would improve information collected in the future and provide increased information about tick exposure. Understanding where people are exposed can allow for improved public health knowledge and control efforts.

Research Grant: Centers for Disease Control (Midwest Center for Vector-Borne Diseases)

Student Support: Office of the Director, NIH, T35 OD011145

Transmission reduction of Marek's disease by HVT vaccination

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Rationale: Marek's Disease is an ongoing problem for the poultry industry that has cost billions of dollars. It is a lymphoproliferative disease that can cause neurological issues, tumors, and death. To keep the poultry population healthy, it is imperative to research ways to reduce the spread and occurrence of Marek's, especially because, with the current vaccine protocol, the birds may appear healthy but shed the virus. The hypothesis of this research is that we can reduce and/or delay transmission of MD by HVT vaccination. Through this research we can generate information on how vaccines influence MDV spread.

Methods: To assess the changes in viral transmission we used the Donor-recipient model. Genetically susceptible White Leghorns were used as donors and recipients of MDV. Donors included groups of HVT vaccinated or unvaccinated birds. We took a portion of the donors and mixed them with recipients at Day 13 and Day 20 post challenge to determine initiation of virus transmission. Birds were necropsied at 8 weeks to determine MD lesions.

Results: We have proven that poultry with HVT vaccines display drastically less transmission at Day 13 and transmit more on Day 20, as opposed to the unvaccinated donors that will transmit highly from Day 13 on. There was also a lower percentage of MD in the recipient population with vaccinated donors.

Conclusions: Understanding how vaccination plays a role in the transmission of the disease will provide a better protocol to reduce Marek's disease in poultry for the industry. Now that we are aware that the HVT vaccination causes a delay in MD transmission as well as a reduction in severity, this will lead to new methods of stopping the transmission of the virus in the poultry industry.

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Student Support: NIH Grant T35OD016477 to MSU and Joan E and Richard L Witter Fund at Michigan State University

Creation of anatomical framework for gene expressions in brain areas implicated in language and communication

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Strong arguments exist that the domestication of the dog is centered around the development of human-dog communicative ability. Recent fMRI studies have found that the canine brain possesses dedicated voice areas for conspecific vocalizations, as well as regions which respond to human vocalizations, including vocal emotions. Similarity between canine and human brain locations of auditory regions that respond to vocalizations and emotional valences could imply involvement of homologous brain structures. Web-based brain databases such as the Brede Database and the Allen Brain Atlas allow free, online public access for regional behavioral analysis of human brain images and gene expression in the adult brain, respectively. Utilization of these databases for an investigation of communication-specific brain regions will serve to build an anatomical framework for further investigations into genes implicated in communication and language. Comparative investigations may be further applied to elucidate gene involvement in animal communication. Our ultimate goal is to develop an anatomically informed gene expression framework that is associated with communication and language to provide insight into the comparative genomics of canine communication and language phenotypes.

Research Grant: N/A

Student Support: Western University of Health Sciences Summer Research Fellowship Program

Dysregulation of the microRNAs let-7a and let-7b in canine urothelial carcinoma

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MicroRNAs (miRs) are molecular regulators of cell development and fate; their dysregulation can lead to carcinogenesis. Studies in human urothelial carcinomas (UC) have shown altered expression of miRs, which can be used as biomarkers for diagnostic testing, prognostic classification, disease monitoring, and as therapeutic targets. However, only a few studies in dogs are available. UC are the most common bladder tumors in dogs; deciphering the role of miRs in canine UC would provide new tools for biomarker discovery and insights into its cancer biology. The goals of this study were to confirm that amplifiable miRs can be isolated from formalin-fixed, paraffin-embedded (FFPE) bladder tissue and to compare the expression of miRs in non-cancerous and UC specimens. Tissue cores were acquired by selecting areas of interest on H&E-stained tissue sections and punching out corresponding areas in the FFPE tissue blocks using an Illinois biopsy needle. MiRs were isolated using miRNeasy FFPE kit (QIAGEN, CA). RNU6B, mir-21, 103, 155, let-7a and let-7b were quantified by RT-qPCR using the miScript PCR system (QIAGEN) on a QuantStudio3 thermocycler (ThermoFisher Scientific, MA). Relative expression was calculated by the $2^{-\Delta\Delta C_t}$ method with RNU6B as the normalizer, and compared by t-test. Let-7a is downregulated while let-7b is upregulated in canine UC tissue, as seen in human UC. The let-7 family is involved in cell cycle and tumor suppression. No expression changes were detected for miR-21, 103, and 155. This study shows that miRs can be isolated from FFPE bladder tissue allowing miR expression studies in archived samples. Moreover, two dysregulated miRs were identified in canine UC, which will be used for future biomarker research.

Research Grant: Boehringer Ingelheim and Purdue Veterinary Medicine

Student Support: Boehringer Ingelheim and Purdue Veterinary Medicine

Biosecurity: Effect of UV germicidal chambers

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Biosecurity is an important foundation for swine operations. By limiting the number of pathogens entering a production system, the animals remain healthier which maintains animal welfare, production standards, and decreases use of antibiotics. Ultraviolet light is known to inactivate pathogens by breaking nucleic acid bonds, so swine producers adopted this technology to decrease pathogen burden on fomites entering the farm. Currently, there are no on-farm standards available to test the effectiveness of UV boxes. Thus, our objective is to develop a protocol to test the inactivation potential of a UV box and provide producers a method to test their UV box. A field study was conducted by sending participants plates with a known concentration of *Escherichia coli* and the participants were asked to perform their current UV protocol. We evaluated a variety of conditions to validate our standards for this study. Aspects that were tested included inoculating plates with different growth stages of *E. coli*, effects of skim milk in freezing inoculum and treating plates, drying and shipping conditions of inoculated plates, variability in the level of UV exposure within the UV box, conditions of the UV emitters before treating fomites, as well as time and dose of UV radiation. Similar to other studies, we found that UV treatment of bacteria in exponential phase was more sensitive than stationary phase. Our results demonstrated that there is variation in UV dose depending on the location within the UV box during the same run and surfaces on multi-dimensional objects are not equally treated. In summary, we developed a testing method using standardized plates to assess the effectiveness of UV boxes used in swine production systems.

Research Grant: University of Minnesota Swine Disease Eradication Center

Student Support: Boehringer Ingelheim Veterinary Scholars Grant

Investigation of bubr1 expression in zebrafish cancers

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The tumor suppressor genes TP53 and BRCA2 are essential for maintenance of genomic integrity, and mutations in these genes result in genomic instability. One consequence of genomic instability is aneuploidy (aberrant number of chromosomes). Genomic stability is dependent in part on the spindle assembly checkpoint (SAC), which ensures accurate chromosomal segregation and is mediated by a complex of proteins that includes the kinase BUBR1. Interestingly, mutations in TP53 and BRCA2 each influence the level of BUBR1 expression in cancers from humans and mice: while mutant TP53 is associated with increased levels of BUBR1, mutant BRCA2 is associated with decreased levels of BUBR1. Levels of BUBR1 expression in cancers have also been linked to diploid versus aneuploid status. To further explore the relationship between BRCA2 and TP53 mutations, BUBR1 expression, and ploidy in cancers, we are characterizing bubr1 expression in zebrafish cancers via immunohistochemistry, Western Blot, and RT-PCR. We will examine cancers from tp53-mutant zebrafish (tp53 m/m) with or without concurrent brca2 mutation (brca2 m/m;tp53 m/m) and compare these findings to ploidy status of cancers. It is expected that cancers will exhibit variable levels of bubr1 expressions that are correlated to both genotype (tp53 m/m or brca2 m/m;tp53 m/m) and ploidy outcome (diploid or aneuploid). Overall this study will provide further insight on the interplay of conserved molecular contributors to carcinogenesis.

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Student Support: NIH - T-35 Interdisciplinary Biomedical Research Training Program (IBRTP)

Mump virus nucleoprotein domain “top” affects defective interfering particle production

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Mumps virus (MuV) is a negative-sense single-stranded RNA virus belonging to the family Paramyxoviridae. A human pathogen, MuV is responsible for acute infection of the parotid glands, and can cause severe cases of encephalitis, meningitis, and deafness. The nonsegmented RNA genome of MuV is encapsidated by the nucleocapsid protein (NP), which forms the ribonucleoprotein (RNP) complex - which serves as a template for RNA synthesis. To make RNA accessible to the viral polymerase, a conformational change within NP must occur. Crystal structure analysis of the NP of parainfluenza virus 5 (PIV5), a paramyxovirus closely related to MuV, indicates that an α -helix close to the RNA genome becomes flexible when RNA is removed. This region of the NP is likely responsible for the conformation change which allows the polymerase to access RNA for transcription and replication. To examine the functionality of MuV's NP, point mutations were made in MuV NP protein corresponding to PIV5 at sites G185P, A197Q, Q200R, and groups denoted as Top (N63G, P139D, A197Q), Tip (P109E, N121G, A124R), and Bottom (G21S, S29T, P43N, R93Q, R304Q). The “Top” MuV mutant exhibited normal growth kinetics at low multiplicity of infections (MOIs); however, at high MOI's the virus could not efficiently replicate. Further analysis indicates that production of defective interfering (DI) particles was enhanced in the mutant virus. Understanding the production of DI particles, which can lead to increased interferon production, will invariably lead to a better understanding of MuV pathogenesis as well as its replication/transcription process.

Research Grant: NIH R01 AI106307

Student Support: None

Tumor suppressor gene copy number loss in horse lymphomas

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During every cell division there is a risk of DNA mutation and thus an accumulated risk of cancer development over the lifetime of an animal. Thus, very large, long-lived animals should be at higher risk of developing cancer, but this is not always the case. Elephants and whales are examples of very large, long-lived animals that have a much lower cancer incidence than humans; a phenomenon referred to as Peto's Paradox. A potential explanation for this phenomenon is an increase in the number of copies of tumor suppressor genes in these animals, and this has recently been documented in elephants which have multiple copies of TP53. We are interested in finding out if less extreme examples of Peto's paradox, such as horses, also have increased copies of tumor suppressor genes and whether copies of these genes are lost during tumorigenesis. Horses are thought to carry 2 copies of the MAL2 tumor suppressor gene, and are large and relatively long-lived. MALL (Mal like) is a gene related in function to MAL2. We examined pathology records from 1000 horses with tumors across various breeds, and chose lymphoma as it was present at all age ranges and in most breeds. We isolated DNA from formalin fixed paraffin embedded cores of matched tumour and normal tissue from each case that met our inclusion criteria: sufficient tissue in the block, no contaminating neoplastic cells in the normal, and < 10% normal cells in neoplastic samples. Forty horses met our inclusion criteria, and copy numbers of MAL2 and MALL are being examined using digital droplet PCR (ddPCR). This work will reveal the range of copy numbers of these 2 genes in normal horse tissue and whether copies are lost during development of lymphoma.

Research Grant: NONE

Student Support: Andrea Leger Dunbar Summer Research Assistantship

***Sos1* as a novel oncogene in canine leukemia**

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B-cell acute lymphoblastic leukemia (B-ALL) is the most common form of childhood cancer. It is characterized as being a rapidly progressing cancer of the blood and bone marrow, caused by the dysregulation and expansion of immature B-cells. In murine B-ALL disease models, increased STAT5 activation is an important driver for oncogenesis. Previous research used Sleeping Beauty (SB) transposon insertional mutagenesis to identify genetic modifications that cooperate with STAT5 activation to induce leukemia. The most commonly targeted gene was *Sos1*. SB insertions generated a truncated SOS1 protein due to an intrachromosomal microinversion, which lacked the amino terminal portion of the protein. Similar alterations in *Sos1* transcripts were observed in spontaneous mouse leukemias. The presence of this mutated *Sos1* correlated with poorer outcome in mouse progenitor B-ALL. We continued the characterization of this novel *Sos1* transcript with new mouse samples using 5'- and 3'-rapid amplification of cDNA ends (RACE). Subsequently, we cloned both the 5'- and 3'- truncated transcript into a stable vector and have isolated the full-length transcript. We identified the same RIKEN-*Sos1* fusion found in previous studies. Further examination of human ALL samples suggests this RIKEN-*Sos1* fusion may also occur in human ALL. Malignant lymphoproliferative diseases also account for approximately 40% of canine neoplasia, leading us to propose that a similar phenomenon may occur in the dog. We looked at the expression levels of *Sos1* in both canine leukemia and osteosarcoma samples using real-time polymerase chain reaction (RT-PCR) and 5'-/3'-RACE. RT-PCR data suggests that the truncated *Sos1* transcript is not present in any samples but that there is an overexpression of the endogenous *Sos1* in osteosarcoma and some leukemias when compared to the control samples. This could lead to a better understanding of the role of *Sos1* in mice, humans, and dogs.

Research Grant: Unknown

Student Support: The Office of Graduate Programs, College of Veterinary Medicine, University of Minnesota

Investigating miRNA-29a as a biomarker for increased intestinal permeability in dogs

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MicroRNAs (miRNAs) are short, 18-22 base pair, non-coding RNAs that can inhibit messenger RNA (mRNA) translation. Previous studies in humans and pigs have demonstrated an association between increased miRNA-29a expression and increased intestinal permeability. The aim of this study was to determine the association between miRNA-29a expression and intestinal disease in dogs with low folate and cobalamin serum concentrations. A low folate and cobalamin concentration suggests small intestinal disease in dogs. The expression of miRNA-29a in serum samples from dogs with normal folate and cobalamin concentrations (n=23) was compared to miRNA-29a expression in serum samples from dogs with low folate and low cobalamin concentrations (n=17). Total RNA from 200 µl of serum was isolated using a Qiazol-based protocol, and the RNA was then reverse transcribed into cDNA. The expression of miRNA-29a in serum was measured using real-time quantitative polymerase chain reaction (qRT-PCR) in all of the 40 samples. The Mann-Whitney test was used for statistical analysis. Compared to the normal folate and cobalamin dog group, there was no significant difference in miRNA-29a expression in dogs with low folate and cobalamin concentrations ($P > 0.999$). The results show that although miRNA-29a has shown increased expression in humans and pigs with increased intestinal permeability in previous studies, there was no difference between this gene expression in dogs with low cobalamin and folate serum concentrations. Other miRNAs should be investigated for their potential to act as biomarkers for increased intestinal permeability in dogs. A miRNA of interest is miRNA 16, due to its increased expression in humans with intestinal disease.

Research Grant: None

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Mechanism and cross-resistance patterns of methicillin resistance in *Staphylococcus* in companion animals

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Methicillin-resistant *Staphylococcus* (MRS) are leading causes of skin, ear, and wound infections in companion animals with zoonotic potential. Methicillin resistance in *Staphylococcus* is typically mediated by the *mecA* gene that encodes PBP2a, a protein with low affinity for most β -lactams. MRS often have concurrent resistance to other antibiotics, resulting in limited treatment options. The goal of this study was to determine prevalence, species distribution, and mechanism of resistance of MRS from clinical cases of companion animals submitted to the Veterinary Diagnostic Laboratory at ISU from 2012-18. The phenotypic methicillin resistance rate of *Staphylococcus* was 26.9% (790/2933) overall, 22.1% (432/1955) in *S. pseudintermedius*, 40.6% (307/757) in coagulase negative *Staphylococcus* (CoNS), and 23.1% (51/221) in coagulase positive *Staphylococcus* (CoPS). Cross resistance to other β -lactams was common among MRS isolates, especially when oxacillin MIC was $> 4 \mu\text{g/mL}$. PBP2a was detected by agglutination in MRS at 81.0% (606/748) overall, 93.4% (384/411) in *S. pseudintermedius*, 61.3% (176/287) in CoNS, and 92.0% (46/50) in CoPS. PCR testing of oxacillin resistant and PBP2a negative isolates (n=48) found *mecA* in 25 isolates, but no other *mec* genes including *mecC*, *mecB*, or *mecD* were detected. A subset of MRS isolates (n=186) were tested for susceptibility to antibiotics that included last-line drugs with high importance in humans. Non-susceptibility was found in one isolate to tigecycline and four isolates to daptomycin. These results indicate a high prevalence of MRS in companion animals that may impair treatment of clinical cases, and warrants routine susceptibility testing of *Staphylococcus* isolates from clinical infections.

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Towards safer and more effective NSAID dosage regimens for birds

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When birds are treated in a veterinary hospital, zoological park, or wildlife rehabilitation center for orthopedic, internal, or traumatic injuries, veterinarians often turn to an NSAID. There is wide variation in the pharmacokinetics and dosage regimens between different bird species (Goessens et al, 2016). This may be explained by the species differences in metabolizing enzymes. In the human liver, metabolism of meloxicam involves cytochrome P450 2C9 and 3A4 (Chesne et al, 1998). In birds, selective constraint variations in CYP2 subfamilies have been linked to different feeding habits, habitats, and migratory behaviors (Almeida et al, 2016). The working hypothesis for this project is that meloxicam dosage regimens are the same within, but differ between, groups of avian species with similar feeding habits, habitats, or migratory behaviors. Historical data was compiled from the international zoological information management system (ZIMS) across ten major orders of birds and examined at different taxonomic levels within those orders. Representation of data in box and whisker plots allowed for identification of species for which the median dose was higher or lower than the median overall for the order. Species of importance were chosen for their prevalence in zoological collections, wildlife rehabilitation centers, the pet trade, and production environments. Significant patterns that would implicate statistical testing could not be identified due to high variability in the data. As a next step, the data will be reclassified by demographic factors for analysis (diet, habit, and migratory behavior).

Research Grant: None

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Evaluation of the feline ocular microbiome before and after treatment with topical antibiotic therapy

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The ocular surface microbiome of veterinary species has not been thoroughly characterized using molecular-based techniques, as only culture-based studies have been reported. Furthermore, alterations in the ocular microbiome after topical antibiotic treatment are unknown. The objective of this study was to describe the ocular microbiota of healthy cats and determine whether antibiotic therapy results in subsequent changes over time. Twelve adult female spayed domestic short-haired cats from a research colony served as subjects. Erythromycin ointment (0.5%) was applied to the ocular surface of one randomly assigned eye per cat three times daily for 7 days, while the fellow eye served as an untreated control. The ocular surface was sampled for bacteria by swabbing the ventral conjunctival fornix of both eyes at the following time points: days 0 (baseline), 7 and 30. DNA was extracted from the swabs (MoBio Powersoil Kit, Carlsbad, CA) and the 16s rRNA gene was sequenced using an Illumina platform. Data were analyzed using QIIMEv2.0 and multivariate analysis of bacterial taxa was performed using a Friedman's and Dunn's post-test. At baseline, the major bacterial phyla included Proteobacter, Firmicutes, Bacteroidetes and Actinobacteria with no significant difference between eyes. Over time, control eyes had significantly more Proteobacter ($P=0.0087$, $q=0.0006$) at day 30 compared to baseline. Treated eyes had decreased Actinobacteria ($P=0.0489$, $q=0.0366$), Firmicutes ($P=0.0183$, $q=0.0366$) and increased Proteobacter ($P=0.0006$, $q=0.0366$) on day 30 compared to baseline. In conclusion, the major bacterial phyla on the feline ocular surface changes over time and with erythromycin treatment.

Research Grant: GINN Research Fund

Student Support: Boehringer Ingelheim VSP, Texas A&M College of Veterinary Medicine & Biomedical Sciences

Evaluation of cytokine production by human natural killer cells expressing engineered Fc receptors

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There has been great progress in developing cancer immunotherapies, but resistance in patients and the non-responsiveness of some malignancies still present challenges. This is the case for ovarian cancer in which antibody-based and cell-based immunotherapies have not been successful for this solid tumor. Natural Killer (NK) cells, innate lymphoid cells, are efficient at recognizing antibodies attached to tumor cells and killing these cells by a process known as Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC). Human NK cells exclusively recognize tumor-bound antibodies by their receptor FcγRIIIA (CD16A). CD16A, however, is a low affinity receptor, which may limit the anti-tumor activity of NK cells. The FcγR CD64 binds to antibodies with much higher affinity than CD16A, but CD64 is not expressed by NK cells. We generated a novel chimeric FcγR composed of the extracellular region of CD64 and the intracellular region of CD16A, referred to here as CD64/16A. We hypothesized that NK cells expressing CD64/16A would produce higher levels of cytokines than NK cells expressing CD16A upon engaging antibody-bound ovarian cancer cells. One of the most important anti-tumor cytokines produced by NK cells is interferon gamma (IFNγ). Cytokine production was evaluated following ADCC and collection of NK cell supernatants. Extracellular cytokines were quantified by the use of a Cytometric Bead Array ELISA and flow cytometry. These data are the focus of my poster presentation. Future implications of my studies are the use of NK cells expressing CD64/16A as a based immunotherapy for ovarian cancer and other malignancies in combination with therapeutic antibodies that induce ADCC.

Research Grant: NIH T35 Summer Scholars

Student Support: National Institutes of Health T35 Training Grant

Prospective evaluation of orthoses in canine patients

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As more canine owners express interest in non-surgical treatments of orthopedic injuries, the use of orthoses (braces) has expanded in veterinary medicine. Currently available research is limited to retrospective studies and surveys but appears to indicate that the application of orthoses may be beneficial for canine cruciate disease, Achilles tendinopathy and collateral ligament injuries. All available studies relied solely on subjective outcome measures. Additionally, few studies recorded data on complications, such as skin sores that dogs may develop from wearing an orthosis. The goal of this study is to determine the outcomes associated with the application of an orthosis in dogs. We plan to enroll consecutive cases presented to the CSU-VTH in a prospective study using objective outcome measures to determine the outcome and complications associated with this technology. We propose to track patients for a 12-month time period, however all patients with a 3-month follow-up will be included in the data analysis. We will analyze the number of skin complications, how often the devices are used and utilize objective gait analysis to determine change in lameness severity. We hypothesize that gait analysis data (Vertical Impulse) will show a significant improvement at the 3-month recheck in comparison with baseline data. This information will help veterinarians with the necessary information when prescribing orthoses for patients with orthopedic injuries.

Research Grant: Young Investigator Grant

Student Support: None

Evaluation of the role of IFN- γ , IL-10 and TNF- α in treatment of heartworm-infected dogs

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Dirofilaria immitis is a parasitic roundworm that leads to severe heartworm disease, vasculitis, lung disease and heart failure. While infection leads to an immune response, we are uncertain as to the dynamics of this response during treatment. This study evaluates the levels of three different cytokines, TNF- α , IFN- γ and IL-10, in serum samples of heartworm-positive dogs throughout the American Heartworm Society treatment. Serum samples from 43 dogs of various breeds were taken at 6 different time points with 3 study groups: 11 positive control dogs (heartworm-positive not undergoing treatment), 3 negative control dogs (non-infected), and 29 treatment dogs (heartworm-positive and undergoing treatment). Baseline serum samples were taken prior to initiation of treatment, with serum samples taken throughout treatment at the following time points: post-tetracycline administration, first melarsomine injection, second melarsomine injection, one-month post-third melarsomine injection, and when no antigen was detected in the individual dog. For control dogs, serum samples were taken at the same time intervals, but no treatment was administered. The samples were prepared using a commercially available canine-specific multiplex immunoassay (Milliplex CCYTOMAG-90k) according to the manufacturer's protocol and analyzed on a Luminex MAGPIX with xPONENT software. Preliminary results demonstrate a potential decrease in IFN- γ from baseline to post-tetracycline treatment with dog specific variability. TNF- α and IL-10 levels are low throughout the treatment timeline. These results may indicate that these cytokines do not participate in any immune response mounted during heartworm treatment.

Research Grant: None

Student Support: Unknown

An intranasal vaccination strategy to improve protection against respiratory pathogens

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Intranasal vaccination is an alternative route of vaccine administration that is needle-free and, in addition to inducing systemic immunity in the bloodstream, has the potential of promoting immunity at the mucosal surfaces of the airways. However, most licensed vaccines against respiratory pathogens are injected vaccines that often require multiple doses in order to achieve protective immunity. However, in times of a crisis, it may be necessary to have readily available and easy administer vaccine that can provide expedited protection. Previous studies in our lab have shown that recruitment of neutrophils in sublingual tissues and cervical lymph nodes of mice immunized by the sublingual route blunts the subsequent immune responses and prevents the development of mucosal IgA responses. We also found that supplementation of a sublingual vaccine with a neutrophil elastase inhibitor helps the development of mucosal IgA, but also regulates the kinetic of antibody responses. In this study, we tested whether pharmacological inhibitors of neutrophil elastase can regulate the immune responses to an intranasal vaccine. Our preliminary results show that a single intranasal vaccination of C57BL/6J mice in the presence of a neutrophil elastase inhibitor results in high titers of antigen specific systemic IgG1. The complete profile of B and T cell responses induced by intranasal immunization of wild-type C57BL/6J mice in the presence of a neutrophil elastase inhibitor is being characterized. This work is expected to demonstrate that the efficacy of current experimental intranasal vaccines can be regulated to quickly provide protection against infection with a respiratory pathogen such as anthrax.

Research Grant: NIH R01 DK101323

Student Support: NIH T35 Training Grant T35OD010977

Safe method for digital enlargement of the epiploic foramen

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A specific type of equine colic, epiploic foramen entrapment, is the focus of this study. A portion of small intestine that has migrated through the epiploic foramen can become strangulated and the tissue can undergo necrosis. Surgical correction of an epiploic foramen entrapment has a risk of intraoperative or postoperative hemorrhage from the portal vein, which can lead to sudden death or necessary euthanasia. This is primarily due to the portal vein's close proximity to the site of correction while dilating the epiploic foramen to relieve intestinal entrapment. This research, as part of a larger study, is investigating the effect of manual dilation of the epiploic foramen by simulating mock correction of epiploic foramen entrapment. Eight horses, free of gastrointestinal disease, have been donated for research purposes. They are placed under anesthesia for the surgical procedure and euthanized at the end of the procedure. The epiploic foramen is digitally enlarged to twice its size while keeping the portal vein intact. The equine subjects are euthanized while under general anesthesia. The portal vein, as well as other venous structures, and the measurement of the enlarged epiploic foramen are evaluated during a subsequent necropsy. The overall goal of this research is to obtain objective data to determine if manual dilation of the epiploic foramen results in compromise of the surrounding venous structures. Data for this study have not yet been collected at the time of this abstract submission.

Research Grant: Island Whirl Equine Colic Research Lab

Student Support: None

The role of the dorsal hippocampus in early stage memory reconsolidation

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Cocaine addiction is linked with a high risk of relapse. Re-exposure to a drug-associated context can trigger craving and relapse upon the recall of cocaine-context memories. Upon recall, such memories destabilize and must undergo protein synthesis-dependent reconsolidation to be maintained. We have hypothesized that the dorsal hippocampus (DH) stores such labile cocaine memories. Here, we examined whether interference with DH functional integrity during the first hour after recall (at peak memory instability) can disrupt memory reconsolidation and subsequent context-induced cocaine-seeking behavior. Male rats (n=11) received infusions of a viral vector expressing halorhodopsin (inhibitory opsin) or yellow fluorescent protein (control construct) and all got optic fibers aimed at the DH. They were assigned to self-administer cocaine (0.15 mg/ml, IV) in one context (context A, > 10 2-hour sessions) and to receive extinction training in another context (context B, 7 sessions). Next, the rats were re-exposed to context A for 15 min (to trigger memory reactivation) or remained in their home cages (no reactivation control, ongoing). Immediately after the session, the rats received laser stimulation of the DH (i.e., optogenetic inhibition) or no stimulation for one hour. Effects on cocaine-memory strength were assessed based on cocaine-seeking behavior in context A. Optogenetic DH inhibition reduced context-induced cocaine-seeking behavior relative to no inhibition. We predict that this effect depends on memory reactivation. These results will suggest that neural activity in the DH during early-stage memory reconsolidation is critical for memory maintenance and continued contextual stimulus control over cocaine-seeking behavior.

Research Grant: NIH R01 DA025646

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Adipose-derived mesenchymal stem cells as a potential therapy for equine recurrent uveitis

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Equine recurrent uveitis (ERU) is an immune-mediated disease causing repeated inflammatory flares which can lead to blindness. Currently, there is no cure for horses with this disease. Mesenchymal stem cells (MSCs) are effective at reducing immune cell activation *in vitro* in many species, making them a good therapeutic option for ERU. The objectives of this study are to define the lymphocyte phenotype of horses with ERU and to determine how MSCs alter T cell phenotype *in vitro*. We hypothesize that horses with ERU will have a pro-inflammatory phenotype and that MSCs will reduce this phenotype *in vitro*. Peripheral blood mononuclear cells were isolated from equine whole blood, and the markers CD21, CD3, CD4, and CD8 were used to identify lymphocyte subsets while CD25, CD62L, Foxp3, IFN γ , and IL10 were used to identify T cell phenotype. Adipose-derived MSCs were incubated with CD4 T cells for four days, after which lymphocytes were collected and analyzed via flow cytometry. The percentages of T cells and B cells in horses with ERU were similar to those from normal horses. However, CD4 T cells from horses with ERU expressed higher amounts of IFN γ and CD62L and lower amounts of IL10, indicating a pro-inflammatory phenotype. When co-incubated with MSCs, activated CD4 T cells reduced expression of CD25, CD62L, Foxp3, and IFN γ . MSCs had a lesser ability to decrease activation when cell-cell contact or prostaglandin signaling was blocked. MSCs continue to show promise as a treatment for ERU as they decreased the CD4 T cell activation phenotype (reducing CD25, CD62L, and Foxp3) as well as the pro-inflammatory marker IFN γ . MSCs are likely exerting these effects through a combination of cell-cell contact and prostaglandin signaling.

Research Grant: Center for Equine Health, UC Davis

Student Support: NIH T35 Training Grant

The influence of engineered nanomaterials on corneal epithelial and fibroblast cell viability and migration

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As engineered nanomaterials (ENMs) become increasingly prevalent in consumer products, human exposure is dramatically increasing. A major interaction site of ENMs is the eye, particularly the cornea. The deposition and retention of ENMs within the eye may induce adverse molecular and pathophysiological changes. Relevant exposure scenarios of diverse ENMs with different physicochemical properties on immortalized human corneal epithelial (hTCEpi) and rabbit corneal fibroblast (RCF) cells were created to determine the concentration-dependent effects of ENMs on cell viability and proliferation *in vitro*. Preliminary studies have demonstrated metallic ENMs, including copper oxide and zinc oxide decrease cell viability and/or migration of corneal fibroblasts and epithelial cells *in vitro*. Thus, we hypothesize that carbon-based ENMs will similarly impair hTCEpi and RCF cell viability and migration *in vitro*. Currently, treatment of carbon-based ENMs including graphene oxide (110nm x 110nm or 250nm x 250nm) and psyllium husk are being investigated for cell viability and cell migration. Cytotoxicity assays (MTT and Calcein AM) were used to evaluate cell viability. A cell exclusion zone assay was used to model cell migration for the hTCEpi cells. Preliminary results demonstrate cell exposure to graphene oxide (250nm x 250nm) treatment for 24 hours decreased cell viability at concentrations ≥ 25 μ g/mL for hTCEpi cells (n=1) and RCF cells (n=3) compared to controls. These findings support the hypothesis that carbon-based ENMs decrease cell viability in hTCEpi and RCF cells. Results from the *in vitro* screening of ENMs will be used to determine which ENMs have the most potential to induce corneal toxicity prior to pursuing *in vivo* experiments.

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Student Support: National Institute of Health

Epidemiology of mastitis pathogens on Amish dairy farms in Missouri

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While the epidemiology of mastitis pathogens is reasonably well understood on machine-milked dairies, our understanding of the major causes of mastitis on Amish dairy farms that milk by hand is lacking. Currently some Amish communities struggle to meet Federal milk quality standards, i.e. somatic cell count (SCC; $\leq 750,000$ cells/ml) and bacteria count ($\leq 400,000$ CFU/ml), and thus have difficulty marketing their milk. The purpose of this study was to investigate the epidemiology of mastitis pathogens on Amish dairies in MO. Mammary quarter milk samples were collected from all lactating cows on 6 farms (3-16 cows/farm) for culture and SCC. All bacterial isolates were identified using MALDI-TOF mass spectrometry and strain-typed, when applicable, by pulsed-field gel electrophoresis. A total of 212 milk samples were collected from 56 cows; 12 quarters on 11 cows were blind. Among these samples, culture yielded 61 (28.7%) no growth (NG), 99 (46.7%) *S. aureus* (SA), 37 (17.5%) non-aureus staphylococci (NAS), 5 (2.4%) misc. other bacteria, and 10 (4.7%) contaminated samples. Among quarters diagnosed with an intramammary infection (excluding mixed and contaminated samples), median (range) SCC $\times 10^3$ cells/ml was 13 (0-650), 221 (0-1715) and 1493 (10-7352) for NG, NAS, and SA, respectively ($P < 0.001$). Given that *S. aureus* was the most prevalent and inflammatory pathogen, all SA isolates were strain-typed. To date, 83 isolates have been strain-typed, and 38 (2-14/farm) strains have been identified; 20 of the 38 strains (53%) have been found in only one mammary quarter. The large number of unique strains suggests that SA infections originate from some environmental reservoir rather than being passed between cows while milking.

Research Grant: None

Student Support: Boehringer Ingelheim

Effect of Enrofloxacin treatment on the prevalence of fluoroquinolone resistant *Campylobacter* in cattle

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Cattle are significant reservoirs for *Campylobacter*, a major foodborne pathogen. Recent studies have shown a rise in fluoroquinolone-resistant (FQ-R) *Campylobacter* in cattle, but it is unclear if this is directly related to FQ use in cattle. The aim of this study is to assess the effect of Enrofloxacin treatment on FQ resistance in cattle. Calves derived from commercial farms were inoculated with FQ-susceptible (FQ-S) strains of *C. jejuni*, followed by treatment with a single dose of Enrofloxacin (12.5 mg/kg by injection). Fecal samples were collected during the course of study for isolation and identification of *Campylobacter*. Interestingly, the calves were naturally infected with FQ-R *C. jejuni* prior to inoculation with the laboratory strains. After the inoculation, FQ-R *Campylobacter* decreased, which was accompanied by increase of FQ-S *Campylobacter*. However, after Enrofloxacin treatment was given, the calves were recolonized by FQ-R *Campylobacter*. Pulsed field gel electrophoresis and multilocus sequence typing revealed genetic diversity of the isolates, but certain genotypes dominated during different stages of the study. Prior to inoculation, the calves were predominantly colonized by FQ-R cluster A (ST982). After inoculation, the predominant genotypes changed to FQ-S clusters C (ST929) and D (ST61). Following the treatment with Enrofloxacin, the primary genotypes shifted to FQ-R clusters A (ST982) and B (ST922). These results indicate that commercial cattle harbor genetically diverse FQ-R *Campylobacter* and a single treatment with Enrofloxacin enriched pre-existing FQ-R populations, but had little effect on de novo selection of FQ-R *Campylobacter* from the inoculated strains.

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Evidence for use of end-expiratory CT in dogs with chronic coughing

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Bronchomalacia (BM) is the weakness and collapse of airways which are supported by cartilage; this condition may be accompanied by tracheal collapse and has been recently described as a single entity requiring bronchoscopy for diagnosis. Chronic cough is the most common complaint for dogs with airway collapse. Many dogs having BM or sublobar bronchial collapse without tracheal collapse are medium to large breeds, which is contradictory to the small-breed signalment usually associated with airway collapse. Because of this and the inspiratory protocols used by most for thoracic computed tomography to evaluate dogs with chronic cough, the sensitivity for detection of BM is likely reduced, especially when present as a single entity, potentially creating a systematic under-diagnosis of bronchial and smaller airway narrowing. Increasingly, combined CT protocols have been used to diagnose presence of excessive airway narrowing in people with good correlation to bronchoscopic results. Our aim is to determine whether the addition of an expiratory CT scan in chronic coughing dogs will detect the presence of BM and whether bronchial narrowing detected on CT will correlate with bronchoscopic findings. Thirteen, client-owned, adult dogs having clinical signs related to chronic airway disease and nine clinically healthy dogs were evaluated using inspiratory and expiratory CT protocols. The airways of coughing dogs were also evaluated via bronchoscopy. We expect that good correlation will be documented between the reductions in luminal airway diameter detected on CT and during bronchoscopic evaluation, and that expiratory CT can be a useful additional protocol in addition to an inspiratory scan to detect those cases having BM.

Research Grant: American College of Veterinary Radiology

Student Support: Louisiana State University, School of Veterinary Medicine, Radiology Service

Comparison of survival among different categories of chronic kidney disease in dogs

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Chronic kidney disease (CKD) is a significant cause of morbidity and mortality in dogs. CKD results from a variety of different underlying disease processes. Previous studies have investigated prognosis in dogs with CKD, however these studies have been limited in that analysis did not include disease category based on a renal biopsy. The objective of this study was to determine if disease category confirmed by renal biopsy was associated with survival time of dogs with CKD. Retrospective evaluation of the International Veterinary Renal Pathology Service (IVRPS) database was performed. This database contains the results of a comprehensive renal biopsy evaluation. Follow-up information, including current clinical status, was obtained for dogs evaluated between 2008-2018. Survival data was obtained for 23% of animals (275/1,191). From this group, nineteen different diagnostic categories were identified. The five most represented diseases in the cases for which follow-up was obtained were: focal segmental glomerulosclerosis, amyloidosis, membranoproliferative glomerulonephritis, membranous glomerulonephropathy, and juvenile nephropathies. On initial univariate evaluation, a diagnosis of renal amyloidosis was associated with a poorer prognosis. Additionally, at the time of biopsy, serum creatinine, serum albumin, and age were associated with prognosis. Multivariate analysis of the data is ongoing. Results from this study provide prognostic information for clinicians and owners that can lead to more informed decisions on therapeutic options, thus improving quality of life.

Research Grant: None

Student Support: Morris Animal Foundation, Texas A&M College of Veterinary Medicine & Biomedical Sciences

Internal parasite loads of wild black bears (*Ursus americanus*) via fecal testing

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Black bears (*Ursus americanus*) are recolonizing their historic range in eastern Oklahoma. As this happens, they also spread their parasites, some of which could be impactful on both human and domestic animal populations. Last year, an outbreak of demodectic mange occurred in the northern population and is now being seen in the southern population. This could indicate significant physiologic stresses on the bears. Surveys of wild black bears for parasites are mostly outdated, and need to be revisited to keep up with changes in both the bear and parasite ecology. The goal of our study was to take fecal samples from bears obtained as part of a larger, ongoing study and evaluate their gastrointestinal parasite loads via fecal testing to try to establish a baseline infection summary and determine if infection could be high enough to be part of the significant stressors on the bears. That testing was comprised of a Wisconsin sugar float, a centrifugal sedimentation, and the Baermann technique. These three tests were picked to increase chances of finding a variety of parasites including nematodes, cestodes, trematodes, and protozoa. Preliminary results have identified a capallariid species and strongyle-type species in over half the bears surveyed, with no co-infections. Overall, the infections look to be mild with low numbers of eggs shedding in the feces.

Research Grant: AG-18-RS-176 Spatial ecology and expansion of recolonizing black bear populations in eastern Oklahoma

Student Support: Oklahoma State University CVHS Summer Research Training Program

Effects of liver cell surface glycoconjugates on invasion by *Plasmodium berghei*

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The initial interaction between malaria sporozoites and host liver cell has become the focus for a novel malaria vaccination. The current recombinant vaccine (RTS,S/AS01) targets the point of hepatocyte invasion, but fails to block parasite transmission reliably. Parasite invasion is still poorly understood. Interaction between the sporozoite circumsporozoite protein and host hepatocyte heparin sulfate proteoglycans (HSPGs) is believed to facilitate invasion; however, other currently unknown redundant pathways involving glycoconjugates targeted by sporozoites for invasion are suspected. To investigate these pathways, HepG2 human hepatic carcinoma cells were grown in the presence of swainsonine, a Golgi α -mannosidase inhibitor, or *HS2ST1* siRNA, a silencer of the heparin sulfate 2-O-sulfotransferase 1 gene. These were chosen to evaluate how glycoconjugates interact with sporozoites. Determining a dose that would yield reduced HSPG / N-glycan levels while maintaining high cell viability was a critical first step. Cells were dosed with a range of 0.02 $\mu\text{g/mL}$ - 10 $\mu\text{g/mL}$ of swainsonine to determine changes in cellular morphology and cell viability. No changes were detected with microscopy at these concentrations. Cells were also incubated with 100 $\mu\text{g/mL}$ swainsonine or the siRNA and stained with fluorescent lectins to evaluate changes in cell surface glycoconjugates. Real time qPCR was used with a suite of genes whose products are known to be involved in sporozoite invasion to observe the global effects of silencing *HS2ST1*. While this project is still in its beginning stages, it is expected that altering the cell surface glycoconjugates will have a negative effect on plasmodium invasion.

Research Grant: USDA NE1443

Student Support: NIH 5T35OD010432-17

Bovine antibody response to immunization against *Dermacentor andersoni* midgut and salivary gland

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Ticks and the numerous microbes they host are a serious burden on the cattle industry. Tick vaccines may be a cost-effective way to reduce herd infestation levels, which reduces the need for pesticide use and decreases tick disease incidence in the herd. Previous work has shown that immunization with crude homogenates of tick midgut or salivary glands tissues can have different effects on tick performance. The objective of this study was to measure the humoral immune response of calves immunized with midgut or salivary gland extracts from *Dermacentor andersoni* ticks. Sera from cattle immunized with *D. andersoni* salivary gland or midgut extract were collected weekly for quantitative antibody assessment using an ELISA. We expect immunization to increase anti-immunogen antibody levels and subsequent immunization boosters will further raise these antibody levels. Antibody levels will likely remain sufficiently elevated for several weeks. The level of cross-reactivity between salivary gland and midgut is expected to be significant, but modest compared to reactivity to matched immunogen. These results are expected to lead to further studies to determine immunoglobulin isotypes and to identify antigenic targets associated with reductions in different parameters of tick feeding and fecundity.

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Do California sea lions with temporal lobe epilepsy have fewer CB1⁺ inhibitory inputs onto granule cells?

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Temporal lobe epilepsy (TLE) is common and difficult to treat. Seizures usually start in the hippocampus. Previous studies have shown reduced GABAergic synaptic inhibition of hippocampal dentate granule cells, but the mechanism is unclear. Cannabinoid receptor type 1 (CB1)-expressing GABAergic interneurons normally inhibit granule cells. We hypothesized that granule cells of epileptic animals receive less CB1⁺ GABAergic synaptic input compared to controls. California sea lions were used as a model of human TLE. Sea lions develop TLE after algal blooms that produce domoic acid, a potent glutamate receptor agonist that causes hyperexcitability and neuron death. Sea lions were intracardially perfused with formaldehyde immediately upon euthanasia after failed response to treatment and poor prognosis. Brains were sectioned coronally. Hippocampi were Nissl-stained and immunoprocessed for CB1. The optical fractionator method was used to estimate the number of granule cells and CB1⁺ boutons per hippocampus. Control sea lions (n=6) had 129 ± 26 (mean $\pm$ sem) boutons/granule cell (2.22 ± 0.12 million granule cells, 281 ± 52 million CB1⁺ boutons). Epileptic sea lions (n=13) had 70% fewer granule cells (0.67 ± 0.16 million) and 55% fewer CB1⁺ boutons (126 ± 28 million), but an average of 2.8x more boutons/granule cell (361 ± 86) than controls. These findings suggest that decreased inhibition of granule cells in TLE is not due to a loss of CB1⁺ interneuron innervation, but rather that CB1⁺ interneurons might be compensating and forming more synapses in a homeostatic attempt to control seizures.

Research Grant: NSF & NIH

Student Support: NIH/ORIP T35 OD010989

Delineating the enzymatic versus non-enzymatic roles of fibroblast activation protein in collagen remodeling

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Fibroblasts are the primary cell type involved in extracellular matrix (ECM) synthesis and remodeling in health and disease. Fibroblast activation protein (FAP) is a serine protease that is expressed by a subset of fibroblasts and plays an important role in collagen proteolysis and ECM remodeling. FAP has also been reported to have protease-independent effects on cell behavior. The objective of this study was to determine to what degree FAP protease activity specifically contributes to ECM remodeling. Using CRISPR technology, we generated mice that express a form of FAP (FAP^{S624A}) with a point mutation that abrogates FAP enzymatic activity. Flow cytometry analysis was used to confirm that FAP^{S624A} is expressed at levels comparable to the levels of FAP in wild-type (WT) mice. Primary lung and dermal fibroblasts were isolated from WT, FAP^{S624A} and FAP knockout mice and used to generate fibroblast-derived matrices (FDM). Two photon second harmonic generation microscopy will be used to compare fibrillar collagen accumulation and architecture in FDMs from each of these genotypes. We hypothesize that FAP enzymatic activity is at least in part required for collagen remodeling, degradation and turnover. If our hypothesis is correct, more collagen should accumulate in FDMs generated by FAP^{S624A} than in WT fibroblasts. In the future, it will be important to address the functional impact of the loss of FAP activity and whether the data from this study is recapitulated *in vivo*. These studies will help identify mechanisms involved in regulating desmoplastic responses in pathophysiologic matrix remodeling in settings including stromagenic tumors and fibrosis and may elucidate potential therapeutic targets in veterinary medicine.

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Equine intestinal microbiome; the future of enteric disease prognostics

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Colic is a common cause of morbidity and mortality in horses and often has no etiology identified. Although colic is often idiopathic, the microbiota of the intestinal tract is thought to play a role in many cases. In addition, previous studies have demonstrated the importance of the equine intestinal microbiome for the sequestration of energy from the digesta, which is essential for the health of the horse and gut maintenance. To date, these studies have all been performed in geographically constrained populations with relatively small sample sizes. Moreover, no studies have included pre and post colic samples. To address these limitations, we have established a multi-center consortium of seven veterinary teaching hospitals including those in Missouri, Kansas, Illinois, North Carolina, Alabama, Louisiana, and Minnesota. These centers will collect fecal samples at the time of admission and every 1-2 days thereafter during hospitalization, from both affected and unaffected horses entering the clinic, with the goal of achieving 6,000 samples over the first two years. DNA will be extracted from fecal samples and sequenced using a well-established pipeline. Multi-factorial characteristics of the microbiome will then be associated with disease using machine-learning techniques, e.g., “random forests”. Results from our previous studies have associated a decrease in microbial richness and diversity with acute and chronic intestinal disease. Furthermore, preliminary analyses have revealed relatively conserved community structure across geographic regions, and disparate composition in affected horses, suggesting compositional characteristics of the microbiota could serve as diagnostic or prognostic indicators of colic.

Research Grant: University of Missouri Metagenomics Center program funds

Student Support: Boehringer Ingelheim

Insulin resistance and its impact on circadian rhythms of glucose metabolism in hibernating grizzly bears

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Diabetes mellitus is a common but poorly understood endocrinopathy and its incidence is increasing in both humans and companion animals. Grizzly bears (*Ursus arctos horribilis*) are a useful model for diabetes mellitus research as they become insulin resistant during hibernation but regain insulin sensitivity during their active season. The circadian system is strongly linked to metabolism and disturbances of circadian rhythms are recognized as negatively impacting glucose disposal. In this study, captive, hibernating grizzly bears were fed dextrose over a two-week period and their glucose disposal monitored as an index of insulin sensitivity. Fat biopsies were taken at the beginning and end of the two-week period and mesenchymal stem cells were differentiated into preadipocytes and cultured. We will compare the rhythms of glucose uptake in cultured cells as well as rhythmic expression of the circadian gene BMAL1 and glucose transporter, GLUT4 from before and after the oral administration of dextrose. We expect that the glucose uptake and gene expression of the cells before and after dextrose administration should be similar to those seen in hibernating and active season cells, respectively. We hypothesize that glucose uptake of bear adipocytes will be higher following oral repeated administration of dextrose over a two-week period and will be reflected in an altered rhythm of glucose uptake and relevant gene expression indicating reversal of insulin resistance at the cellular level. A better understanding of the molecular and genetic basis of blood glucose regulation in bears may provide useful insight into the pathophysiology and management of diabetes mellitus in other species including humans and companion animals.

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Student Support: Department of Integrative Physiology and Neuroscience, CVM, Washington State University

Transstadial transmission of *Mycobacterium leprae* in experimentally infected *Amblyomma maculatum* ticks

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Although zoonotic transmission of *Mycobacterium leprae* from armadillos has been well documented, and despite many new leprosy patients leading active outdoor lifestyles, few of these patients report significant contact with these animals. As a possible arthropod vector, ticks were investigated for their ability to harbor and transmit *M. leprae*. *Amblyomma maculatum* nymphs injected with *M. leprae* retained bacteria through the molt and into the adult life cycle stage. In order to infect a new host, viable *M. leprae* must be present in the adult *A. maculatum* salivary glands. Thus, the infection kinetics of the *M. leprae* within a tick host were examined. Adult female *A. maculatum* that were infected as nymphs, were fed for either 1, 3 or 6 days before manual removal. Ticks were then dissected and their gut and salivary glands were prepared for infection analyses by both immunofluorescence assay (IFA) and qPCR. Of the twelve females that successfully fed, only one gut was PCR-positive for *M. leprae* and IFA was non-definitive. Further studies are planned to examine the role of arthropods in the ecoepidemiology of *M. leprae* infection.

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Student Support: NIH T35 Training Grant 5T35OD011151

Genetic characterization of *Cryptosporidium* in wild chimpanzees

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Cryptosporidium is an important cause of parasitic gastrointestinal diarrheal disease in humans, especially in young children and immunocompromised individuals in developing countries. Because these obligate intracellular protozoa are well adapted for zoonotic transmission via fecal-oral route of the *Cryptosporidium* oocyst, it is important to investigate possible wildlife host populations that may overlap with at-risk human populations. Previous studies identified *Cryptosporidium* infections in fecal samples from chimpanzees and baboons in Gombe National Park, Tanzania. However, no genetic characterization of the parasites was performed. Here, we used nested PCR to probe fecal DNA from these same Gombe communities, targeting a 780 bp segment of the 18s small subunit rRNA (SSU). Testing a total of 365 samples, we amplified *Cryptosporidium* DNA from 5 individuals and identified 9 variants, only 1 of which appeared to be closely related to *C. hominis*. Remarkably, all of these represented samples are from SIVcpz infected individuals. Further experiments are underway to amplify a 550bp fragment of the oocyst wall protein 1 gene (COWP1) and the 800 bp gp60 gene by nested PCR, in order to further characterize *Cryptosporidium* infection genotypes.

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Calcium regulation of NF- κ B signaling in lymphocytes

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The pro-inflammatory transcription factor NF- κ B is associated with many diseases, including autoimmunity and cancer. In lymphocytes, NF- κ B exists in the cytosol as heterodimers of p50/p65 or p50/c-Rel associated with inhibitory I κ B proteins. Upon lymphocyte activation, intracellular-Ca²⁺ levels increase, leading to activation of the I κ B kinase complex, which phosphorylates and targets I κ B proteins for proteasomal degradation. NF- κ B is then released and localizes to the nucleus to initiate gene expression. The precise mechanisms by which Ca²⁺ regulates NF- κ B signaling remain poorly defined; however, previous work from our labs determined that site-specific Ca²⁺-dependent serine phosphorylation of p65 at S536 controls its transcriptional competence and specificity. A second serine residue (S316) was also identified by unbiased mass spectrometry to be a novel Ca²⁺-dependent phospho-acceptor. To study the role of this phosphoserine on lymphocyte activation, a S316A mouse model was created using CRISPR-Cas9. Immunophenotyping of p65 S316A mice was performed using flow cytometry to characterize lymphocyte populations, B cell maturation, and T cell development. Interestingly, while no significant differences were identified in these populations, we found that the granulocyte population in S316A mice was consistently elevated. We further characterized these cells using markers for monocytes, macrophages, and neutrophils. Finally, the role of S316 phosphorylation on T cell proliferation was investigated *in vitro* using WT and S316A CD4⁺ T cells. Taken together our findings provide new insight into the mechanisms of Ca²⁺ regulation of NF- κ B activity and function.

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Association between dysregulated non-canonical NF- κ B signaling and eosinophilic esophagitis pathogenesis

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Noncanonical NF- κ B signaling is a pathway involved in organ development and immune cell differentiation, and trafficking. Little is known about this pathway, especially its role in the gastrointestinal tract. Previous studies conducted by our lab found an association between dysregulation of the noncanonical NF- κ B signaling pathway, especially of NF- κ B inducing kinase (NIK), and development of an immune-mediated and often allergy associated disease called eosinophilic esophagitis (EoE). Most commonly diagnosed in children, disease pathology is localized to the esophagus, causing symptoms such as difficulty eating, dysphagia, food impaction and failure to thrive. Pathogenesis is thought to be driven by Th-2 cell activity; however, as our previous data suggests, noncanonical NF- κ B dysregulation may also play a role through breakdown of NIK at the protein level by its negative regulators. This study aims to further elucidate the relationship between the noncanonical NF- κ B pathway and EoE at both the gene and protein levels in human patients and to determine if NIK has an effect on eosinophil migration. mRNA analysis revealed a concurrent increase in NIK expression and decrease in downstream chemokine expression in EoE patients when compared to controls, agreeing with the preliminary data. In combination with the results of Western blot band intensity analysis, these findings supported the hypothesis that noncanonical NF- κ B activity is reduced in EoE patients due to NIK destabilization and lack of effector chemokine activity.

Research Grant: VMCVM IRC

Student Support: NIH T35 Training Grant T35OD011887

Cross-sectional second harmonic generation (SHG) histology of healing equine flexor tendons

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Superficial digital flexor tendon (SDFT) injuries are among the most common injuries in racehorses. Although longitudinal collagen alignment has been described, cross-sectional histology focused on fascicle pattern and distribution of interfascicular matrix during tendon healing is lacking. We hypothesize that second harmonic generation (SHG) histology analyses will provide objective, quantitative measurements of collagen fiber organization and cellular morphology in cross-section of healing tendons, at micro-scale resolution and with high specificity. Collagenase-induced tendinitis was created in the superficial digital flexor tendons of eight horses. Tendon tissue was collected at 1-, 2-, 4-, 6-, and 16-weeks following injury. Paraffin-embedded tendon tissue sectioned in cross-section at 10 microns, counter-stained with DAPI were imaged through a confocal microscope with 2-photon laser source to obtain SHG images. High resolution images of tendon cross-section depicting the fascicle pattern/arrangement, interfascicular matrix and nuclear morphology can be obtained with SHG imaging. Preliminary evaluation suggests that although the fascicle pattern in healing tendons is restored overtime, the fascicle size remains larger with decreased interfascicular matrix compared to normal tendons. Our current efforts are focused on developing quantitative indices to represent these subjective findings. Given that restoring the hierarchical structure of tendons is paramount to regaining their functional strength, this study will determine the utility of cross-sectional tendon SHG imaging, the current industry standard for longitudinal tendon histology.

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Detecting early cartilage degradation by nuclear magnetic resonance spectroscopy in a mechanical injury model

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Osteoarthritis (OA) is a common degenerative joint disease. It is currently detected by radiography or magnetic resonance imaging (MRI), but pathology is often undetectable until later stages of disease when treatment is difficult. Magic angle spinning nuclear magnetic resonance spectroscopy (MAS NMR) has been studied as a method to better inform MRI protocols for earlier diagnosis of cartilage injury by observing changes in biomarkers of cartilage degradation and permitting intervention prior to development of OA. Previous MAS NMR studies have shown decreased levels of N-acetyl and amino acids (glycine, alanine) correlated to loss of glycosaminoglycans (GAGs) and collagen respectively in end stage osteoarthritic cartilage compared to healthy tissue. However, this technique has not been used to study early cartilage injury. To simulate acute joint damage, this study uses a novel bovine *ex vivo* mechanical model of cartilage injury. It is hypothesized that decreases in N-acetyl, alanine, and glycine will be found by MAS NMR analysis in the mechanical model compared to healthy tissue. So far, an enzymatic digestion protocol has been developed to prepare positive control samples for NMR alongside the mechanical model. Either collagenase or papain will degrade cartilage to provide comparison points for GAG loss and collagen degradation respectively. MAS NMR data was collected on a Bruker AVANCE spectrometer with an 11.74 Tesla wide bore magnet and an NMR protocol was optimized for bovine cartilage samples allowing identification of the N-acetyl (2.063 ppm), glycine (3.4688 ppm), and alanine (1.4836 ppm) peaks. Further experiments comparing healthy, mechanically injured, and enzymatically digested cartilage are in progress.

Research Grant: This research is supported by the University of California, Davis Center for Equine Health, which receives funds from the State of California Pari-Mutuel Fund and contributions from private donors

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Immunoprofile of a novel soluble PD-L1 and PD-L2 inhibitor for use in cancer

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PD-1 is an immune checkpoint receptor that, when bound to its ligands PD-L1 and PD-L2, induces immunosuppressive phenotype. Inhibitors of the PD-1 pathway are now widely used in the treatment of cancers. PD-1 and PD-L1 inhibitors prevent cancer cells from evading immune surveillance by preventing the binding between the ligands and receptor. Using a unique mutational screen, we have developed a high affinity decoy trap that binds PD-L1 and PD-L2 10,000x and 200x times greater respectively than the native receptor-ligand interaction. Our molecule has shown significant anti-tumor activities in both mouse models of colorectal and melanoma cancer models. In this study, we sought to determine the activation of immune cells upon the treatment of our molecule in comparison with the anti-PD-1 antibodies. We hypothesized that the anti-tumor activity was mediated through enhanced immune response in tumors treated with our molecule. To test this hypothesis, 3 cohorts of C57Bl/6 mice were injected with a melanoma cell line (B16-OVA), followed by treatment with our molecule, mouse anti-PD-1, or vehicle. The tumors were collected along with blood and the draining lymph node for FACs analysis, evaluating the expression and the number of CD4 T cells, CD8 T cells, NK cells, dendritic cells, and macrophages. The proportion and total number of immune cells based on FACs analysis will provide insights into the immune landscape upon treatment with molecules that inhibit either the ligands or the receptor of the PD-1 pathway.

Research Grant: NIH R3J CA197713, NIH R01 CA198291, and NIH R01 CA067166

Student Support: NIH/ORIP T35 Training Grant OD010989

TolC and other *in vivo* virulence factors implicated using *Ehrlichia* HF transposon mutants

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Ehrlichia spp. are obligatory intracellular bacteria that infect monocytes, macrophages, and endothelial cells. *Ehrlichia chaffeensis* is the causative agent of human monocytic ehrlichiosis (HME). *E. chaffeensis* does not induce disease in immunocompetent mice, precluding them from being used in investigating *E. chaffeensis* pathogenesis factors. *Ehrlichia* HF was previously determined to contain 16S rRNA sequences that are 98.2% similar to *E. chaffeensis*, and unlike *E. chaffeensis* induces a fatal dose-dependent disease in mice. C57BL/6J mice (5-8 weeks old, male and female, 5 mice per group) were intraperitoneally inoculated with *Ehrlichia* HF mutants selected from a HimarI transposon random mutagenesis library, and compared to wild-type *Ehrlichia* HF. Mice were euthanized by CO₂ inhalation and blood specimens were collected by cardiac puncture, and spleens were aseptically excised, weighed, and then stored at -80°C. Sections of spleens were frozen in RNAlater for RNA extraction and cDNA synthesis and qRT-PCR analysis of bacterial load. Other sections were used for DNA purification and PCR for confirmation of transposon insert. A mixture of mutants of TolC type I secretion system outer membrane protein and hypothetical protein, a mutant clone of a type IV secretion system needle protein, and a clone-like mutant of ComEC family protein were tested in mice. The type I secretion system and hypothetical protein mixture showed significant reductions in spleen bacterial load when compared to wild-type HF. TolC and/or a hypothetical protein may function as virulence factor in *Ehrlichia* HF *in vivo*, thus may be implicated in HME. Further studies are needed to verify and define *in vivo* virulence factors of *Ehrlichia* sp.

Research Grant: NIH

Student Support: NIH T35 Training Grant T35OD010977

Preliminary retrospective review of important pathogens in small poultry flocks in Ontario, Canada

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The recent popularity of non-commercial small poultry flocks calls for an assessment of the prevalence of infectious diseases among these birds. To this aim, a retrospective study of small flock postmortem submissions to the Animal Health Laboratory (Guelph and Kemptville, Ontario) from January 1998 to September 2015 was conducted. To be included, flocks had to reside in Ontario and either contain ≤ 300 birds or be listed as “exhibition flock”. Preliminary results include a total of 313 retrieved submissions. Chickens submissions were most common (65%), followed by turkeys, ducks, and game birds. Bacterial tests were performed on 37% of submissions. *Escherichia coli*, *Pasteurella* spp. (including *P. multocida*), *Erysipelothrix rhusiopathiae*, and *Salmonella* spp. were diagnosed in 53, 10, 5, and 6% of tested submissions. The two submissions positive for *Salmonella* spp. were serotype as *S. Heidelberg*. Mycoplasma tests were conducted on 11% of submissions, with Mycoplasma spp. detected in 10% of tested submissions. Virology tests were conducted on 26% of submissions. Infectious laryngotracheitis virus and infectious bronchitis virus were detected in 21, and 19% of tested submissions. Avian influenza virus (H3 subtype) was isolated from one turkey submission. Histomoniasis was detected in 32% of turkey submissions and 2% of chicken submissions. Overall, bacterial agents were the most commonly detected infectious agents. In contrast to previous small flock studies, the prevalence of *Salmonella* spp. isolates was low. The information provided by this study will contribute in determining the historical prevalence of infectious agents among small flocks in Ontario, and will aid in developing disease prevention and control measures.

Research Grant: OMAFRA-University of Guelph Partnership

Student Support: Andrea Leger Dunbar Summer Research Studentships

Idly infected: a review of infectious agents in populations of two-toed sloths (*Choloepus* spp.)

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The two-toed sloth (*Choloepus* spp.) has become a popular species utilized in zoological and wildlife institutions. Thus, their contact with the public has increased, but not much is known about what health risks they may pose to humans, nor which diseases they are susceptible to. This study reviewed all published literature from 1809 to 2018 that examined infectious agents affecting two-toed sloths in both captive and wild populations. Online databases were selected and electronically searched for relevant articles using strings of inclusion and exclusion terms, resulting in identification of 1,242 articles. After conducting two relevance screenings, 36 articles were deemed appropriate for inclusion in the review. A total of 1,676 two-toed sloths were accounted for in the included studies, with evidence of infection in 577 individual sloths. Approximately 77% of those identified were cryptic fungal, parasitic, and viral infections, and the remainder presented with clinical cases of bacterial, fungal, and viral infections. The infectious agents reported were parasitic (12), viral (8), bacterial (4), and fungal (3). Of the parasitic infections reported, only one was of clinical significance to the animal; however, in geographical areas where human populations have shown a high prevalence of leishmaniasis, coexisting sloths have exhibited a corresponding prevalence of subclinical *Leishmania* infections, indicating they are likely vectors for the disease. Significant gaps remain regarding clinical and subclinical infectious disease prevalence in sloths in captivity, which should be further investigated while sloths continue to be an important species in the eyes of the public.

Research Grant: None

Student Support: Boehringer-Ingelheim Veterinary Scholars Program and Purdue Veterinary College

Evaluating biomarkers for pharmacodynamic monitoring of glucocorticoid therapy

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Glucocorticoids are frequently used in veterinary medicine to achieve the suppression of inflammation and immune responses necessary for the successful treatment of a wide range of diseases. These drugs are known to be clinically effective, however the variation in the extent of efficacy in individual patients requires careful monitoring of therapy with appropriate dose adjustment so that patients are not overly immunosuppressed. Developing a pharmacodynamic assay to assess biomarkers of glucocorticoid efficacy would be clinically valuable and allow for more reliable monitoring of therapy. The aim of this study is to compare the expression of interleukin 2 (IL-2), interferon gamma (IFN- γ), chemoattractant protein 1 (MCP-1) and lipocortin as measured by quantitative reverse transcription polymerase chain reaction (qRT-PCR) in canine whole blood treated *in vitro* with dexamethasone, methylprednisolone, and prednisolone. We will determine which of these biomarkers is the most sensitive to the concentration-dependent effects of glucocorticoids, and therefore holds the most promise as a pharmacodynamic marker. We hypothesize that the expression of lipocortin will increase, and the expression of IL-2, IFN- γ , and MCP-1 will decrease in a concentration-dependent manner in canine whole blood treated with dexamethasone, methylprednisolone, and prednisone *in vitro*. Once developed, these pharmacodynamic assays will allow clinicians to more accurately monitor individual response to glucocorticoid therapy with the goal to improve patient outcomes.

Research Grant: None

Student Support: NIH Training Grant 5T35OD010963-09

Loss of transcription factor FoxJ1 alters basal behavior of mice in a novel environment

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Movement and circulation of cerebrospinal fluid (CSF) is critical for maintenance of homeostasis in the brain. CSF flow through the ventricular system is in part driven by motile cilia on the ventricular surface of ependymal cells (ECs), the major epithelial layer in the brain. EC function is vital in maintaining proper intracranial pressure in both humans and animals, with disruptions in this process resulting in varying degrees of hydrocephalus. The transcription factor FoxJ1 is a critical regulator of gene expression and essential for development of motile cilia in ECs. Genetic deletion of *Foxj1* results in severe early onset hydrocephalus due to inability of ECs to mature. However, the impact of early onset hydrocephalus on development of behaviors in mice lacking Foxj1 remains unknown. The aim of this study is to analyze the behavior of conditional Foxj1-null mice in which Foxj1 is absent only in brain ECs. Experimental groups consisting of FoxJ1 conditional-knockout (FoxJ1-cKO, n=5) and controls (cWT, n=10) underwent recorded behavioral testing that consisted of placement in a novel environment. Basal behaviors over 30 minutes were measured and classified as sniffing/digging, rearing, mobile, and grooming behaviors. Preliminary data shows that FoxJ1-cKO mice display significant increases in time spent in rearing and mobile behaviors compared to cWT mice (Mann Whitney test: $P=0.0127$, $P=0.0117$). In conclusion, FoxJ1-cKO mice display differences in locomotive and cognitive behavior compared to cWT mice. Current plans include continuing behavioral analyses of FoxJ1-cKO mice, and immunohistological and biochemical analyses to assess differences between the experimental groups.

Research Grant: NIH R01NS098370 and NIH R01NS089795

Student Support: NC State CVM Boehringer Ingelheim Veterinary Scholars Program

Risk of respiratory and parasitic disease in shelter dogs that participate in playgroups

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The relationship between canine welfare and social interactions is proven beneficial, but there are concerns regarding infectious disease, which may limit playgroup implementation in shelter environments. With the complex interplay of host, agent, and environment in the epidemiologic triangle, it is unclear how playgroups might influence the risk, severity, and duration of infectious disease within a shelter environment. The major hypothesis is that the risk of infectious disease in canines is independent of playgroup participation in shelters. This study is a prospective randomized controlled trial consisting of 100 large breed dogs admitted to Animal Care and Control Team Philadelphia. Eighty will participate in playgroups while 20 will not. Respiratory samples collected on day 3-5 and 12-14 after admittance to the shelter will be tested by polymerase chain reaction (PCR) for respiratory coronavirus, pneumovirus, and *Bordetella bronchiseptica*. Fecal samples will be tested by PCR for giardia. Fecal and respiratory clinical signs will also be logged daily. The benefits of playgroups to the mental health of shelter groups is supported by several studies. This study will examine both quantitative data of primary interest to researchers and qualitative clinical data of primary interest to shelter management regarding infectious disease in shelter dogs exposed to playgroups. It will generate novel data that will allow shelter management to make informed decisions regarding the balance between physical and mental health of shelter dogs.

Research Grant: PetSmart Charities Research Fellowship and Dr. Kreisler Startup Funds

Student Support: PetSmart Charities Veterinary Scholarship Program

High vector-borne *Haemosporidia* prevalence in eastern Sandhill Cranes (*Antigone canadensis*) over two decades

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Haemosporidians are a diverse group of vector-transmitted blood parasites. In birds, the impacts of haemosporidian infection can range from asymptomatic to acute mortality or population-level impacts on fecundity and survival. These parasites have been found in Sandhill Cranes (*Antigone canadensis*), a migratory species that may serve as a sentinel for understanding health threats to the endangered Whooping Crane (*Grus americana*). We used molecular techniques to compare prevalence and diversity of Haemosporidia in wild Sandhill Cranes from the eastern flyway population that migrates annually between Wisconsin and Florida. Using archived blood samples collected over a twenty-year period (1997-2017), we amplified the mitochondrial cytb gene of *Plasmodium* and *Haemoproteus*. Phylogenetic analysis is being conducted to identify parasite lineages. Overall the infection prevalence in birds (n=175) was 71.4%, with no difference across early versus late time period sampled ($P=0.43$) or between sexes ($P=0.53$). Infection prevalence in young (hatch year) birds (80.7%; n=109) was significantly greater than that of older birds (66%; n=66; $P=0.0009$); this may be related to the immune status or relative lack of anti-vector defense of immature individuals. In at least one recaptured individual, PCR and sequencing revealed evidence of co-infection with both a *Plasmodium* sp. and a *Haemoproteus* sp. The baseline data gathered from this study will contribute to our understanding of the factors that influence Haemosporidian prevalence in Sandhill Cranes over time and inform directions for future epidemiologic studies and conservation efforts.

Research Grant: Schubot Exotic Bird Health Center

Student Support: Boehringer Ingelheim VSP, Texas A&M College of Veterinary Medicine & Biomedical Sciences

Enhancing human NK Fc receptors to improve tumor cell conjugation and killing

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Natural killer (NK) cells are a crucial player of the innate immune system. Functioning as cytotoxic lymphocytes, NK cells rapidly kill virally infected cells and tumor cells without prior sensitization. A key anti-tumor function of NK cells is their ability to kill tumor cells via antibody-dependent cell-mediated cytotoxicity (ADCC), which is exclusively mediated by the IgG Fc receptor CD16A (FcγRIIIa). Fc-mediated engagement of CD16A results in a series of downstream signaling cascades that induce the release of cytoplasmic granules containing perforin and granzyme, which lead to tumor cell lysis. Many clinically successful therapeutic antibodies utilize ADCC as a mechanism of action; however, CD16A binds IgG with low affinity, thereby limiting the efficacy of therapeutic antibodies. Additionally, ADCC requires intracellular adhesion and stable conjugate formation between the NK cell and its target cell. We hypothesize that increasing the IgG binding affinity of CD16A will enhance both the speed of intracellular adhesion and the formation of a long lasting stable conjugate between the NK cell and its target cell. We have engineered a unique chimeric receptor, CD64/16A, which consists of the extracellular region of the high affinity Fc receptor CD64 (FcγRI) and the transmembrane and intracellular regions of CD16A. Through two color flow cytometry, we are quantitatively measuring the formation of NK:target cell conjugates using the therapeutic antibody Herceptin, our enhanced CD64/16A receptor and the low-affinity wild-type CD16A receptor as a control. These results could lead to the formation of an “off-the-shelf” cellular therapy that can be combined with therapeutic antibodies for the treatment of many tumor types.

Research Grant: None

Student Support: University of Minnesota College of Veterinary Medicine

Assessing the readability of veterinary clinical trial consent forms

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“Readability” of hospital consent forms is vital to the informed consent process, particularly in veterinary clinical trials where owners agree to enroll their pets in studies of investigational therapies. Studies have shown that the average human hospital consent form is written at a 10th grade reading level or above, while the average American adult reads at only an 8th grade level. While signed consent is considered a cornerstone of informed consent, limited information currently exists regarding the readability of veterinary general medical or clinical trial consent forms. The goal of this study is to assess the readability of client consent forms in veterinary clinical trials from five different CTSA One Health Alliance (COHA) veterinary centers recently involved in a working group focused on veterinary clinical trial review and consenting (The Ohio State University, Tufts University, University of Florida, University of Missouri, and Colorado State University). We hypothesize that consent forms for veterinary clinical trials are not optimized for client comprehension and are written above the CDC-recommended 6th grade reading level. Each hospital was asked to submit the two most recently approved study consent forms from the following clinical specialties: cardiology, equine medicine, internal medicine, oncology, food animal, neurology, and surgery. Readability was assessed using three methods readily available to veterinarians, the Flesch-Kincaid Grade Level and Flesch Reading Ease (Microsoft Word) and the Readability Test Tool (free web-based program). Aggregate data will be evaluated by assessing mean grade level and reading ease and compared between specialties. Data analysis is currently ongoing.

Research Grant: Internal Funds, The Ohio State University

Student Support: Georgetown College Science Honors Program

Phytocannabinoid metabolism by canine cytochrome P450s

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Phytocannabinoids (pCBs) are natural compounds found in *Cannabis* plants (marijuana). Recent popularity of pCB use in human and veterinary medicine, combined with an insufficient understanding of pCB interactions on animal homeostasis, warrants investigation of pCB metabolism and pharmacokinetics. Cytochrome P450s (CYPs) are a class of enzymes involved in phase 1 drug metabolism playing a significant role in pCB biotransformation. In this study, metabolism of selected pCBs by canine CYPs 3A12 and 3A26 enzymes was explored and compared to metabolite formation by the human ortholog CYP3A4. We focused on two medically relevant pCBs found in *Cannabis*: Δ^9 -tetrahydrocannabinol (Δ^9 -THC) and cannabidiol (CBD). These enzymes may produce similar metabolites due to conserved substrate and protein structures. To analyze human metabolism, we recombinantly expressed and purified CYP3A4 and its redox partner, cytochrome P450 reductase (CPR) in *E. coli*. Due to differences in expression between CYPs 3A12 and 3A26 in canine liver and peripheral tissues, native canine liver microsomes (containing both enzymes) and CYP3A12-specific batosomes were obtained. Experiments will involve incubation of rCYP3A4, canine liver microsomes and batosomal CYP3A12, with Δ^9 -THC and CBD. Resulting metabolites will be extracted prior to high performance liquid chromatography-mass spectrometry (HPLC-MS), so that fragmentation analysis can enable structure identification. Since dogs are commonly used in drug trials and pCBs are becoming increasingly popular in veterinary medicine, the comparison and discovery of human-versus-canine-derived pCB metabolites will create a foundation for future studies supporting *Cannabis*-based therapy.

Research Grant: American Heart Association Scientist Development Grant 15SDG25760064 (A.D.), National Institutes of Health (NIH) Grant R01 GM1155884 (A.D.), and R03 DA042365 (A.D.)

Student Support: Office of the Director, NIH, T35 OD011145

Phage display for identification of peptides that bind Zika virus surface exposed protein E

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ZIKA virus (ZIKV) is not a newly identified virus since it was first isolated almost 70 years ago from a sentinel monkey in the Zika forest in Uganda. ZIKA has been assigned to the Flaviviridae family of positive-stranded RNA viruses along with yellow fever, Dengue, West Nile, and Japanese Encephalitis virus. The World Health Organization (WHO) declared ZIKA a public health emergency of international concern due to its rapid global spread, combined with accumulating evidence linking prenatal ZIKV infection to an increased rate of babies born with microcephaly and other neurodevelopmental abnormalities. Zika virus has 3 structural and 7 non-structural proteins, the structural protein of focus is the E-protein. The objective of this study is to identify those phages that display peptides which bind with high affinity to the E-protein, in the anticipation that these peptides will block virus entry into human cells. To do this, we grew M13 phages in *E. coli* bacteria, after three rounds of panning against recombinant E-protein, and performed phage ELISA binding assay with direct target E-protein coating to identify those phages that bound with strong affinity. Bound phages were verified by using a monoclonal antibody to M13 phage followed by a conjugate. Phages that displayed optical density readings equal or greater than 0.4 were selected. During the phage ELISA binding assay we observed high O.D. readings of up to 1.0, which are excellent indicators of high affinity binding. We are currently in the process of determining those peptide sequences. Future work will evaluate peptide consensus sequences that are able to block zika virus infection of human cell lines.

Research Grant: Summer Research Veterinary Scholars, College of Veterinary Medicine

Student Support: NIH T35 Training Grant

Identification of pathogenic bacteria derived from natural water sources

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The pathogens *Edwardsiella tarda*, *Edwardsiella ictaluri*, *Streptococcus iniae*, and various species of *Salmonella* have proven to have a significant impact on the fishing industry and to public health on a global scale. *Edwardsiella ictaluri* and *Edwardsiella tarda* both have a significant impact on fish farming industry. *Salmonella* remains a major cause of human disease, and both *E. tarda* and *Streptococcus iniae* have exhibited zoonotic potential. All of these bacteria are known to reside in climates with warm water temperatures. The purpose of this research was to sample natural freshwater sources in the Macon and Lee County areas of Alabama, and test for the occurrence of any of these bacteria. Most of the focus of this research was on Lake Tuskegee in Tuskegee, Alabama; a location mainly used for fishing. The bacteria in the collected samples were isolated in selective enrichment broths and later plated on selective and differential solid media to allow for colony isolation and gross identification based on colony morphology. Successfully isolated colonies that fit the morphological description were then suspended in enrichment broth and, once established, they underwent biochemical testing, most notably the Triple Sugar Iron (TSI) agar test. Samples that produced positive results were finally analyzed using the Biolog GN III system to confirm the identity of the bacteria. It is hoped that the collected information will be used in future research to test other water sources in the area, as well as to determine antibiotic susceptibility of these pathogens.

Research Grant: Boehringer Ingelheim

Student Support: unknown

Effect of medium-chain triglycerides compared to long-chain triglycerides on enterohormones in healthy dogs

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A complex interplay of hormonal and neural mediators control digestive processes such as gastrointestinal motility and digestive enzyme secretion. The hormones cholecystokinin (CCK) and secretin are released in response to the presence of intraduodenal nutrients such as long-chain triglycerides (LCT), and act to promote pancreatic enzyme and bicarbonate secretion, as well as gallbladder contraction. Ghrelin, an orexigenic hormone released in times of fasting, is suppressed in the dog by the ingestion of a meal containing fat or carbohydrate. Previous work establishing the relationship between dietary fat and enterohormone release has primarily utilized LCT, the primary component of most dietary fat. Clinical interest in medium-chain triglycerides (MCT) has increased due to their unique digestion and metabolism. They are already incorporated into canine diets for therapeutic purposes; however, the effect of MCT ingestion on canine enterohormones has not been investigated. Pancreatitis is a common and serious disease and CCK release may exacerbate this disease through further stimulation of an already damaged pancreas. In people with chronic pancreatitis, ingestion of enteral formulas containing MCT with hydrolyzed proteins instead of LCT results in decreased CCK release and decreased post-prandial pain. It is unknown if this approach may also benefit canine patients. The objective of this study is to evaluate the plasma concentrations of the fat responsive enterohormones CCK, secretin, and ghrelin in healthy canine patients ingesting MCT compared to LCT. This will help elucidate the physiologic response to MCT in healthy dogs and whether further investigation into other therapeutic roles is warranted.

Research Grant: Companion Animal Health Fund (CAHF)

Student Support: Boehringer-Ingelheim Veterinary Research Scholars Program

Role of HTLV-1 HBZ in transformation and disease

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Human T-cell leukemia virus type 1 (HTLV-1) is a retrovirus that infects 10-20 million people worldwide. While most infections are asymptomatic, around 5% of patients will develop an aggressive T-cell malignancy called ATL or a neurodegenerative disease termed HAM/TSP over the course of their lifetime. The HTLV-1 protein HBZ is consistently expressed in HTLV-1 infected cells and provides cellular proliferative and tumor maintenance signals. Human T-cell leukemia virus type 2 (HTLV-2) is a closely related retrovirus to HTLV-1. While both viruses encode proteins with similar structure and function, they differ in pathogenicity. HTLV-2 is not associated with disease like HTLV-1. The HTLV-2 equivalent to HBZ is called APH-2. *We hypothesize differences in cellular binding partners of HBZ and APH-2 will translate to differences in viral pathogenicity between HTLV-1 and HTLV-2.* Using affinity capture coupled with shotgun proteomics, we identified and compared cellular binding partners of HBZ and APH-2. Potential HBZ binding partners were confirmed using S-tag pulldowns and coIP experiments. Based on these results, 7 cellular proteins that interacted with HBZ and displayed a connection to HTLV-1 biology or disease progression were selected for further analysis. Ongoing experiments using shRNA and/or over-expression vectors to determine the importance of each candidate HBZ interacting protein in the viral life cycle are in process. Through exploration of HBZ binding partners and their effects within T-cells, we hope to further refine the role of HBZ in T-cell transformation and development of disease. Insight of these roles will allow us to explore HBZ cellular targets as potential targets for disease prevention and treatment.

Research Grant: PO1CA100730

Student Support: T35OD0977

Turning the oligonucleotide on equine osteoarthritis: an evaluation of a novel anti-DEK aptamer

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Osteoarthritis (OA) is a major cause of lameness in horses; current treatments are expensive or cause undesirable side effects. The pro-inflammatory DEK protein is a chemoattractant for neutrophils and maintains neutrophil extracellular traps (NETs), which are found in arthritic joints of mice and humans. An anti-DEK aptamer (ADA; an oligonucleotide with 3-dimensional structure) reduces joint inflammation in mice with zymosan-induced arthritis. This study assesses the safety and efficacy of the ADA when administered intra-articularly in arthritic horses. We hypothesize the ADA will safely alleviate lameness. Three adult horses with middle carpal OA were injected with either 5 ng/joint or 100 ng/joint of the ADA and with the same concentration of random oligonucleotides in a control joint. Joint circumference, range of motion, radiographs, and lameness were monitored. Synovial fluid was taken pre- and fourteen days post-injection for nucleated cell counts, total protein, biomarker analysis (for inflammation, cartilage synthesis and degradation), and identification of DEK and NETs. Tissue samples (synovium and cartilage) were collected postmortem for H&E analysis. One horse received fluorescently labeled anti-DEK aptamer to assess intra-articular location. Statistics included a one-way ANOVA (significant at $P < 0.05$). Two of the three horses showed improvements in lameness after ADA injection: one after receiving the 5 ng/joint dose and one after receiving the 100 ng/joint dose. No significant adverse events were observed when saline was used as a carrier. Histology and biomarker analyses are pending. The ADA appears to reduce the clinical signs of OA at both the 5 and 100 ng/joint doses without negative side effects.

Research Grant: Michigan Translational Research and Commercialization (MTRAC) Award (ID: 011042-00001)

Student Support: Boehringer Ingelheim Veterinary Scholars Program and MSU Internal Funds

Effects of Nerve Growth Factor- β on ovulatory cascade factors from follicular fluid of pre-ovulatory follicles

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Nerve growth factor- β (NGF) is a seminal plasma protein that induces ovulation in camelids. Although NGF is not responsible for ovulation in the bovine species, previous results revealed that it enhances luteal development and markers of maternal recognition of pregnancy. There is, therefore, a critical need to understand what components of the ovulatory cascade are affected by NGF. We hypothesized that administration of NGF to Holstein heifers stimulates ovulatory cascade factors that can help understand the mechanism of action of NGF on pre-ovulatory follicles. Virgin Holstein heifers ($n = 24$) had their estrous cycle synchronized using a 5-day CIDR Synch program. On day 8 of the cycle, heifers with pre-ovulatory follicle (> 12 mm) and no corpus luteum noted by ultrasonography were deemed eligible for the study. Eligible heifers ($n = 17$) were randomly assigned to one of two treatments: CON ($n = 7$), intramuscular injection of 12 mL phosphate buffer solution (PBS, pH=7.4), or NGF ($n = 10$), intramuscular injection of 296 μ g of NGF- β diluted in 12 mL of PBS. Follicular fluid samples were obtained from pre-ovulatory dominant follicles 8 hours after treatment via follicular aspiration, added to TRIzol (Invitrogen, Carlsbad, CA) and flash-frozen in liquid nitrogen. Samples were evaluated for gene expression of estrogen receptor 1 (ESR1) and Prostaglandin E Synthase (PGES) using quantitative real-time PCR. Data were analyzed with the GLIMMIX procedure of SAS. While no differences were revealed for ESR1 ($P = 0.99$) and PGES ($P = 0.89$), additional genes associated with the ovulatory cascade will be analyzed in the near future to provide a further understanding of the mechanism of action of NGF.

Research Grant: Lima Reproductive Biology Research Funds

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Impact of dosing regimen of danofloxacin on intestinal pharmacokinetics and the fecal microbiota of steers

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Understanding the impact of antimicrobials on fecal microbiota in steers is limited by an inability to correlate intestinal drug concentrations with changes in enteric bacteria. Fluoroquinolones are labeled for treatment of pneumonia in cattle and considered critical for human health. The purpose of this study was to determine the impact of danofloxacin dosing regimens on the MIC of fecal *Enterococcus* in steers. Our hypothesis is that the single high dose of danofloxacin will have less of an impact on the MIC of *Enterococcus* spp. Twelve steers were administered danofloxacin as either a single dose of 8 mg/kg or two doses of 6 mg/kg per every 48 hours after implantation of ultrafiltration probes in the intestine. *Enterococcus* spp. were isolated from fecal samples before and after treatment. Eight isolates per steer were speciated and the MIC of these isolates were quantified with microbroth dilution. Fecal *Enterococcus* fluctuates 2-3 log overtime irrespective of treatment regime. *E. hirae* was the predominate isolate in both groups prior to treatment. In the low dose group, *E. casseliflavus* became the predominate isolate at 24 hours. In the high dose group, *E. faecium* predominated after 84 hours. The MIC of the high group initially decreased and then rebounded at day six. The MIC of the low group does not appear to change and peaked 120 hours after treatment. Treatment with danofloxacin minimally impacts the MIC of *Enterococcus* spp. and there does not appear to be any clinically relevant differences between the dosing regimens.

Research Grant: CALS-CVM Strategic Enrichment Grant

Student Support: CVM Veterinary Scholars Program

Comparison of stress abatement strategies for feline friendly housing

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Cats housed in veterinary hospitals are often exposed to stressful environments. In a previous study evaluating stress reduction strategies, cats were placed in a simulated hospital environment and allowed to choose between unshielded cages and cages shielded with ¼-inch clear or grey plexiglass. Cats with higher stress scores spent significantly more time behind the cage front option that allowed for the greatest buffering of external stimuli. Unfortunately, sound abatement was minimal. The purpose of this study was to evaluate different strategies for increasing sound reduction while maintaining adequate light abatement, and then take this information into a clinical trial. Several sound/light abatement strategies were evaluated, including different dimensions, thicknesses, and colors of plexiglass, as well as soundproofing tiles. Noise/light levels in the study cage were measured as the different strategies were evaluated. Sound/light levels within the cage were reported as percent reduction compared to outside levels. Results indicate that the use of soundproofing tiles was the most effective strategy for providing noise reduction; however, it was the least clinically applicable. The use of 1/2-inch plexiglass more than doubled noise reduction compared to the previously used ¼-inch plexiglass while still being cost effective; however, the thicker grey plexiglass resulted in loss of patient visibility within the cage. Bronze and blue 1/2-inch plexiglass sheets proved to have comparable light reduction capabilities while still allowing for patient visibility. A clinical trial evaluating stress reduction in hospitalized feline patients, with 1/2-inch bronze plexiglass shielding, will be performed shortly.

Research Grant: Fund for Discovery, Herbert Benjamin Distinguished Professorship Endowment, Fluorescence Distinguished Professorship Endowment, Office of the Associate Dean for Research and Graduate Studies

Student Support: NIH T35 Training Grant T35OD012199

Assessing the likelihood of epizootic hemorrhagic disease virus-2 infection in *Culicoides sonorensis*

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Epizootic hemorrhagic disease virus (EHDV) is a vector-borne virus that affects livestock and wildlife, such as sheep, cattle, and deer. EHDV transmission occurs when *Culicoides* biting midges blood-feed on an EHDV-viremic animal, become infected, and transmit the virus along with saliva to a new host during their next feeding. Despite the midges' key role in EHDV transmission, the minimum virus concentration in blood required to infect a midge during feeding is not known. As such, this research aims to determine the minimum viral titer of EHDV serotype 2 (EHDV-2) needed in a blood meal to infect *Culicoides sonorensis* biting midges and thereby facilitate EHDV-2 transmission. First, previous literature was analyzed to create a multilevel model that predicts the likelihood of an individual midge becoming infected by feeding on different concentrations of EHDV-2 infected blood. This model will be validated for future use with the data gathered from the following feeding experiments. Groups of three-day-old colonized *C. sonorensis* midges were fed sheep blood mixed with decreasing concentrations of EHDV-2. After each feeding, female midges were sorted and housed for nine days. Midges were then euthanized and individually analyzed for EHDV-2 prevalence and viral load. This research provides insight into the likelihood of EHDV-2 infection of *Culicoides sonorensis* midges after blood-feeding on varied concentrations of virus and into the likelihood of EHDV transmission via these midges, which, together, may aid in establishing a livestock treatment protocol and reducing EHDV transmission in future work.

Research Grant: Research supported by the USDA-ARS NP103 Animal Health National Program and the NP104 Veterinary, Medical, and Urban Entomology National Program

Student Support: Student supported by the Boehringer Ingelheim Veterinary Scholars Program

Effects of therapeutic ultrasound on passive range of motion and lameness in osteoarthritic horses

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Osteoarthritis (OA) is a degenerative joint disease that is a major cause of equine lameness. Therapeutic ultrasound (TU) is used for OA in humans to help increase joint range of motion (ROM) and decrease pain; however, few studies have been conducted in horses. We hypothesized that horses receiving TU would have increased carpal surface temperature, increased joint ROM, and decreased lameness. Ten adult horses with OA and/or restricted joint ROM in at least one carpus received 20 minutes of continuous carpal TU (3.3 MHz, 1 W/cm²) and a sham treatment contralaterally, with each followed by 10 minutes of carpal passive ROM exercises. Surface temperature (thermography) was measured before and after TU, while joint ROM (radiographic and goniometric) and lameness (subjective and objective) were measured at several timepoints (baseline, post-TU and post-passive ROM, that afternoon, the next day, and 7 days post-TU). A subset of horses had their worst carpus treated with TU for 5 days with this protocol. Statistics included: repeated measures ANOVA, paired t-tests, and Spearman's correlations with $P < 0.05$ indicating significance. There was a significant decrease in carpal surface temperature after receiving sham treatment only. There was no statistical improvement in ROM over time in either joint. Goniometry had good correlation to some, but not all radiographic measurements. Clinical lameness improved in the afternoon following treatment in 6 horses (3 TU, 3 sham), but there was no significant improvement in lameness overall. In the subset of horses, overall lameness did not significantly improve, but ROM increased in 2 horses. In conclusion, only temporary improvements in lameness or ROM were seen with TU treatment.

Research Grant: Michigan State University Internal Funds

Student Support: Boehringer Ingelheim Veterinary Scholars Grant to Michigan State University

The use of a head camera for video self-assessment of surgical performance of veterinary students

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Several published studies in human and veterinary medicine have demonstrated that individuals using video footage for self-reflection after performance of surgical procedures has a significant improvement in their basic surgical skills acquisition. The objective of the study is to determine if the use of a head camera to record ovariectomy and orchiectomy by veterinary students followed by self-assessment with video improves subsequent surgical performance compared to students that perform a self-assessment without the video. We hypothesize that veterinary students who use video recordings of the surgical procedures they perform for self-assessment, will have better improved performance on subsequent surgical procedure compared with those that perform a self-assessment without the aid of a video. Third-year students of the DVM 2019 were invited to participate in the study, students randomized into two groups. Students' surgery performance was scored on the first two surgeries. All surgeries were scored by the same evaluator. Students in Group A reviewed their surgery performance of the first surgery using their recorded video. Students in Group B didn't use the video to review their surgery performance of the first surgery. The evaluator scores were compared between the first and second surgery of each student and between the groups. At the end of the study, students completed a 5-point Likert scale survey regarding the use of the head camera for their acquisition of surgery skills. The preliminary results indicated that surgical performance for each student between the first and second surgery improved by 3.11%. Seventy-one percent of the participants felt the GoPro camera video was beneficial.

Research Grant: CVM Research Office Intramural Grant and Western University (WU) Research Office Intramural Grant

Student Support: CVM Research Office and Dr. Henriksen's office

Investigation of Haemosporida parasites of wild turkeys

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While wild turkeys (*Meleagris gallopavo*) are present throughout many ecosystems in the United States, little is known on which blood parasite species are in the turkey populations of the eastern US, the prevalence of these parasites, and what survival and health impacts they have on the birds. Although it is currently thought that these parasites do not cause a large negative impact on wild turkeys that are not immunocompromised, more research must be done to determine what species are present, the level of parasitemia in these bird populations, and which species are more pathogenic than others (Charles-Smith, L.E. et al, 2014). Given the high prevalence of Haemosporida found in wild turkey populations in Florida and in other bird species populations across the southeastern United States, a high number of samples tested positive for Haemosporida from the TN turkey samples. Genetic sequencing is still underway to determine the genera of parasites in positive individuals. Since a high prevalence of infection was found, the next step would be to determine whether or not presence of infection actually negatively impacts the health and survival of these wild turkey populations.

Research Grant: Unknown

Student Support: Boehringer Ingelheim Veterinary Scholars Program

HIV-induced neuronal hyperactivity and neuropathology in the FIV infection model

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Over half of HIV-infected individuals suffer from HIV-associated neurocognitive disorders (HAND), yet the molecular mechanisms leading to neuronal dysfunction are poorly understood. Feline immunodeficiency virus (FIV) naturally infects cats and shares its structure, cell tropism, and pathology with HIV, including wide-ranging neurological deficits. We employ FIV as a model to elucidate the molecular pathways underlying HIV-induced neuronal dysfunction. HIV envelope glycoprotein gp120 triggers elevation of intracellular Ca^{2+} activity in neurons, stimulating various pathways to damage synaptic functions. By quantifying neuronal Ca^{2+} activity in hippocampal neurons, we confirm that FIV envelope glycoprotein gp95 also elevates neuronal Ca^{2+} activity mediated by the chemokine receptor CXCR4, synaptic NMDA receptors (NMDARs), and importantly, cGMP-dependent protein kinase II (cGKII). These results provide a novel mechanism of cGKII-mediated synaptic hyperexcitation in HAND. In addition, FIV-infected feline hippocampal tissues can be stained using the Se10 antibody for amyloid beta ($\text{A}\beta$) and preliminary results may show increased $\text{A}\beta$ in these tissues. Secretion of $\text{A}\beta$ is first promoted by neuronal hyperactivity and its accumulation is associated with synaptic dysfunction and neurodegeneration. This may indicate that cGKII-mediated hyperactivity leads to increased levels of $\text{A}\beta$ in the brain of FIV-infected cats. Finally, we employ ^{18}F FDG-PET/CT imaging of uninfected cats as a preliminary study to be used in the quantification and rescue of brain hyperactivity in FIV-infected cats. These results provide excellent evidence for the use of FIV as a model of HIV as well as providing novel therapeutic targets for HAND.

Research Grant: Colorado State University Department of Biomedical Sciences funds

Student Support: AVMA/AVMF

Survey study to estimate *Trypanosoma cruzi* infection prevalence in domestic dogs and kissing bugs in Oklahoma

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Trypanosoma cruzi is the arthropod-borne protozoan causing Chagas disease in canines and humans in the Americas. Clinical manifestations of the disease range from asymptomatic to acute myocarditis and sudden death to chronic disease with eventual heart failure. Recent data estimating *T. cruzi* infection prevalence in domestic dog populations within the state of Oklahoma are lacking. Also limited are data estimating infection prevalence in native kissing bug species (family Reduviidae, subfamily Triatominae). Here, we surveyed shelter dogs ($n = 55$) in Tulsa, OK for *T. cruzi* reactive antibodies by ELISA (Chagas STAT-PAK Assay) and FFPE cardiac tissues from client-owned dogs diagnosed with myocarditis ($n = 89$) at the Oklahoma Animal Disease Diagnostic Laboratory (OADDL, Stillwater, OK) for molecular evidence of *T. cruzi* microsatellite DNA. In addition, we analyzed submitted kissing bugs ($n = 7$) from several counties in Oklahoma for evidence of *T. cruzi* DNA in insect abdominal extracts, as well as for the molecular identification of remnant vertebrate blood meal source. Seven of 55 serum samples (12.7%) from shelter dogs estimated to be one year of age or greater were positive for antibodies against *T. cruzi*, and one out of 89 cardiac samples was positive for *T. cruzi* DNA. One of 7 submitted kissing bugs (14.3%) was positive for *T. cruzi* DNA; one individual kissing bug each was positive for human and canine DNA. Collectively, these serologic and molecular data collected from shelter dogs and client-owned dogs, respectively, and molecular data from volunteer submitted kissing bugs suggest that *T. cruzi* may be an under recognized pathogen cycling in nature in north-central Oklahoma.

Research Grant: None

Student Support: PetSmart Charities

Role of estrogen receptor in the gestational upregulation of AT₂R in rat uterine arteries

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Maintenance of a successful pregnancy acquires major adaptations of the uterine arteries (UA), leading up to a forty-fold increase of the uterine blood flow. However, the mechanisms effectuating these changes are not fully understood. Studies with pregnant rats demonstrated that the increase in uterine blood flow is mediated by an upregulation of the angiotensin receptor type II (AT₂R) and that estradiol (E₂) dose dependently upregulates AT₂R mRNA in UA. Thus, we hypothesize that E₂ induces upregulation of AT₂R during pregnancy via a mechanism depending on specific estrogen receptor subtype. To test this, we performed an *ex vivo* study on virgin, pregnant at different days of gestation and post-partum Sprague-Dawley rats. We determined plasma E₂ levels by ELISA and expression of estrogen receptor alpha (ERα) and beta (ERβ) mRNA in UA by RT-qPCR. ERα did not change, but ERβ significantly increased as pregnancy progressed (*P*-value < 0.05), with a peak-value near-term and normalized to pre-pregnant levels after delivery. This pattern mirrors the gestational increase of E₂ and correlates well with the AT₂R upregulation (*R*² = 0.8495), which suggests that E₂ upregulates AT₂R through ERβ. To confirm this, we treated UA isolated from virgin and gestation day 20 pregnant rats *ex vivo* with vehicle and E₂ (100 nM) with or without the specific ERβ antagonist PHTPP (10 μM). Results showed that E₂ induces a significant increase in ERβ mRNA in pregnant UA, which is blocked by PHTPP. Whereas in nonpregnant UA E₂ induces no change in ERβ expression. Currently we are examining if PHTPP also blocks the E₂-induced AT₂R increase in pregnant UA, to establish the necessity of ERβ for AT₂R upregulation during pregnancy.

Research Grant: HL119869 and HL134779 from the National Heart, Lung and Blood Institute (NHLBI)

Student Support: Boehringer Ingelheim Summer Scholars Program

Genomic analysis of the *SLC3A1/SLC7A9* renal transporter in androgen-dependent cystinuria across canine breeds

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Cystinuria results from impaired renal tubular reabsorption of cystine leading to the loss of cystine and dibasic amino acids (ornithine, lysine, and arginine), known as COLA, in the urine. Cystine precipitates in urine to form crystals and calculi due to its low solubility at neutral to acidic pH. The 2 genes *SLC3A1* and *SLC7A9* form the renal transporter for COLA. Of the 3 types of cystinuria we previously characterized, the mechanism for androgen-dependent type III cystinuria, which is observed only in mature, intact males, has not yet been elucidated. We compared the genomes of 10 affected, stone-forming dogs across 8 breeds focusing on the *SLC3A1* and *SLC7A9* genomic regions. In the 56kb region containing *SLC3A1*, there are 401 variants with 3 synonymous SNPs and 2 nonsynonymous SNPs. In the 31kb region containing *SLC7A9*, there are 501 variants with 3 synonymous SNPs and 1 nonsynonymous SNP. Despite similar disease presentation, we did not find variants directly associated with these 2 genes common across all of the breeds we sequenced. Several variants were identified in highly conserved regions of the genes, which may affect the control of gene expression. We noted that one English Mastiff haplotype in *SLC3A1* is rare and shared with several dogs of breeds in which cystinuria has been reported. Candidate variants in the English Mastiff and Scottish Deerhound will be genotyped using restriction enzyme and TaqMan assays in other cystinuric dogs of the same breeds. In conclusion, the mechanism for type III cystinuria may be a result of multiple variants across these 2 genes and/or a variant elsewhere in the genome conveying negative long-range androgen effects on the gene expression and function of this renal transporter.

Research Grant: Funded by NIH Animal Referral Center for Hereditary Diseases grant OD010939, the AKC Canine Health Foundation, the Mastiff Club of America, and the Scottish Deerhound Club of America

Student Support: Supported by NIH grant T35OD010919 and a grant from Boehringer Ingelheim

Development of a rapid, affordable system to identify breed-specific progressive retinal atrophy genes in dogs

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Progressive retinal atrophy (PRA) is a disease that affects many breeds of dogs. PRA is a heritable disorder with extensive locus heterogeneity. There are over 50 suspected genes that can cause PRA in different breeds of dogs. There are at least 30 breedclubs that indicate PRA as a disease of concern. Recessively inherited PRA carrier genotypes can present as unaffected, which makes them harder to eliminate from the population. Breeders can run a test for found causative genes in a quicker, cheaper, overall more effective manner than sequencing all of the suspected genes. Microsatellites are highly variable markers near genes that help identify whether affected animals carry the same recessive gene. Microsatellites were examined in order to determine the potential homogeneity of nearby genes of affected dogs. Near candidate PRA genes, microsatellites were chosen from the dog reference genome that have a high Smith-Waterman sequence alignment score (> 500), which is correlated with maximum heterozygosity, which is useful for excluding "innocent" genes. Highly multiplexed microsatellites were PCR-amplified and genotyped by the MSU Genomics core facility. We tested the hypothesis that some microsatellites will share homozygous alleles across all samples of affected dogs. We tested 6 PRA-affected Belgian turvurens. Heterozygosity among microsatellites near eight genes excluded them as being causative; however, near complete homozygosity for a microsatellite near the *BBS2* gene indicates that it deserves further research. Further multiplexes will need to be created in order to assay more genes.

Research Grant: None

Student Support: NIH Grant T35OD016477 to Michigan State University

Effects of prenatal programming on hypertension related genes in the brainstem

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Pregnant women are constantly exposed to a variety of endocrine disrupting chemicals (EDCs) such as bisphenol-A (BPA) and diethylhexyl phthalate (DEHP) during gestation. Exposure occurs because these EDCs are widely used to make plastics and are also found in other everyday items such as lotions or processed foods. Not only do these EDCs affect pregnant women, they are also known to cross the placental barrier, thus affecting the development of the fetus. The aim of this study was to understand how prenatal exposure to EDCs affects fetal programming, specifically of the cardiovascular-brain axis. In this study, pregnant Sprague-Dawley rats were exposed to BPA (5 $\mu\text{g/Kg BW}$), DEHP (7.5 $\mu\text{g/Kg BW}$), and a combination of BPA and DEHP by oral gavage from gestation day 6 to 21. Offspring were then allowed to develop for four months, after which blood pressure measurements were obtained using radiotelemeters. Three months later, the offspring were sacrificed, brain stems removed, frozen and sectioned. The rostral ventrolateral medulla (RVLM) was isolated and RNA was extracted using standard protocols. We are studying the impact of prenatal EDC exposure on the expression of a variety of genes associated with oxidative stress such as NOX2, TNF, SOD, Edn1 etc. We will correlate the changes in gene expression with changes in blood pressure. These results will provide clear evidence that exposure to either BPA, DEHP, or both have effects on prenatal programming that are detrimental to health of the offspring by negatively altering the cardiovascular-brain axis.

Research Grant: University of Georgia Research Foundation

Student Support: Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Analysis of cardiopulmonary values in chemically immobilized white rhinoceros in Kruger National Park

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Cardiopulmonary (CP) responses in chemically immobilized white rhinoceros (*Ceratotherium simum*) are poorly understood. Hence, we designed a randomized crossover study of a group of six sub-adult male white rhinoceros to investigate the CP effects under different immobilization protocols. Etorphine in a dart, or etorphine in a dart followed by IV butorphanol 2 minutes after recumbency, was administered intramuscularly [2.5 mg etorphine, 25 mg butorphanol (1000-1250 kg), or 3.0 mg etorphine, 30 mg butorphanol (1250-1500 kg)]. Additionally, the above protocols were modified by adding azaperone [37.5 mg (1000-1250 kg), or 45 mg (1250-1500 kg)] to the dart. Sampling started at initial recumbency and was repeated at 5-minute intervals for 25 minutes. Arterial blood gases, limb muscle tremors, expired minute ventilation and respiratory frequency were measured at each sampling point. Calculated values included alveolar-arterial oxygen gradient, expected respiratory minute volume (Ve), tidal volume (Vt), oxygen consumption, and carbon dioxide production. Descriptive statistics (mean, standard deviation, range, median, and 95% CI) were used to assess the overall data distribution. Non-parametric analysis (linear regression using ranks and the Wilcoxon Signed Rank test) specifically designed for studies with small sample sizes were used to compare CP values between different protocols and over time within different protocols. The analytical component of this project has been completed and a detailed statistical report has been shared with veterinarians in Kruger National Park, South Africa. A field visit is scheduled for November of 2018 to further discuss, evaluate, and understand the clinical relevance of the obtained results.

Research Grant: None

Student Support: Applied Veterinary Epidemiology Research Group

Molecular prevalence of *Neorickettsia risticii* in aquatic invertebrates in Ontario

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Neorickettsia risticii (formerly *Ehrlichia risticii*) is an obligate intracellular, gram-negative bacteria that causes Potomac Horse Fever (PHF). PHF is a severe and potentially fatal disease which is associated with the signs of fever, diarrhea, depression, toxemia, abortion in pregnant mares, and sometimes laminitis. The bacteria live in parasitic flukes whose life-cycle includes aquatic snails, amphibiotic insects, birds, and bats. Horses appear to become infected with the bacteria by ingesting insects on pasture that carry the bacteria, or by consuming water that contains the metacercaria of the parasitic fluke that are infected with the bacteria. Upon ingestion, the bacteria replicates in the epithelium of the horse's gastrointestinal tract, causing the reported clinical signs. The disease currently exists in pockets in Ontario, near bodies of fresh water. The aim of this research is to detect the presence of *N. risticii* in the intermediate hosts near farms in Ontario that have had positive cases of PHF in order to determine its presence. This will be accomplished by collecting and extracting DNA from aquatic snails and the larva of amphibiotic insects. The presence of *N. risticii* DNA will be determined using nested PCR and primers selecting for the 16S rRNA of *N. risticii* at two different time points in the summer (early and late).

Research Grant: Equine Guelph

Student Support: Andrea Leger Dunbar Summer Research Studentship

Total cell counts of equine bronchoalveolar lavage samples using ImageJ software

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Inflammatory Airway Disease (IAD) and Recurrent Airway Obstruction (RAO) are variants of Equine Asthma Syndrome. Accurate diagnosis affects prognosis, choice of appropriate treatment, and patient welfare. Evaluating neutrophil percentages on cytocentrifuged samples of bronchoalveolar lavage (BAL) fluid aids in diagnosis. No standard location on the cell dot (periphery, center or random) exists for counting cells and results may vary, potentially resulting in misdiagnosis and inappropriate treatment. To determine accuracy of each counting location, total cell counts using ImageJ software are being performed on 54 BAL samples previously counted by each method. To date, 7 samples have been evaluated by comparing neutrophil percentages obtained by the 3 methods to that of the total counts. Preliminary data indicates good agreement between the total counts and each of the three methods with concordance correlations of 0.95 (random vs total), 0.99 (periphery vs total) and 0.98 (center vs total). Based on the initial 7 samples, neutrophil percentages obtained with the periphery method are closest to those of the total counts (mean difference (Md) and standard deviation (sd) = $-0.43 \pm 0.79\%$) followed by the center method (Md and sd = $1.0 \pm 2.38\%$) and the random method (Md and sd = $3.14 \pm 2.67\%$). Small differences in neutrophil percentages could impact diagnoses at or near the established cutoff values for differentiating IAD and RAO. Differential counts done at the periphery may minimize diagnostic errors but further work is needed.

Research Grant: None

Student Support: Atlantic Veterinary College Veterinary Student Research Award

Effect of cocoa flavanols on endothelial nitric oxide synthase (eNOS) expression in brains of aged mice

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Cocoa flavanols improve peripheral vascular function in humans, both acutely and chronically, through increases in the bioavailability of nitric oxide (NO) in circulation. Recent work in aging populations suggests that such beneficial effects on the vasculature might extend into the central nervous system (CNS). Our preliminary work showed that feeding a high-cocoa-flavanol diet in aged mice starting at approximately 12 months old for 6 months prevented age-associated declines in blood perfusion throughout the brain, compared to a control (low-cocoa-flavanol) group. Our current study aimed to determine the mechanism for these beneficial effects. We hypothesized that improved blood perfusion in the high-cocoa-flavanol group was due to higher levels of endothelial nitric oxide synthase (eNOS), the enzyme responsible for NO production. Brains from the original study ($n=5$ cocoa and $n=5$ control) were sectioned in the coronal plane at 40 microns. A 1-in-6 series (with 240 microns separating adjacent sections) was stained using an anti-eNOS primary antibody with Diaminobenzidine (DAB) as the chromogen. Area fraction covered by eNOS-positive labeling was quantified using stereological methods in the granular and molecular layer of the dentate gyrus and CA1 regions of the hippocampus using Image J software. Results showed similar levels of eNOS expression between control and cocoa treated mice for all 3 brain regions. Taken together, results suggest that increased eNOS expression is an unlikely mechanism for increased blood perfusion in the high-cocoa-flavanol group.

Research Grant: Abbott Nutrition (Center for Nutrition, Learning and Memory at the University of Illinois)

Student Support: Office of the Director, NIH, T35 OD011145

***Chlamydia*-activated B cells prime CTL activity that controls tumor growth in mice with HPV-associated cancer**

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B cells, macrophages, and dendritic cells (DC) are professional antigen-presenting cells (APC) that coordinate responses between innate and adaptive immunity. Using tumor antigen-loaded DC to boost endogenous antitumor immunity is well explored, but our lab developed a vaccine platform using *Chlamydia*-activated B-cells (CABs). We now sought to define immune effector mechanisms induced by injection of tumor antigen-loaded CABs in a mouse model of human papilloma virus (HPV)-associated cancer. First, mice were s.c. injected with 2×10^5 TC-1 tumor cells (cells that express HPV-18 E6 and E7 proteins). Unloaded CABs or CABs loaded with E6 and E7 epitopes were i.v. injected 10 days later. Flow cytometric analysis identified significantly increased numbers of tumor-specific CD8⁺ T cells secreting IFN- γ upon antigen in mice treated with antigen-loaded CABs vs. unloaded CABs, and implied CD8⁺ T cell immunity induced tumor regression. To confirm this possibility, we injected HPV-antigen loaded CABs into wild type mice pretreated with antibody that depleted CD4⁺ or CD8⁺ T cells or mice genetically deficient in perforin or NOS2 (identically treated wild type mice and mice administered rat IgG provided controls). Mice were evaluated for tumor growth every other day until study completion or mice met criteria for euthanasia. We saw that antigen-loaded CABs induced full tumor regression in wild types and mice depleted of CD4⁺ T cells or deficient in NOS2, whereas similar treatment did not enhance tumor regression in mice depleted of CD8⁺ T cells or perforin deficient mice. Taken together, our findings revealed HPV antigen loaded-CABs promote increased cytolytic activity in tumor-specific CD8⁺ T cells that mediates tumor regression.

Research Grant: The Stanford University School of Medicine and the Ohio State University College of Medicine provided financial support for this work

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Effects on mitochondrial respiration of rat gut epithelium chronically exposed to angiotensin II

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Hypertension is a growing health concern in the United States, and the number of cases continues to increase each year. Due to this prevalence, the pathophysiology of hypertension has been extensively studied. Our area of interest relates to the over activation of the renin-angiotensin system (RAS) in patients with hypertension, and how this specifically affects gut cells. The gut is often an organ that is overlooked in terms of its importance to hypertension, and this is concerning as it is intimately connected to normal physiologic function. It is unknown how a chronic exposure to angiotensin II can affect mitochondrial respiration of cells within the gut. To determine the effect of angiotensin II on the gut microbiota, an *in vitro* system was developed. Intestinal crypt cells from the proximal colon of Sprague-Dawley rats were isolated to establish primary cell lines. We will test the effects of angiotensin II on these cells by performing a 72-hour exposure, and then running a cell mitochondrial stress test to measure mitochondrial respiration.

Research Grant: Unknown

Student Support: Florida Veterinary Scholars Program

Effects of cells and proteins on cerebrospinal fluid MRI FLAIR signal

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Fluid-attenuated inversion recovery (FLAIR) magnetic resonance imaging (MRI) is an important diagnostic tool for several types of brain lesions because it differentiates between healthy and pathologic tissues, including cerebrospinal fluid (CSF). Healthy CSF appears hypointense, whereas abnormal CSF can appear hyperintense on FLAIR MRI. Two chief pathologic factors affect CSF composition: white blood cells (WBC) and protein. Previous research suggests that proteins have a positive effect on CSF MRI FLAIR signal, but no studies yet have examined the effect of WBC concentration on signal. Understanding these factors can improve the diagnostic utility of FLAIR for neurological diseases. In this study, we investigated the effects cells and protein have on FLAIR MRI signal *in vitro* and, retrospectively, *in vivo*. We hypothesized that there would be a synergistic interaction between cells and proteins and this would be reflected in FLAIR MRI signal intensities. Our *in vitro* results indicated that proteins had a positive effect on CSF MRI FLAIR signal ($P < 0.001$) and cells had a slightly negative effect ($P < 0.001$). Our *in vivo* results also indicated that proteins had a positive effect on CSF MRI FLAIR signal ($P=0.009$), but WBC concentration did not have a significant effect ($P=0.611$). Patients with infectious inflammatory, noninfectious inflammatory, neoplastic, idiopathic, and other diseases differed in their CSF protein and WBC concentrations, but did not differ in their CSF FLAIR signals. Thus, protein concentration seems to be the predominant factor increasing CSF MRI FLAIR signal intensity, and WBC concentration does not seem to contribute. Clinically, CSF MRI FLAIR signal intensities alone cannot differentiate disease types.

Research Grant: None

Student Support: Boehringer-Ingelheim Animal Health

The effect of simvastatin on CD44 shedding in canine osteosarcoma

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Osteosarcoma (OS) is the most common primary bone tumor in dogs. Despite multimodality therapy with surgery and chemotherapy, 90% of dogs develop distant metastases and die from disease progression. Thus, there is a need to identify novel therapeutics that slow metastatic progression and increase survival time. Simvastatin belongs to a drug class called statins and is used primarily for controlling hypercholesterolemia in people. Some studies suggest that statins also exert anticancer effects. These effects may be mediated through shedding of CD44, a transmembrane receptor that normally mediates cell adhesion by interacting with hyaluronan and also contributes to tumor progression and metastasis. Simvastatin stimulates CD44 shedding in human cancers and decreases cell migration, but this effect has not yet been evaluated in veterinary oncology. We hypothesize that simvastatin stimulates CD44 shedding in canine immortalized OS cells. Following simvastatin treatment for 48 hours at concentrations of 100 nM, 1 μ M, and 10 μ M, flow cytometry was used to assess CD44 expression on the canine OS cells. Exposure to simvastatin decreased median fluorescent intensity of detectable CD44 in treated cells compared to untreated controls. ELISA will be used to quantify the amount of CD44 that is shed, and a transwell assay will be used to assess the effect of simvastatin on cell migration. In addition, an outcome-linked tissue microarray will be used to evaluate the relationship between basal CD44 expression and patient clinical outcomes. Demonstrating that simvastatin induces CD44 shedding would make it a promising candidate for future *in vivo* studies.

Research Grant: Internal Research Funds

Student Support: Office of the Director, NIH, T35 OD011145

Impact of gut microbiota on oligodendrocyte development and maturation

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Under healthy conditions, the central nervous system and gut microbiota have a symbiotic relationship involving bidirectional communication. Gut dysbiosis can negatively impact brain function and behavior and is associated with diseases such as Autism spectrum disorders (ASDs). Dysfunction in the prefrontal cortex (PFC) along with alterations in white matter (WM) tracts have been demonstrated in ASD patients; however, the cellular mechanisms underlying these phenomena remain elusive. Unlike rodents, neonatal piglets possess gyral WM in the PFC and share a similar WM developmental trajectory to their human counterpart. The aim of this study is to determine the impact of gut microbiota on the production and maturation of WM producing cells-oligodendrocytes. Germ free (GF) piglets were compared to normal piglets during early postnatal brain development-a time of rapid brain growth. Our findings revealed that GF piglets weighed significantly less than controls, though their presumably delayed growth was not at the expense of brain weight as there were no differences between the two groups. We found no significant differences in gliogenesis within the subventricular zone in GF conditions; however, GF piglets displayed a significant reduction in the number of mature oligodendrocytes in the PFC. Because mature oligodendrocytes are capable of myelinating axons, reduced numbers may be indicative of reduced myelination in the PFC. Further analyses will be necessary to determine if the reduction in mature oligodendrocytes is due to cell death, or delayed/impaired maturation. Together, our findings demonstrate that gut microbiota influences oligodendrocyte maturation in the PFC, a critical brain region often altered in ASDs.

Research Grant: None

Student Support: Boehringer Ingelheim Veterinary Scholars Program (BIVS)

Cloning and expression of feline calicivirus P domain protein

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Feline Calicivirus (FCV), a single-stranded positive sense RNA virus of the family *Caliciviridae*, is a highly infectious and genetically diverse virus. FCV mainly causes respiratory disease in cats. Outbreaks of the related virulent systemic FCV, which causes disease of greater severity, occur throughout the world. Our recent sequencing data indicated that currently circulating FCV strains showed only 80% nucleotide identity to those deposited into GenBank. The high percentage of nucleotide disparity will possibly contribute to false negatives in conventional molecular diagnosis, and vaccination failure in cats. The viral P protein forms a protruding domain of the FCV capsid and is responsible for immune recognition and host receptor interaction. The goal of this project is to clone and express P domain protein of FCV. We produced two different constructs: one with the hinge region that connects the S and P domain of the capsid, and one without the hinge region. The constructs were expressed via the pGEX-4T-1 vector in *E. coli* strain BL21. Recombinant proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The recombinant protein will be purified using chromatographic methods. This work provides the foundation for FCV diagnosis and perhaps disease prevention through vaccination.

Research Grant: University of Illinois College of Veterinary Medicine

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Development of novel vaccines and immunological reagents for pathogenic *Brucella* species

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Highly pathogenic *Brucella* species include *B. melitensis*, *B. abortus*, and *B. suis*. These organisms cause brucellosis, the most common zoonotic disease in the world. Currently, no vaccine exists for humans and licensed veterinary live attenuated *Brucella* vaccine strains provide incomplete protection, interfere with diagnostic tests, and are pathogenic to humans. Our ultimate goal is to develop safe and cross-protective vaccinations for humans and animals against brucellosis. To accomplish this, we are developing a novel vaccine platform using a viral vector delivery system expressing *Brucella* high value target antigens BatE, BatF, and Omp25. BatE and BatF are autotransporter proteins involved in host colonization, while Omp25 is an outer membrane protein previously shown to provide protection in subunit vaccines. The goal of this project is to develop immunological reagents to validate viral constructs expressing *Brucella* vaccine targets and immune responses evoked by vaccination with these constructs. To achieve this, gene fragments encoding BatE, BatF, and Omp25 proteins were cloned in the expression plasmid vector pETcoco-1, which specifies an N-terminal His-tag for affinity purification. Recombinant proteins were overexpressed in *E. coli*, purified under denaturing conditions, refolded, and used to generate murine antibodies (polyclonal and monoclonal) specific for BatE, BatF, and Omp25. These antibodies will be used to verify expression of the vaccine targets by the viral vector delivery system and to study expression of these high value antigens by *Brucella* species during infection. Recombinant proteins will also be used as reagents to study immune responses elicited by vaccines (e.g. antigen recall assay, ELISA).

Research Grant: GALVmed-AgResults Brucellosis Prize awarded to ERL.

Student Support: NIH Office of Research Infrastructure Programs, Grant Number 5T35OD010433-12

BONSAI 2: Effect of Non-steroidal anti-inflammatory drugs on bone infection and response to antibiotic therapy

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Non-steroidal anti-inflammatory drugs (NSAIDs) are used in orthopedic medicine for management of pain and swelling with fractures, but these drugs can produce adverse effects toward bone healing. Inflammation facilitates the process of fracture repair through important cytokines and prostaglandins, while NSAIDs target this pathway by blocking cyclooxygenase 1 and 2. The objective is to test if concurrent administration of NSAIDs with antibiotics would reduce the body's ability to combat implant-related infection. Six groups of six skeletally mature female rats underwent surgical screw implantation at the proximal portion of the tibia; two groups received sterile screws and remaining screws were inoculated with *Staphylococcus epidermidis* to induce osteomyelitis. The infected group received the following regimens: NSAIDs [Carprofen] with antibiotics [Rifampicin, Cefazolin] (A), only NSAIDs (B), only antibiotics (C), or no medication (D). The non-infected group received either only NSAIDs (E) or no medication (F). Micro Computer Topography was performed immediately after operation and subsequent days after surgery to compare temporal changes in the percent of bone-implant-contact (BIC) and of bone-volume-to-total-volume (BV/TV). Post mortem suspensions from the screw and surrounding tissue are cultured to quantify bacterial loads. Results indicate all infected rats reached lowest BIC% day 14, then increase toward baseline, except group A, which was lowest at day 28. BV/TV% dipped below baseline for all infected rats; group C and D were able to recover by day 28, but group A remained below this level. Cultures indicated 5/6 rats from group C were able to clear infections, while 5/6 rats from A were still infected.

Research Grant: AO Trauma

Student Support: Western University of Health Sciences, Office of Research & Biotechnology (intramural funding)

Intestinal stem cell reparative response to severe ischemic injury

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Ischemic injury is a common life threatening gastrointestinal condition. Illness and death occur when the intestinal epithelial protective barrier is damaged. Two intestinal stem cell populations (ISC), actively cycling (aISC) and reserve (rISC) constantly renew the epithelial barrier. Interestingly, immediately following severe ischemic injury, the number of aISC decreases whereas rISC are maintained. Preliminary data demonstrates that despite this loss of aISC, the epithelium can fully regenerate. However, the dynamics that control this recovery process, and the contribution of each ISC population, is not known. We hypothesize that rISC are the source of epithelial regeneration following severe ischemic injury. We therefore aim to identify the peak time point of ISC activation to determine which population contributes to the reparative process. 8-10 week old Yorkshire Cross pigs were subjected to reversible mesenteric vascular occlusion for 3 and 4 hours (hr), then allowed to recover for 18, 40, or 72 hr. Villus height and crypt depth were measured. ISC activation was evaluated using immunofluorescence with protein biomarkers SOX9 and HOPX to identify aISC and rISC, respectively. Co-localization with Ki67 was used to determine ISC proliferation. Crypt depth increased at 40 and 72hr recovery. The numbers of proliferating aISC increased at 18hr but were reduced at 40hr recovery. Interestingly, the number of proliferating rISC increased at both 18 and 40hr of recovery. This data suggests rISC are critical to regeneration and may be the source of new aISC and crypt expansion. Data gathered from this study will help determine the contribution of each ISC population to the regeneration of severe ischemic injured intestine.

Research Grant: Research Grant: NIH K01 OD019911 (LMG)

Student Support: Student Support: Boehringer Ingelheim Veterinary Scholars Program and NCSU CVM

Quantitative PCR assay development for the detection of *Ambystoma tigrinum tigrinum* in eDNA samples

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The presence of animals can be detected by the DNA from cells that they shed in their environment. This environmental DNA, or eDNA, is low- quantity and low- quality DNA that can be detected by species- specific quantitative PCR (qPCR) assays. These assays target and amplify sequences in a target animal's DNA; making this method useful for sensitive, non-invasive detection of elusive, endangered animals like the Eastern Tiger Salamander (*Ambystoma tigrinum tigrinum*). We developed a qPCR assay to detect the presence of *A.t.tigrinum* in eDNA samples collected from areas of predicted species occurrence. The mitochondrial genome (mt-DNA) for *A.t.tigrinum* was targeted for species-specific assay development and analyzed with NCBI BLAST. The genes NADH dehydrogenase subunit 5 (ND5) and NADH dehydrogenase subunit 6 (ND6) had the greatest differentiation between *A.t.tigrinum*, salamanders in the Ambystomidae family, and other New York-native species. Candidate PCR primers and probes were developed from ND5 and ND6 using PrimerBlast, NCBI Primer BLAST, and Primer3Plus. Candidate assays were evaluated *in silico* for species specificity by individually analyzing probes and primer sets using NCBI PrimerBlast, until a final assay with predicted optimal specificity was achieved. Further *in vitro* testing of the assay against tissue samples of all New York Ambystomidae will determine whether the PCR primer set and probe were species specific. Our final optimized assay will be tested for sensitivity on eDNA samples collected from *A.t.tigrinum* breeding pools during May-July 2018. If successful, this test could improve conservation efforts and develop a better understanding of the geographic distribution of *A.t.tigrinum* along the east coast.

Research Grant: New York State Department of Environmental Conservation Return a Gift to Wildlife Fund: Environmental DNA monitoring of Amphibian Species and Pathogens

Student Support: Cornell University VIP Program, National Institute of Health Training Grant

The transcriptional response of endothelial cells to statin treatment *in vivo*

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Statins inhibit hydroxymethylglutaryl-CoA reductase, an enzyme in the cholesterol synthesis pathway, and are widely prescribed for cardiovascular risk reduction. Besides lowering cholesterol, statins have been proposed to further reduce cardiovascular risk via other pleiotropic anti-inflammatory and pro-angiogenic effects, potentially mediated through the endothelium. To study the effects of statin treatment on the endothelium, 3 male mice were treated orally with atorvastatin daily for one week, and 3 untreated males were used as controls. To characterize changes in the endothelial cell (EC) transcriptome induced by statin treatment, we applied the Translating Ribosome Affinity Purification (TRAP) technique, tagging the ribosomal protein RPL22 with hemagglutinin (HA) in all ECs in the mouse *in vivo* by crossing a conditional Rpl22-HA allele with a Tek-Cre recombinase transgene. After cycloheximide perfusion to freeze polysome:mRNA complexes, tissues (brain, heart, kidney and liver) were harvested. EC-specific mRNA was isolated through immunoprecipitation and subjected to high throughput RNA sequencing analysis. In addition to the identification of pan-endothelial and vascular bed-specific EC transcripts, unsupervised hierarchical clustering of EC genes indicated clear (tissue-specific) effects of statin treatment. Sixty-six genes were affected by atorvastatin in ECs of all tissues, of which several were validated using *in situ* hybridization assays. In conclusion, we are able to isolate endothelial-specific mRNA *in vivo* and observed distinct heterogeneity between expression profiles of different vascular beds, as well as in response to treatment with atorvastatin.

Research Grant: NIH R35-HL135793, American Heart Association, Howard Hughes Medical Institute,

Student Support: Burroughs Wellcome Fund and Howard Hughes Medical Institute Medical Fellowship

The role of the golgi complex in *Trypanosoma cruzi* calcium homeostasis

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The eukaryotic parasite *Trypanosoma cruzi* is the etiological agent of Chagas disease. Ca^{2+} signaling plays an essential role in its life cycle, bioenergetics, and in host cell invasion. In mammalian cells, the Golgi complex (GC) is known to play a heterogeneous role in Ca^{2+} signaling and homeostasis, but little is known about its role in *Trypanosoma cruzi*. The purpose of this study was to characterize the function of the GC in *T. cruzi* Ca^{2+} homeostasis. Recently, a protein termed GDT1 was identified in yeast and mammalian GC as a $\text{Ca}^{2+}/\text{H}^{+}$ antiporter. An ortholog for GDT1 is found in *T. cruzi* and localizes to the parasite GC. To characterize this protein, we deleted TcGDT1 using a CRISPR/Cas9 strategy. We found that the GDT1 mutant grew much slower than control cell lines (wild type and transfected with a scrambled sgRNA). While wild type and scrambled parasites invaded about 24% of the host cells, GDT1 mutant invaded only 4.5% ($n = 3$, $P < 0.03$). Additionally, the replication rate of the GDT1 mutant amastigotes was reduced by 74% when compared to that of control cells. We are planning experiments using a mouse model to test the importance of this protein during *in vivo* infections. Additionally, both yeast and mammalian GDT1 contain an N-terminal signal peptide. But such signal peptide could not be detected in *T. cruzi* GDT1 protein. We are conducting experiments to test the presence of an unconventional signal peptide and to see if its sequence can be exploited to target a genetically encoded Ca^{2+} sensor to the *T. cruzi* GC. Together, these data will determine the role of GC in overall *T. cruzi* Ca^{2+} homeostasis. Additionally, these experiments may also lead to the discovery of new drug targets in *T. cruzi*.

Research Grant: NIH 1008222

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Indirect impacts of invasive Burmese python introduction in South Florida on the spread of Everglades virus

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The direct impacts of invasive predators are usually observable and generally understood. However, indirect impacts, such as disease transmission risk, are more difficult to predict. Everglades virus (EVEV), subtype II of the Venezuelan equine encephalitis complex, is transmitted exclusively by the mosquito, *Culex cedecei*. EVEV is endemic to Florida and can cause nonfatal neurological disease in humans. The Burmese python, *Python bivittatus*, was introduced to south Florida over 20 years ago, and has altered ecosystems by decreasing large and medium sized mammal populations with rodent populations remaining stable. In particular, the population of the hispid cotton rat, *Sigmodon hispidus*, a main reservoir host of EVEV, has remained the same or increased slightly in the Everglades. The hypothesis was investigated that the Burmese python creates an ecological disturbance that increases the contact between the *Cx. cedecei* vectors and the reservoir hosts, thus increasing the prevalence of EVEV in *Cx. cedecei*. The goal of this research was to quantify EVEV mosquito infection in three field sites in Florida classified as high, medium, and low for python invasion. Mosquitoes of *Cx. cedecei* species were collected, pooled, and analyzed for the presence of EVEV using a pan-alphavirus real-time RT-PCR. Data thus far indicate that EVEV is only present in sites with high and medium python invasion. Additional mosquito pool testing and sequencing of the virus detected in the positive mosquito pools are underway.

Research Grant: 2017 UF Opportunity Fund, Emerging Diseases Arbovirus Research and Testing Laboratory

Student Support: Florida Veterinary Scholars Program

Is parenting associated with changes in structural and synaptic plasticity in the prairie vole?

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Prairie voles (*Microtus ochrogaster*) are one of the few monogamous mammalian species to express biparental care to their offspring. Thus, because both males and females contribute to the rearing, it makes it an excellent model to study parenting. Furthermore, this species displays a natural variation in the total amount of parental care devoted to the pups, allowing us to distinguish between high-contact (HC) parents and low-contact (LC) parents. Previous work has established that the offspring of HC and LC parents show differences in behavior, cortical organization, neuroanatomical connections, and gene expression. In this pilot study, we investigate in parents another aspect of neuroanatomical organization: dendritic spine density. The question is whether these differences in parenting style are associated with synaptic and structural plasticity in brain areas related to parenting. To address this question, we will quantify the dendritic spines in two brain regions of HC and LC parents: the medial preoptic area (mPOA) and the primary somatosensory cortex (S1). This will be done using Golgi-Cox staining, a neuroanatomical staining technique. We predict a higher density of dendritic spines in HC parents than in LC parents. We expect some variation between males and females as well. These findings will allow a better understanding of the mechanisms underlying natural variation in parenting styles.

Research Grant: NIH HD071998

Student Support: Boehringer Ingelheim Veterinary Research Scholars Program

Correlation of anti-canine parvovirus serum immunoglobulin G and fecal immunoglobulin A in vaccinated dogs

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Canine parvovirus 2 (CPV-2) causes a highly contagious and severe enteric disease that affects domestic dogs. Prophylaxis of CPV infection relies on modified live virus vaccines that are highly effective at producing long term immunity. Current tests for measuring a dog's level of immunity to CPV depend on measurement of serum anti-CPV-2 antibodies using the serum neutralization assay, hemagglutination-inhibition assay, or ELISA. For dogs with questionable vaccination status or in puppies at any point during the CPV vaccination series, a simple and rapid test using available fecal samples would be a highly useful tool. The aim of the study is to investigate the correlation between serum IgG and fecal IgA levels from dogs vaccinated with CPV. Enzyme-linked immunosorbent assays were used to quantify the levels of serum IgG and fecal IgA in approximately 21 dogs. After coating the plates with mouse anti-canine parvovirus antibody, a canine parvovirus isolate and goat anti-canine IgG or IgA were utilized to form the sandwich immunoassay. Selected samples for IgG and IgA were analyzed using 4 parameter nonlinear regression to determine the best fit dose response curves in the serum and fecal supernatants, respectively, to obtain standard curves. Half maximal effective concentration (EC50) was calculated for serum IgG and fecal IgA from these curves. Antibody titers of serum IgG and fecal IgA were interpolated for the remaining samples using one to two dilution points with OD readings in a range close to the EC50 of the standard curves. Overall, our results suggest that quantification of IgA levels in fecal samples may be a useful marker of immunity against CPV-2 in dogs.

Research Grant: Merck & Co., Inc., USA

Student Support: CVM 2018 Summer Research Scholars Program

Validating Cr-EDTA as an in vivo measure of barrier function in feed-restricted lactating dairy cows

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Objectives were to evaluate intestinal permeability following 5 d of severe feed restriction using the paracellular permeability marker chromium (Cr)-EDTA and to validate the use of plasma Cr concentrations as opposed to total Cr recovery in urine alone. Cows ($n = 6$) were enrolled in a study containing 2 experimental periods (P); during P1 (4 d) cows consumed feed ad libitum and baseline data was collected. During P2 (5 d) all cows were restricted to 40% of their P1 voluntary feed intake. On P1 d 3, P2 d 1 and P2 d 3 1.5 L of a 180 mM Cr-EDTA solution was orally pulsed and plasma was collected hourly for 12 h following delivery. Total urine was collected for 2 cows throughout the study using urinary catheters. By experimental design, DMI decreased (60%) relative to P1 ($P < 0.01$). Relative to P1 milk yield decreased (29%) throughout P2 ($P < 0.01$). Overall and relative to P1, milk fat, protein, lactose content, and somatic cell count were unaffected by feed restriction ($P > 0.10$). Throughout P2, rectal temperature was decreased (0.2°C) relative to P1 ($P = 0.02$). Platelets tended to be decreased (31%) on d 5 of P2 relative to P1 ($P = 0.10$). Urinary Cr recovery did not differ on P2 d 1 or P2 d 3 relative to baseline ($P > 0.10$). Peak plasma Cr concentrations were observed at 4 h relative to Cr-EDTA administration on P1 d3 and P2 d 1 and at 8 h post-infusion on P2 d 3. Relative to P1, plasma Cr area under the curve did not differ on P2 d 1 and tended to be reduced on P2 d 3 ($P = 0.08$). In summary, patterns of urine Cr recovery mirrored plasma Cr concentrations, however, intestinal permeability did not appear to be increased (based upon this evaluation technique) during the first 3 d of feed restriction.

Research Grant: Jacobson Endowment

Student Support: Boehringer Ingelheim Veterinary Scholars Program

MLN4924 (pevonedistat) inhibits growth and induces apoptosis in canine oral melanoma cell lines

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MLN4924 (Pevonedistat), a small molecule inhibitor of the NEDD8-activating enzyme (NAE) has proven efficacy as a targeted therapy drug, disrupting proteasome degradation and DNA replication. MLN4924 induces apoptosis and DNA damage in both round cell and solid tumors, both human and canine. We hypothesize that MLN4924 has an anti-cancer in canine oral melanoma, an aggressive disease that is characterized by rapid tumor progression, high rates of metastasis and poor clinical outcomes. Two well characterized canine oral melanoma cell lines, CML-1 and CML-6M were treated with 0.25 μ M, 0.5 μ M, and 1.0 μ M and drug vehicle (DMSO) for 24, 48 and 72 hours, and quantified via Trypan Blue Exclusion Assay. Four cell lines, CML-1, CML-6M, 17CM98, and CML-10C2 were cultured and treated for 72 hours with varying concentrations of MLN4924 and drug vehicle to determine growth inhibition. IC50 of MLN4924 was determined in all four cell lines via CellTiter 96 Aqueous One Solution Cell Proliferation Assay. CML-6M and CML-1 cell lines were analyzed via flow cytometry for proliferation and apoptosis. MLN4924 exerts significant growth inhibition in all four investigated canine oral melanoma cell lines, with all cell lines displaying susceptibility to the drug at concentrations < 1.0 μ M. MLN4924 acts by inducing cell cycle arrest and apoptosis in CML-6M and CML-1. MLN4924 displays significant inhibition of growth in all investigated canine melanoma cell lines, and induces apoptosis and DNA damage as in human melanoma. These data support further investigation of MLN4924 in patient-derived melanoma samples and *in vivo* studies of MLN4924's anti-cancer efficacy.

Research Grant: Veterinary Cancer Society Resident Research Fund

Student Support: NIH T35OD01107 (PI: Bjorling)

Monitoring for arbovirus in mid-Missouri by local tick sampling and viremia in domestic and wild animals

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Fatal human cases of infection with novel tick-borne viruses have emerged in recent years and continue to increase in incidence. Heartland virus (HRTV), a phlebovirus in the family *Bunyaviridae* was first reported in 2009 and was associated with severe fever, leukopenia, and thrombocytopenia in affected individuals. Bourbon virus, a Thogotovirus in the family *Orthomyxoviridae*, was first detected in 2014. Surveillance studies have identified these viruses in ticks collected around the geographical locations of cases in the states of Missouri and Kansas. Although, limited serological survey demonstrating exposure of wild and domestic animals in Missouri to these viruses suggested that vertebrate hosts may play a role in the transmission cycle, there is paucity of information on active infection of these animals in the state. These types of emerging viruses have already demonstrated a serious threat to human and animal health - and increasing prevalence may be related to the growing popularity of outdoor recreation. It is currently unknown whether these viruses are present in ticks found in peri-urban parks in Missouri. The aim of this study is to determine the incidence of tick-borne *Phleboviruses* and *Thogotoviruses* in diagnostic samples submitted to the VMDL and in ticks collected from urban parks around Columbia, Missouri. Investigation will include (1) retrospective analysis of stored RNA, extracted from domestic and wild animal samples, submitted to the VMDL from 2012-2017 and (2) collection and analysis of ticks from urban parks and recreation areas around the city of Columbia in the summer of 2018. Family, genus, and species-specific RT-PCR will be used to probe tick and animal samples for arbovirus.

Research Grant: None.

Student Support: University of Missouri College of Veterinary Medicine Office of Research

Effect of intravenous Maropitant on blood pressure in critically ill dogs and cats; preliminary results

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Maropitant Citrate (Cerenia), is a neurokinin-1 antagonist approved for the prevention and treatment of vomiting from a variety of clinical etiologies in dogs and cats. Subcutaneous administration has the disadvantages of a relatively long onset of action (45-60 minutes) and eliciting pain on injection. Recent approval of intravenous (IV) administration decreases the time to onset of action but clinical experience indicates that it may have an adverse effect on blood pressure (BP), especially in clinically ill patients. The objective of this study is to determine the effect of IV administration on BP in clinically ill canine and feline patients. Twenty-four client owned patients prescribed maropitant IV were enrolled from the ICU and small animal surgery departments of Lloyd Veterinary Medical Center at Iowa State University. Oscillometric blood pressure (systolic (SAP), mean (MAP) and diastolic (DAP)) was measured in triplicate at baseline, prior to injection, and at 0, 5, 10, 15, and 20 minutes post injection. Data was analyzed using a repeated measures ANOVA with a Greenhouse-Geisser correction to determine the extent of the effect. Preliminary results show a significant decrease in SAP between 10 and 20 minutes ($p < 0.01$). The maximum decrease was at 15 minutes post injection with an average decrease of 9.208 mmHg. By 20 minutes, SAP had recovered to 5.708 mmHg less than baseline. There was also a significant drop in MAP at 15 minutes post injection with an average decrease of 6.694 mmHg ($P < 0.05$). This preliminary evidence demonstrates a significant decrease in SAP and MAP in critically ill dogs and cats that may warrant monitoring of BP during IV maropitant administration and for 15-20 minutes post injection.

Research Grant: Funding provided by Boehringer Ingelheim National Veterinary Scholars Program 2018

Student Support: Funding provided by Boehringer Ingelheim National Veterinary Scholars Program 2018

Comparison of in vitro susceptibility of *Tritrichomonas foetus* to metronidazole and fenbendazole

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Tritrichomonas foetus is a sexually transmitted protozoan that causes reproductive failure in cattle. Trichomoniasis does not cause overt disease but causes early abortion and temporary infertility. This results in a significant decrease in the calf crop coupled with a negative impact on calving distribution, resulting in an overall loss in the operation's revenue. Currently, there is no legal efficacious treatment for *T. foetus*. Metronidazole has been proven effective against *T. foetus* but is currently illegal under the provisions of Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA) and 21 Code of Federal Regulations (CFR). This study aimed to determine if fenbendazole is as effective as metronidazole in the in vitro inhibition of *T. foetus*. The first objective was to characterize a growth curve at various seeding densities of a wild-type strain of *T. foetus*. The second objective was to compare the susceptibility of *T. foetus* to metronidazole and fenbendazole in vitro. Metronidazole or fenbendazole were added at dilutions ranging from 0.5 to 0.0313 $\mu\text{g/mL}$. At multiple time points motile trichomonads were counted with a hemocytometer. Results from the growth curve indicated *T. foetus* reached peak density at an average of 11×10^6 trichomonads per mL. At 0.5 $\mu\text{g/mL}$ metronidazole completely inactivated *T. foetus* by 48 hours; 0.25 $\mu\text{g/mL}$ treatment led to a transient decrease in motility that rebounded by day 7. Lower concentrations of metronidazole also suppressed motility but did not completely inactivate *T. foetus*. At the same concentrations fenbendazole had much less effect on motility. These findings indicate that under the conditions of this study, fenbendazole is less effective than metronidazole to inhibit *T. foetus*.

Research Grant: Boehringer-Ingelheim

Student Support: Boehringer-Ingelheim

Reducing captive bird stress using videos and reward-based positive reinforcement

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Animals welfare is decreased in captive animals that are under stress because it could lead to self-harm behaviors. Neophobia, a fear of new stimuli, can cause stress when non-domesticated animals interact with new people. Birds have the ability to recognize human faces and objects on tablet screens, which opens the possibility of using scheduled reinforcement to modify their behavior towards these novel stimuli. In my undergraduate preliminary study, I showed birds a video of a novel person and paired this with a small food reward. After 21 days of watching the video, the six experimental birds showed a 98% reduction of stress-associated behaviors, which merited further investigation. For the current project, I hypothesize that a tablet-based system playing video and dispensing rewards will train the birds to be calm when encountering new people and novel objects. In the current study, 33 cages each containing male-female pairs of Indian Ring-Necked parakeets were visually isolated from each other. Each cage received a tablet and dispenser. Tablets were randomly assigned a video of Intern A, Intern B, or a novel object. Pre and Post bird reactions to the interns were recorded before and after the 21-day training period. Response to the novel object was scored only after the 21-day training period. By the end of the experimental period, I expect that the birds will show far fewer stress related behaviors in the presence of the person or object featured in the video they watched.

Research Grant: National Science Foundation

Student Support: Texas A&M College of Veterinary Medicine and Biomedical Sciences

Anti-inflammatory properties of firocoxib in equine pregnancy and placentitis

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Placentitis, a leading cause of pregnancy loss in mares, is often caused by ascending bacterial infection. The ensuing inflammatory response to infection, and prostaglandin production, induces preterm delivery. Survival rates in preterm foals are poor; therefore, treatment strategies for placentitis that control infection and inhibit inflammation are vital for foal survival. The goals of this study were: 1. Determine the anti-inflammatory effects of firocoxib, trimethoprim sulfamethoxazole [TMS] and altrenogest administered to mares with placentitis; 2. Determine the efficacy of firocoxib, TMS and altrenogest for improving foal outcome in mares with placentitis. We hypothesized that this treatment would significantly decrease pro-inflammatory mediators in fetal fluids and tissues when compared to untreated, infected mares. We further hypothesized that this treatment would improve foal survival. Placentitis was induced in 13 pregnant mares (DGA 270-300; intracervical inoculum of *S. zooepidemicus*). Mares were randomly assigned to: infected, untreated group (n=6); or infected, treated group (n=7; firocoxib, TMS, and altrenogest administration from clinical signs of placentitis until abortion/delivery). Fetal fluids, placental and fetal tissue samples were obtained at abortion/delivery for assay of TNF- α , IL-1 β , and IL-10, IL-6, IL-8, PGE2 and PGF2 α using ELISA and real-time PCR (data pending). Gestation length and foal survival were significantly higher in treated mares (mean days post-infection = 40; 7/7 live foals; $P < 0.05$) than untreated mares (mean days post-infection = 14; 2/6 live foals). Cytokine and prostaglandin data will further characterize treatment impact on inflammation in mares with placentitis.

Research Grant: Grayson Jockey Club Research Foundation (grant 057845-01)

Student Support: Grayson Jockey Club Research Foundation; UGA College of Veterinary Medicine

Use of intradermal syringe adapter on bovine skin samples

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Tuberculosis testing of cattle is a test of regulatory importance and serves to protect the public health. The test has a known false positive rate anecdotally reported to be 1-4%, yet record inspection reveals cattle marked as ‘suspect’ at rates that suggest under-reporting. Problems of under-reporting may be multifactorial, but this study was constructed to investigate and emphasize the importance of accurate intradermal delivery of the test antigen. We collected skin samples from deceased or slaughtered cattle by harvesting skin from the caudal tail fold and cervical neck, sites involved in the regulatory testing. We enrolled 20 veterinarians and 20 veterinary students as voluntary participants who performed a total of four injections into the skin samples. Using a 1cc tuberculin syringe with 28 gauge needle, each participant injected 0.1mL of green tattoo ink into caudal tail fold specimen using an unmodified syringe and needle. They then injected ink into the same skin specimen with a novel ID adapter attached. Volunteers then performed the same tasks with cervical neck skin. Tissues were harvested in a site-specific manner and were placed in 10% formalin for 48 hours. Biopsies were taken from each injection site and reviewed under histopathology. Each biopsy was viewed to determine which dermal space the needle was placed at time of injection and scored via a numerical system enhanced by visual analog spectrum. Results are still being processed at this time. No discussion available until final reports are submitted.

Research Grant: None

Student Support: Oklahoma State University Center for Veterinary Health Sciences Department of Pathobiology

Protein conservation: urea nitrogen salvage by gut microbiota in the hibernating 13-lined ground squirrel

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Seasonal hibernation in the 13-lined ground squirrel (GS) (*Ichthyomys tridecemlineatus*) involves a prolonged fasting period that affects the gut environment and resident microbiota and eliminates exogenous sources of amino acids. Our previous results showed the microbiota undergoes taxonomic shifts during hibernation, but functional consequences of those shifts for host-microbe symbiosis are unclear. It has been proposed that protein conservation during winter fasting in hibernators is facilitated by urea nitrogen salvage (UNS), the liberation of urea nitrogen by urease-expressing gut bacteria and subsequent host absorption of ammonia or bacterial amino acids, which are then available for protein synthesis. The purpose of this study is to demonstrate UNS in GS and to test the hypothesis that UNS contribution to protein conservation differs in summer and hibernating GS. We used immunoblotting and immunohistochemistry to determine abundance and localization of host UT-B urea transporters and stable isotope assisted labeling using ^{13}C , ^{15}N -labeled urea to detect $^{13}\text{CO}_2$ in GS breath and enrichment of ^{15}N in GS tissues. Preliminary results suggest no difference in UT-B transporter abundance between summer and hibernating GS cecum, indicating persistence of transporter expression despite winter metabolic depression. We predict that after ^{13}C , ^{15}N -labeled urea treatment, tissues from hibernating GS that lack dietary proteins for several months will be more highly enriched in ^{15}N than those from summer GS, when such proteins are plentiful. Comparing UNS in summer and hibernating GS will provide a better understanding of the host-microbe symbiosis with respect to protein conservation during prolonged periods of mammalian fasting.

Research Grant: NSF Award IOS1558044

Student Support: NIH T35 Training Grant T35OD01107 (PI: Bjorling)

Molecular analysis of *Cryptosporidium* spp., *Giardia lamblia*, and *Entamoeba* spp. in asymptomatic humans

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Diarrheal disease is the leading cause of morbidity and mortality in all ages across the globe. Three of the most common and important parasites causing diarrheal disease in humans are *Cryptosporidium* spp., *Giardia lamblia* and *Entamoeba* spp. (Haque et al., 2007). It is becoming increasingly evident that not all infected individuals exhibit overt disease suggesting that species and genotype may impact disease and asymptomatic carriers could potentially serve as reservoirs. With minimal work done to understand the prevalence and genetic diversity of these parasites this study aimed to broaden this knowledge. DNA was extracted from fecal samples collected from 115 asymptomatic human adults from Colombia, Ecuador, and Egypt. For an initial screening of *Giardia*, *Cryptosporidium*, and *Entamoeba*, samples were analyzed using RT-qPCR for the 18S gene. *Cryptosporidium* spp. DNA was detected in 3/115 (2.6%), *Giardia lamblia* in 12/115 (10.4%) and *Entamoeba* spp. in 4/115 (3.5%) of the samples. *Cryptosporidium*-positive PCR products were further analyzed using a nested multiplex PCR for the 18S rRNA and 60 kDa glycoprotein (gp60) genes and DNA sequencing. Two nucleotide sequences were obtained from two Ecuadorian samples and were identified as *Cryptosporidium meleagridis* and *Cryptosporidium canis*. *Giardia lamblia* positive PCR products remain to be analyzed by conventional PCR and DNA sequencing.

Research Grant: National Institutes of Health Intramural Research Program

Student Support: Summer Cancer Research Training Award from the NIH CBSTP, NIAID and NCI

Engineering 3D model to study metastasis of breast cancer to bone

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Breast cancer is the leading form of cancer occurring in women, with 70% of breast cancer metastases developing in bone. 2D tissue culture models poorly mimic the cellular microenvironment and gene signaling networks in human metastasis. The aim of this study is to develop a physiologically relevant 3D tissue culture model to identify the gene networks perturbed during breast to bone metastasis. To do so, human breast cancer cell lines MCF10A (non-metastatic control) and MCF10A-a (highly metastatic), and MG63 (human osteosarcoma) were encapsulated using a photo crosslinking strategy in 5%, 8%, and 10% in Gelatin modified with methacryloyl side groups (GelMA) for optimizing cell viability. Our results showed that 5% GelMA provided the optimal microenvironment condition with > 95% viability. Furthermore, applying Ingenuity Pathway Analysis software (IPA, USA) we performed in silico gene mining to identify signal networks in breast cancer metastasis. When all breast metastatic sites were evaluated through IPA gene expression data analysis the top 3 signaling networks altered were FGFR, EGFR and Keratin filament pathways, whereas, when only breast to bone metastasis was evaluated the top 3 pathways affected were ESR1, EGFR and MAPK1 pointing both to the commonality (EGFR) and specificity (FGFR, Keratin vs ESR1 and MAPK1) of signaling pathways involved in breast metastasis. Overall, the findings from this study demonstrate that 5% photo cross linked GEIMA will be optimal for encapsulation of both breast and osteosarcoma cell lines in a cell responsive microengineered hydrogel environment. The result from this study will contribute to design experiments to understand breast cancer metastasis and therapeutics.

Research Grant: None

Student Support: NIH 5T35OD010991-14

Role of gut metabolome in the colonization of *Clostridium perfringens* in black-footed ferrets

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Clostridium perfringens is an anaerobic bacterium that can cause acute gastroenteritis and endotoxemia in domestic and wild animals, similar to that seen in humans secondary to *Clostridium difficile*. *C. perfringens* type A gastroenteritis is a documented cause of morbidity and mortality in endangered black-footed ferrets (*Mustela nigripes*) housed in captivity. Factors that may indirectly contribute to sporulation of this bacteria and result in clinical disease include stress, parasitism, eating contaminated food items, and over-eating (excess carbohydrates). Host-specific metabolome factors are also suspected, but have yet to be investigated in this species and many gaps still exist in our understanding of the pathogenicity of this disease. To better understand the gut factors potentially involved in the colonization of *C. perfringens*, fecal samples were collected from six healthy (control) and six previously infected (abnormal) black-footed ferrets held at a single institution and submitted for quantification of gut metabolites (amino acids, short-chain fatty acids, bile acids). Interestingly, levels of tyrosine, chenodeoxycholic acid (CDCA), taurochenodeoxycholic acid (TCDCA), taurodeoxycholic acid (TDCA), and taurocholic acid (TCA) were considerably increased in the fecal samples from previously infected ferrets. Results from this study indicate that differences in these gut metabolites exist and they correlate with alterations in the levels of these metabolites in *C. difficile*-infected humans. Future studies should be performed to validate the metabolomics results, which can positively impact the preventive medicine strategies utilized for this species in captivity.

Research Grant: Midwestern University College of Veterinary Medicine Research Fund

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Cultivation and maturation of feline enteroids utilizing trans-well inserts and WRN-conditioned media

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Enteroids, in vitro miniature replicas of the small intestine, provide a medium for interested parties to study enteropathy causing agents, such as *Toxoplasma gondii* or *Cryptosporidium parvum*, in their natural sites of infection. Currently, it is possible to cultivate partially matured enteroids via WRN-conditioned media, TGF- β inhibitor, rock inhibitor, and nicotinamide in a 24-well plate, simulating only the luminal surface of the small intestine. The aim of this study is to develop a method of this cultivation that results in more differentiated enteroids, to the point of goblet cell and paneth cell maturation as well as tubular formation of the small intestinal cells. This will be achieved by utilizing these same 24-well plates with trans-well inserts, with this same media suspend beneath and above the enteroids so that the luminal and basal surfaces of the intestine may be simulated. This method has led to the visual production of mucus, insinuating the maturation of goblet cells. To further support and prove that this differentiation has occurred, western blots and immunofluorescence assays (IFAs) will be performed. These procedures are still ongoing, thus they cannot confirm nor deny that this trans-well method promotes further differentiation than that of the prior method.

Research Grant: None

Student Support: NIH T35 Grant

The role of *Enterococcus faecalis* in the modulation of avian pathogenic *E. coli* infections of poultry

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E. coli and *Enterococcus* spp. co-evolved as commensal microbes in the gastrointestinal tracts of many species including domestic poultry. Interestingly, these bacteria are also frequently co-isolated from young birds with colibacillosis, a fatal septicemic disease. While the role of avian pathogenic *E. coli* (APEC) in colibacillosis has been extensively studied, the contributions of co-isolated Enterococci to the pathogenesis of colibacillosis has largely been ignored. In this preliminary study we characterized 16 pairs of APEC and *Enterococcus faecalis* (EF) co-isolated from colibacillosis lesions using antimicrobial resistance phenotyping, whole genome sequencing, and a chick embryo lethality assay of virulence. Antimicrobial resistance was frequently detected in both APEC and EF isolates with tetracycline resistance commonly shared by co-isolates. However, in APEC, resistance was mediated by tetA and tetB compared to tetM and tetL in EF. Virulence genes identified in APEC isolates varied in number with 1 to 9 identified per isolate. The siderophore receptor *iroN* gene was found in 13 of the 16 APEC isolates. As EF has been shown to facilitate iron acquisition by *E. coli*, co-isolates were cultured as individual macrocolonies with increasing proximity to one another on iron-depleted medium. APEC colonies most proximal to EF macrocolonies exhibited increased growth compared to the more distal APEC macrocolonies ($P \leq 0.01$). Co-infection with APEC and EF increased mortality in the embryo lethality assay compared to APEC or EF alone. These data suggest that EF may play a significant role in modulating APEC virulence during polymicrobial infections of poultry.

Research Grant: Unknown

Student Support: NIH T35 Training Grant T335OD012199

PCR identification of specific BRD pathogens and their integrative conjugative elements

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Mannheimia haemolytica and *Pasteurella multocida* are important pathogens of bovine respiratory disease (BRD). These pathogens have been shown to harbor antimicrobial resistance (AMR) genes to at least three classes of drugs on integrative conjugative elements (ICEs). ICEs have previously been identified through whole-genome sequencing, but a more rapid and affordable method is needed to facilitate field studies of factors that contribute to the dissemination of multidrug resistance (MDR). The purpose of this study was to develop a PCR assay to differentiate between two common BRD pathogens while identifying ICEs they might carry. Two transcriptional regulatory genes (*Pm0762* and *Pm1231*) were targeted to identify *P. multocida*. Two regions of a leukotoxin gene (*lkt*) were used to identify *M. haemolytica* and rule out related species. Primers were designed to target *M. haemolytica* specific ICE amplification (ICE), the *rpoB* gene in *M. haemolytica* (*rpoB*), and a gene encoding an integrative conjugative element protein in both pathogens (ICE4). The PCR assay positively identified *M. haemolytica* isolates known to be MDR. A non-MDR *M. haemolytica* isolate tested negative for an ICE based on PCR results. Primer *rpoB* identified *M. haemolytica* but PCR conditions require further optimization to confirm specificity. The PCR assay detected an ICE in *M. haemolytica* from a calf not treated with antimicrobials, confirming that cattle can harbor bacteria with genetic elements encoding MDR before they receive antimicrobials. Further research is warranted to identify how ICEs are being disseminated in cattle populations, and to determine methods to mitigate their spread.

Research Grant: None

Student Support: Boehringer Ingelheim – Merial

Validity and reliability of heart rate variability measurement to assess stress in horses

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Equine assisted therapy (EAT) is reportedly effective in many human disorders. EAT studies rarely assess horses' stress responses during EAT. Those that do use invasive means to measure cortisol or only do behavior observation, which can be unreliable. Heart rate variability (HRV) can be a measure of physical and emotional stress. HRV has been measured with Polar RR HRV recorders previously. However, prominent T-waves in horses' ECG readings can be mistaken for ectopic beats and distort HRV measured by a Polar RR. Moreover 40% of horses have a second degree AV heart block which could impact the validity of Polar RR HRV readings (Reef, et. al, 2010). The purpose of this study is to test the validity of a Polar RS800CX device against a typical ECG to measure HRV in horses in 3 different settings. We compare HRV data with a validated video behavior recording to measure horses' stress in horses during EAT. We hypothesized that HRV is not significantly different between the Polar RS800CX device and a typical ECG, and that HRV data are an adequate, noninvasive measure of stress. HRV data will be collected with both methods while the horse is tied, free in a field, and during EAT. Data will be analyzed to answer two main questions: to what extent are Polar HRV readings valid and reliable compared with an ECG and to what extent is HRV data reliable to determine stress in horses compared with observed stress behaviors. We expect to find that Polar HRV is not significantly different than an ECG when corrected for artifact via the Kubios HRV premium computer program. We also expect to find that HRV is a valid measure of stress in horses during EAT when correlated with observed stress behavior analysis.

Research Grant: None

Student Support: University of Missouri College of Veterinary Medicine Office of Research and PetSmart Charities

Blood coagulation assessment of captive Asian Elephants (*Elephas maximus*): a pilot study

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One of the most significant causes of captive Asian elephant (*Elephas maximus*) mortality, especially in juveniles, is elephant endotheliotropic herpesvirus (EEHV). Over 80 cases of EEHV-associated disease have been identified since 1995. The virus is named for its specific targeting of the endothelial cell lining of capillaries, resulting in vascular dysfunction, causing hemorrhaging and excessive clotting. Development of new diagnostic tests, which can aid in both diagnosis and management of the disease, are needed. Newer methodologies including, viscoelastography (Sonoclot), thromboelastography (TEG), and rotational thromboelastography (ROTEM) mimic the coagulation process *in vitro*. Coagulation is induced by adding clot stimulators and by placing a probe into the blood sample, which measures resistance as the clot forms. These methodologies, if proven to be feasible in Asian elephants, may prove to be useful for the assessment of EEHV infections. This study plans to investigate the feasibility of Sonoclot, TEG, and ROTEM in healthy, captive Asian elephants. Blood will be collected and fresh samples (without anticoagulant) will be analyzed immediately. The remaining blood will be placed in sodium citrate blood tubes and analyzed at 30 min, 2 hrs, and 4 hrs after being collected. One additional blood tube will be transported to the research facility and analyzed at 24 hours to mimic a routine blood sample delivery to a specialized laboratory. Data will be analyzed and the effect of activator, time, and sample preservation will be investigated. The data produced by this study will provide information regarding optimal methodology and feasibility of these methodologies in healthy captive Asian elephants.

Research Grant: Kirkpatrick Foundation

Student Support: Morris Animal Foundation

Developing a vaccine against *Brucella*

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Brucella is a gram-negative intracellular pathogen that infects both humans and animals and causes a variety of clinical diseases. The three species of human importance are *B. melitensis*, *B. abortus*, and *B. suis*. There is not currently a vaccine that provides complete protection against *Brucella* on the market for animals and there is no licensed human vaccine available. The goal of this study is to create a vaccine for human and animal use that can cross protect against *Brucella* in humans and animals. Parainfluenza Virus 5 (PIV5) has been shown to be safe and efficacious as a vector for human and animal vaccines. In this study two antigens of *Brucella* were cloned into the PIV5 genome. These antigens were selected based on prior research into *Brucella* pathogenesis and are conserved. Recombinant PIV5 viruses expressing these antigens have been obtained, confirmed, and analyzed. In future studies we will test the efficacy of these vaccine candidates in animals.

Research Grant: GALVmed and AgResults Brucellosis Vaccine Competition

Student Support: NIH Office of Research Infrastructure Programs, Grant Number 5T35OD010433-12

Clinical utility of obtaining both right and left lateral abdominal radiographs in cats

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Evidence in veterinary literature supports adopting a three-view abdominal radiographic protocol as a standard of practice in dogs due to the influence of recumbency on redistribution of luminal gas allowing increased visibility of the pyloric antrum and duodenum. This has not been established in cats. This study evaluated antral and duodenal gas redistribution on abdominal radiographs between both lateral views in twenty healthy cats and in a cohort of 110 cats from the LSU imaging database. Radiographs from both groups were reviewed and the location of gas was assessed in the pyloric antrum and duodenum in all views. Comparisons of the frequency of luminal gas were made between the different views and between cats with gastrointestinal disease vs cats without. Additionally, the effects of sedation in the retrospective group and the effects of gastric dilation in all cats were considered. Chi-Square analysis was performed with $P = < 0.05$ being considered significant. The results showed that there was no significant difference in the presence of luminal gas in left vs right lateral views in all cats regardless of the order in which the views were obtained or in cats with GI disease vs cats without. Cats with gastric dilation had a higher overall frequency of gas; those with dilation with homogeneous material were higher than those with heterogeneous material. A higher frequency of gas was found in sedated cats than non-sedated. It was concluded that a three-view abdominal study as a standard of practice may not be necessary in cats, but obtaining the additional left lateral view when gastric dilation is present might allow better visualization of the pyloric antrum and duodenum.

Research Grant: none

Student Support: Boehringer Ingelheim Veterinary Summer Scholars Program

Molecular epidemiology of enteric pathogens among livestock in small-scale Sri Lankan farms

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Community-acquired gastrointestinal infections are an important source of mortality and morbidity in south Asia, and increasing levels of antimicrobial resistance among gastrointestinal pathogens threaten to compromise the efficacy of antimicrobial therapy. Enteric bacteria, including *Campylobacter* spp. and members of the *Enterobacteriaceae* family, are important causes of diarrheal disease and human cases often result from zoonotic transmission. In southern Sri Lanka, small-scale livestock farms are common, and livestock owners and their families live in close quarters with their animals. However, the epidemiology of enteric bacteria and antimicrobial resistance among humans and livestock in this population is poorly understood. This pilot study investigated the prevalence of extended spectrum β -lactamase (ESBL)-producing *Enterobacteriaceae* and *Campylobacter* spp. colonization among livestock and identified risk factors for transmission among livestock caretakers in southern Sri Lanka. Twenty small-scale farms were sampled. Rectal swabs or fecal samples were collected from each species of livestock present on the farm - including dairy cattle, buffalo, goats, pigs, and chickens. One out of 49 samples cultured (2%) for *Campylobacter* spp. tested positive; and eight out of 36 samples cultured (22.2%) for ESBL-producing *Enterobacteriaceae* tested positive. No livestock owners or handlers reported antimicrobial usage among people or livestock on the farm. Most interviewees did not report using biosecurity practices (e.g. foot baths, dedicated farm clothes) or regular farm disinfection. Susceptibility profiles and whole-genome sequences will be obtained from isolates after they are shipped to North Carolina, USA.

Research Grant: Comparative Medicine Institute and Triangle Center for Evolutionary Medicine seed grant

Student Support: NIH T35 Training Grant T35OD011070, NCSU Study Abroad Office Scholarship

Developing a point-of-care diagnostic test for viral hemorrhagic septicemia

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Viral Hemorrhagic Septicemia virus (VHSV), a highly-pathogenic, OIE-listed, RNA virus affects > 40 freshwater and marine fish species, including several with high economic value. Of the four VHSV genotypes (I - IV) identified, a recent variant genotype IVb caused multiple mass fish mortalities in the Laurentian Great Lakes. The gold standard for VHSV detection is virus isolation, which takes up to one month for completion and requires a second validation assay. The present study aims to develop an antibody-based point-of-care diagnostic test for VHSV with similar or better sensitivity to available diagnostic methods. Antibodies were generated by immunizing rabbits and mice with purified VHSV IVb. Two high titer antisera and 15 monoclonal antibodies (mAb), positive for VHSV by ELISA, were selected for further Western Blot analysis. Antisera bound to proteins from all VHSV genotypes but not to mock-infected EPC cell lysates or other fish Rhabdoviruses. Of the 15 mAb tested, supernatants from clones 11C6 and 10D9 bound to VHSV matrix protein (M), and 5C6 and 12H1 bound to glycoprotein G (G) from all genotypes. Clones 11H4 and 12F10 bound specifically to M and Nucleoprotein (NP) of genotype IV, respectively. Studies are underway to develop an ELISA test to detect VHSV in clinical samples. The test will be evaluated for sensitivity and specificity, compared to virus isolation and a standard PCR test, using known positive and negative samples. This assay will ultimately be implemented in a hand-held point-of-care device that can be used for quick detection of VHSV in the field, dramatically decreasing the time necessary for fish health management decisions or regulatory approval for fish movement.

Research Grant: MN Drive: OVPR STEMMA

Student Support: Department of Veterinary Population Medicine

Evaluation of aseptic techniques used to collect bovine milk samples

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Aseptic technique is thought to be vital when collecting milk samples for culture to detect intramammary infection in dairy cows. As aseptic teat preparation techniques have not been evaluated, the objective of this study was to determine if there were differences among teat end preparation techniques with regard to potential contamination of milk samples for culture. Mammary quarter-level foremilk samples were collected from 168 dairy cows. Teat preparation techniques were randomized at the quarter level, such that each teat on a given cow was assigned to 1 of 4 treatment groups: 1) no preparation (control), 2) iodine based pre-dip followed by drying the teat with an individual towel, 3) scrubbing the teat end with an alcohol-soaked cotton pad only, and 4) iodine based pre-dip, drying with an individual towel, and scrubbing with an alcohol-soaked cotton pad. Milk samples were cultured using standard methods, and the number of different bacterial colony morphologies were quantified. A Kruskal-Wallis one-way analysis of variance on ranks was performed to compare the median number of colony types isolated from milk samples among the groups with post-hoc pairwise comparisons made using the Dunn's method. The median (range) number of colony types for groups 1-4 were 3 (0-10), 3 (0-8), 1 (0-7), and 0 (0-6), respectively ($P < 0.001$). Median numbers of colony types for groups 1 and 2 were significantly higher than groups 3 and 4 ($P < 0.001$), but groups 1 and 2 ($P = 1.000$) and 3 and 4 ($P = 0.210$) did not differ from each other. Overall, these data confirm that scrubbing the teat end with alcohol minimizes contamination of the milk sample, but application of pre-dip prior to scrubbing with alcohol may not be necessary.

Research Grant: University of Missouri, College of Veterinary Medicine

Student Support: University of Missouri, College of Veterinary Medicine

A case of necrotizing purulent pleuropneumonia in a White-tailed Deer

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A necropsy was performed on a White-tailed deer with a poor body condition score. Numerous *Amblyomma americanum* ticks were noted. Grossly, a severe necrotizing purulent pleuropneumonia with multifocal abscesses was observed. Lung cultures isolated *Trueperella pyogenes*. *Dictyocaulus viviparus* larvae were seen histologically in the right lung lobes. Multiple whipworms and roundworms were seen within the large intestine. Diagnostic testing was performed to ensure the animal did not harbor disease that could be spread to cattle in the same area. Both Epizootic Hemorrhagic Disease and Chronic Wasting disease diagnostic tests were negative. The animal was found to be a faint positive for *Ehrlichia chaffeensis*.

Research Grant: Auburn College of Veterinary Medicine

Student Support: Merial Summer Scholars Program

Determination of famciclovir concentrations in compounded formulations

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Famciclovir is an anti-viral medication that has revolutionized control of diseases associated with feline herpesvirus, a disease of world-wide significance to the cat population. The recommended dose of famciclovir is 90 mg/kg BID, and compounded forms are commonly employed to achieve this dosing regimen. The purpose of this study was to determine famciclovir concentrations in formulations compounded into oral oil suspension, tiny tabs, chew treats, and oral paste. We hypothesized that all products would be within 10% of the labeled concentration and that concentrations would remain stable over 2 months. Famciclovir was obtained as oral oil suspensions in concentrations of 25 mg/ml to 400 mg/ml and in unflavored, tutti frutti, and anchovy flavors. Additional formulations included fish flavored oral paste in concentrations of 125-400 mg/ml, tiny tabs, and chew treats of varying concentrations. Drug was extracted from each compound, and famciclovir concentration was measured by liquid chromatography tandem mass spectrometry. To date, 6/12 oral suspensions and 3/3 oral pastes formulations had greater than 10% variation in measured famciclovir concentration compared to the stated concentration on day 0. On day 7, 8/12 oral oil suspensions and 3/3 oral paste samples varied by more than 10% of the reported value. On day 14, again 8/12 oral oil samples and 3/3 oral pastes varied by more than 10% of the stated concentration. Values for chew tabs and tablets are pending validation of an extraction procedures. Preliminary results suggest that there is > 10% variability in the concentration of famciclovir in compounded oral oil suspensions and pastes.

Research Grant: Student Training in Advanced Research program at the University of California SVM
and the Bailey Wrigley Fund

Student Support: NIH T35 Training Grant T335OD012199

Effects of long-acting GLP-1 receptor agonist on glucose and insulin metabolism in alpacas

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Camelids with diabetes mellitus-like conditions, such as persistent hyperglycemia or lipid mobilization, currently require intensive care that frequently involves hospitalization and intravenous treatments, such as insulin. It is postulated that camelids' susceptibility to these disorders relates to their relatively poor pancreatic response to hyperglycemia, and that this poor response may in turn relate to low GLP-1 concentrations. Unfortunately, the current intensive treatments are not practical for all camelids, especially when required long-term, and have the potential for complications. This study seeks to address whether supplementing healthy camelids with a long-acting GLP-1 agonist (exenatide) will improve their pancreatic function enough to improve their glucose regulation. By extrapolation, we hope also to demonstrate that long-acting GLP-1 analogs hold promise for the treatment of camelids with clinical illnesses involving deranged energy metabolism. Test subjects included eight, healthy adult gelded alpacas from the Oregon State University teaching herd. A baseline glucose tolerance test was performed on all animals after an overnight fast. Four randomly selected alpacas, forming the test group, were then injected once weekly with 1 mg of extended release exenatide subcutaneously for up to eight treatments. Glucose tolerance tests were repeated on all test subjects nine days after the first treatment and again after resting glucose had dropped. Blood samples were analyzed for glucose, insulin and GLP-1 concentrations. We expect exenatide-treated alpacas will demonstrate improved glucose tolerance, insulin response and overall glucose regulation.

Research Grant: North West Camelid Foundation

Student Support: Boehringer Ingelheim Veterinary Scholar Program

Fatal pneumonia in bighorn sheep lambs: the critical role of *Mycoplasma ovipneumoniae* carrier ewes

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Mycoplasma ovipneumoniae is a primary etiologic agent of pneumonia outbreaks in bighorn sheep (*Ovis canadensis*). Introduction of *M. ovipneumoniae* into bighorn sheep populations results in all-age epizootics. These epizootics are followed by years of recurrent pneumonia-induced mortality in lambs, a phenomenon that significantly impairs population growth. It is hypothesized that ewes that are chronic nasal carriers of *M. ovipneumoniae* transmit this pathogen to their lambs, and initial dam-lamb infections are amplified by lamb-lamb infections within lamb social contact networks, exposing all lambs in the group to the pathogen even if only a small proportion of dams are carriers. Captive bighorn sheep were used to test the hypothesis that the presence of *M. ovipneumoniae* carrier ewes within nursery groups precipitates lamb pneumonia outbreaks. Post-epizootic bighorn ewes (n=6) were longitudinally sampled for *M. ovipneumoniae* carriage over a 2-year period. One carrier and five non-carriers were identified. In 2017, these ewes were placed into two pens, with pen C1 (carrier 1) containing the carrier and one non-carrier, and pen N (non-carrier) containing four non-carriers. Both lambs born in pen C1 developed pneumonia, while the four lambs born in pen N remained non-pneumonic. In 2018 we moved the non-carrier in pen C1 to pen N, and replaced her with two non-carriers previously held in pen N. We also added one additional pen (C2) containing four carriers. We predict all lambs born in the carrier pens will develop pneumonia, while all lambs born in pen N will remain non-pneumonic. If this prediction holds true our hypothesis for the role of carrier ewes in precipitating pneumonia outbreaks in lambs will be strongly supported.

Research Grant: AVMA/AVMF 2nd Opportunity Research Scholarship, Wild Sheep Foundation, US Forest Service

Student Support: WSU CVM Veterinary Student Summer Research Fellowship

Genomic characterization of canine squamous cell carcinomas

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Many spontaneous canine cancers closely recapitulate the clinical presentation, histological features, and molecular profiles of the corresponding human cancers. Consequently, the study of cancer emergence and progression in client-owned dogs not only enables us to better understand and treat canine cancers, but also has the potential to translate into advances in human medicine. Since little is known about the molecular pathogenesis of canine squamous cell carcinoma (SCC), we have commenced genomic analysis of a cohort of 44 SCCs from various anatomical sites. Through histopathological and genomic analysis of each tumor, we can identify and explore potential associations between their cellular appearance, clinical behavior and molecular profile. For example, prior studies have shown that the V595E mutation in the canine *BRAF* gene is detected in approximately 85% of canine urothelial and prostatic carcinomas, both highly aggressive and metastatic cancers. We interrogated our SCC cohort for V595E and observed this mutation in only 5/44 cases (11.4%). Interestingly, all five V595E-positive SCC specimens were obtained from the oral cavity. Histopathological analysis revealed that four of these five tumors had invaded adjacent bone, consistent with more advanced disease. Future work will be directed at screening additional canine oral SCC tumors for V595E, and other mutations commonly found in human oral SCC. This comparative study should provide a deeper understanding of the genomic landscape of both canine and human oral SCC, and ultimately may identify clinically actionable targets that are common to both species.

Research Grant: NC State University Canine Cancer Genomics Fund

Student Support: NIH-T35 Interdisciplinary Biomedical Research Training Program (IBRTP)

Role of annexin A2 in plasmin induced macrophage activation

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Acetaminophen is one of the most frequently used pain relievers and antipyretics. Although this provides great benefit, acetaminophen overdose is the number one cause of acute liver failure in the US. We recently demonstrated that the fibrinolytic enzyme, plasmin, is critical for liver repair after acetaminophen overdose in part through activation of macrophages. The mechanism by which plasmin activates macrophages after acetaminophen overdose, however, remains unknown. In the present, we tested the hypothesis that plasmin directly activates macrophages. Treatment of Kupffer cells, the resident macrophages of the liver, or bone marrow-derived macrophages with plasmin increased expression of several pro-inflammatory cytokines. Upregulation of these cytokines was prevented by the plasmin inhibitor tranexamic acid, indicating that cytokine induction required plasmin enzymatic activity. Recent studies suggested that annexin A2 is a plasmin receptor expressed by macrophages. To determine the importance of annexin A2 in activation of macrophages by plasmin, bone marrow-derived macrophages were isolated from wild-type and annexin A2 knockout mice. Treatment of macrophages from wild-type mice and annexin A2 knockout mice with plasmin increased cytokine expression to a similar extent, indicating that annexin A2 is not required for activation of macrophages by plasmin. Collectively, these findings demonstrate that plasmin activates both hepatic and bone marrow-derived macrophages and that this occurs independent of annexin A2. Further insight into the mechanism by which plasmin activates macrophages could lead to new therapies to stimulate liver repair in patients suffering from acetaminophen induced acute liver failure.

Research Grant: NIH RC105297

Student Support: NIH R25HL103156

The clinical utility of a targeted smart phone application in a veterinary setting

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Calculation of medical therapies such as constant rate infusions (CRIs) with a non-programmable calculator (NPC) is a common source of errors in veterinary and human medicine. These errors are time-consuming and can be harmful, or even lethal, to patients. The objective of this study is to evaluate the efficiency and accuracy of a targeted smart phone application (TSPA) when compared to conventional methods (i.e. NPC) when calculating CRIs. A randomized crossover clinical trial was used to collect data from 48 fourth year veterinary students during their anesthesia rotation. Students were presented two scenarios, ten minutes apart, which both required calculating a CRI. Students were placed in 1 of 4 parallel groups that determined the order these scenarios were presented and the means (TSPA or NPC) by which to complete them. All students completed both scenarios, and used a TSPA and NPC one time. Upon completion, students completed an online survey, providing demographic information and feedback on the TSPA. Duration to complete the calculations and the percent deviation from the calculated drug amount to the correct amount were both analyzed using a paired t-test ($P < 0.05$ considered significant). Median (range) duration of completion was significantly shorter for a TSPA [212 s (101-551)] when compared to a NPC [423 s (104-944); $P < 0.0001$]. Drug calculation errors were significantly higher using a NPC [77% (0-5900)] when compared to a TSPA [1% (0-908); $P < 0.0001$]. Of those who completed the survey, 80% and 95% preferred using TSPAs and thought that TSPAs would be helpful in a clinical setting, respectively. Overall, TSPAs offer a more efficient, more accurate, and safer means of calculating CRIs than conventional methods.

Research Grant: None

Student Support: Boehringer Ingelheim VSP, Texas A&M College of Veterinary Medicine & Biomedical Sciences

Validation of PFA-100 to measure platelet function in equine whole blood and platelet-rich plasma

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Measuring platelet function in blood of horses can be important in disease and to evaluate drug effects on platelets. The PFA-100 is a rapid, inexpensive and simple test of platelet function. This method has been used to assess platelet function in equine blood using protocols validated for human blood. The broad objective of this study is to validate a protocol specific for horse blood and platelet-rich plasma (PRP) using the PFA-100. Our hypotheses are that horse blood must be analyzed within a time frame less than allowable for humans, and that this technology is a valid method for platelet function analysis in PRP. 80 ml of anticoagulated blood was collected from 6 horses and 60 ml was used to prepare PRP. The packed cell volume (PCV), platelet, and white blood cell (WBC) concentrations of blood and PRP were determined using manual techniques. The PFA-100 closure time (CT) was determined at 10, 30, 60, 120, 180, and 240 minutes for blood and 30, 60, 120, and 240 minutes for PRP. Paired t-tests were used to analyze PCV, platelet and WBC concentration in blood and PRP, and CT was analyzed using a repeated measures ANOVA, $P < 0.05$. Preliminary results based on 3 horses are as follows. PRP was successfully generated from whole blood with a significant increase in platelet ($P=0.0128$) and WBC ($P=0.0282$) concentration and a significant decrease in PCV ($P=0.0809$). There was no significant difference in the CT of blood over 4 hours. PRP had a significantly shorter CT at 4 hours than blood. 50% of PRP runs resulted in error messages. Equine blood can be evaluated on the PFA-100 up to 4 hours after blood draw. PRP results in frequent errors and may not be reliable for PFA-100 analysis.

Research Grant: None

Student Support: Florida Veterinary Scholars Program

Role of HMGB1 in angiotensin II induced inflammation: in-vitro study using murine neuronal cells

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Angiotensin II (AngII) is a hormone that regulates the blood pressure homeostasis and fluid balance. Though there have been studies focusing on the inflammatory effects of AngII in the central nervous system (CNS), there is still much to learn regarding the mechanism behind AngII-mediated inflammation. Previous studies have shown that an insult to brain cells induces neuronal release of High Mobility Group Box 1 (HMGB1) and subsequent inflammatory reaction. HMGB1 acts as a damage associated molecular pattern (DAMP) signaling molecule for the innate immune system. With the objective to elucidate the mechanism of AngII induced inflammation in the CNS, we started to look into TLR4 and HMGB1 signaling in AngII challenged Murine neuronal 2a (N2a) cells. To accomplish this goal we treated N2a cells with AngII for 6 as well as for 24 hours. Real time PCR was performed to see the variable mRNA expression of inflammatory markers as well as TLR4 and HMGB1 and found the increased expression of TLR4, HMGB1 and proinflammatory cytokines. We are planning to pretreat N2a cells with Losartan, an angiotensin II receptor type 1 (AT1 receptor) blocker, followed by AngII treatment which should attenuate AngII-induced expression of TLR4, HMGB1 and inflammatory molecules. This study may be confirmed by using TLR4 or HMGB1 silencing approach to check the role of TLR4 and HMGB1 in AngII induced inflammation in N2a cells. Increased expression of HMGB1-TLR4 and proinflammatory markers $\text{TNF}\alpha$ and $\text{IL-1}\beta$ in mRNA expression analysis shows these biomolecules may have important regulatory roles in AngII-induced inflammation in neuronal cells.

Research Grant: American Heart Association

Student Support: LSU School of Veterinary Medicine and NIH T35

Diamond microelectrodes for electroanalytical measurements of catechol amine neurotransmitters and metabolites

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Diamond Microelectrodes have been proven to be one of the most convenient tools for a wide variety of applications in electrochemistry and in the biological environment in general. Current and previous research has already exposed just how beneficial boron-doped diamond electrodes (BDD) can actually be when it comes to performing electrochemical measurements in biological media. Our research is fueled by studies that have shown diamond microelectrodes to have the potential to measure particular neurotransmitters such as dopamine in the brain. To date, scientists have been able to perform in vivo analysis of dopamine in the brains of mice, however scientists have yet to fabricate a microelectrode small enough to perform in vivo analysis of biological tissue without the electrode being invasive to the tissue. The scope of our research focuses on making measurements with different types of electrodes using various electrolytes to examine the responsiveness of these electrodes to three analytes of interest, then comparing their performance to that of the BDD microelectrodes. The systems being investigated are dopamine and some of its metabolites, with a specific focus on the charges associated with each system. The experiments performed will allow scientists as a whole to understand how diamond microelectrodes perform in relation to conventional electrodes when charge is introduced as a variable of interest.

Research Grant: Unknown

Student Support: NIH R25HL103156

Proteomic profiling of Warmblood horses with myofibrillar myopathy

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Myofibrillar myopathies (MFM) in humans are caused by mutations in genes that encode sarcomere Z-disc proteins and present with progressive skeletal muscle weakness. MFM has recently been identified in Warmblood horses (WB) with exercise intolerance based on desmin immunohistochemistry and Z-disc and myofibrillar disruption in muscle biopsies. We hypothesized that specific Z-disc proteins identified through proteomic analysis would be differentially expressed in MFM WB and that their encoding genes would be candidate genes for equine MFM. The objective of this study was to compare proteomic profiles of 5 MFM WB and 4 healthy control WB. Protein was extracted from snap frozen gluteal muscle, digested in trypsin and labeled with isobaric tags (iTRAQ 10-plex) for multiplexed MS/MS quantitative analysis. Proteins were identified with the Equus caballus complement of UniProtKB and significance was determined by a Mann-Whitney test with *P* values adjusted for multiple testing using a Bonferroni correction. 1532 proteins were identified and 105 were differentially expressed in MFM vs. controls ($P < 0.00003$). Gene Ontology (GO) enrichment analysis for cellular processes identified 22 sarcomere proteins ($P = 0.0005$), 15 I-band proteins ($P = 0.026$), 13 Z-disc proteins ($P = 0.041$), and 14 oxidoreductase mitochondrial proteins ($P = 0.047$) that were overrepresented in MFM WB (FDR < 0.05). Proteins encoded by genes known to cause MFM in humans were amongst significantly upregulated proteins and represent candidate genes for further investigation of MFM in horses. With their large muscle mass and selection for athletic performance, WB horses could serve as a unique model for human MFM.

Research Grant: Mary Anne McPhail Endowment

Student Support: AVMF 2nd Opportunity Research Scholarship, MSU CMIB Graduate Group

A genetic-based vaccine overcomes maternal antibody inhibition of immune responses

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Infants are particularly vulnerable to infections and severe disease, including from influenza virus. One increasingly promising strategy to protect them during this period is through maternally derived immunity transferred to the neonate. Maternal antibodies (matAb) can protect the infant soon after transfer but wane over time, leaving the infant vulnerable again. Therefore, active immunity via vaccination must also be generated in the infant. Here, using a mouse model we show that influenza virus-specific matAb inhibit the development of infant antibody responses after infection with live influenza virus or vaccination with inactivated virus. However, a novel mRNA-based vaccine expressing an influenza virus protein was able to generate strong antibody responses in mice that possessed influenza virus-specific matAb, leading to long-lasting protection. This vaccine overcomes matAb inhibition through long-term antigen expression and sustained germinal center activity and does not require cell-surface antigen expression. Together, these results suggest that genetic vaccines can overcome matAb inhibition and elicit potent immune responses in infants.

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Student Support: NIH Training Grant 5-T32-AI055428

Development of a clinical electrophysiological protocol to separate cone subclass function in dogs

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Electroretinography (ERG) is used to differentiate the function of rod and cone photoreceptors by measuring electrical responses to white light stimuli. Although useful to identify rod and cone disorders in dogs and humans, white light ERGs cannot differentiate the function of different cone subtypes. ERG recordings to color stimuli have been reported in humans, and may be useful in identifying cone disorders. The goal of this study was to differentiate the function of long/medium- (L/M) and short- (S) wavelength cone subtypes in dogs using a multicolor electroretinography. L/M and S cone-driven responses were isolated in dogs using sine wave stimuli and silent substitution methods using a ColorDome Ganzfeld stimulator with multicolor LED stimuli. Three normal canines were rented from the NCSU CVM LAR and dark-adapted for one hour. Under general anesthesia, ERGs were performed using different light wavelengths, intensity, and frequency of stimuli. Peak rod driven responses were achieved with low frequency and low luminance stimuli. In contrast, peak L/M cone responses were achieved with high frequency and high mean luminance. Maximal S-cone responses were derived from low frequency stimuli and the maximal luminance achievable with the instrument. These data provide a method to objectively differentiate photoreceptor subclass function, which is challenging to test clinically in nonverbal patients such as dogs. Dogs suffer from similar photoreceptor disorders to humans and form important large animal models in which to develop and test therapies. This work will allow more detailed evaluation of therapeutic effect on photoreceptor function.

Research Grant: None

Student Support: NCSU CVM Veterinary Scholars Program

Comparison of bupivacaine HCl and liposomal bupivacaine for post-operative pain with dental extractions

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Dental disease is one of the most prevalent problems encountered in a veterinary practice. Along with dental prophylaxis, treatment can include extraction of a diseased or broken tooth. A new liposomal bupivacaine formulation has been developed and can last up to 72 hours after administration. The purpose of this study is to compare the effect of bupivacaine HCl and liposomal bupivacaine on post-operative pain following dental extractions in dogs. The hypothesis is that the liposomal bupivacaine will provide superior pain relief and reduce the need for rescue analgesia compared to standard bupivacaine. Dogs were selected for participation in the study based on their need for dental extractions and having only two-rooted teeth extracted. Patients also must have been able to follow a standardized anesthesia protocol and were not having any other procedures done that day. Prior to extractions, infraorbital and/or inferior alveolar nerve blocks were administered with either bupivacaine HCl or liposomal bupivacaine, chosen randomly. Patients were assessed in hospital for pain at six time-points, using the Glasgow Composite Measure Pain Scale-Short Form. The owners then were given a two-day survey regarding perceived pain at home. Out of 25 potential patients, 8 animals were enrolled in the study. Patient exclusion was due to owner decline, comorbidity, cancellations, or not needing extractions. There were 3 male neutered and 5 female spayed participants, ranging from 4.0 kg to 10.0 kg and averaging 8.5 years old. The average number of teeth pulled was 4.5, with the most being 17. Due to pending client surveys at the time of this publication, results have not yet been analyzed.

Research Grant: Midwestern University College of Veterinary Medicine faculty start-up funds

Student Support: Boehringer Ingelheim Veterinary Scholars Program

Blood parameters and oxidative damage in the aging zebra finch

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In the study of aging, avian species offer unique qualities that make them attractive model organisms. In addition to having erythrocytes with functional mitochondria, birds live far longer than mammals of a similar size. Because of these characteristics, avian erythrocytes are particularly well suited to investigate the oxidative stress theory of aging, a theory in which the deleterious symptoms associated with age are hypothesized to be due to the accumulated effects of reactive oxygen species formed during oxidative phosphorylation. The aim of this study was to investigate age-related differences in oxidative damage as well as other blood parameters in Zebra Finches (*Taeniopygia guttata*), a common laboratory model organism and companion animal encountered in veterinary medicine. Older birds were lighter in body mass ($r=-0.39$, $P=0.0026$) with a lower mean cell volume than younger birds (MCV; $r=-0.28$; $P=0.042$). Neither cell counts ($r=0.26$; $P=0.055$) nor the mean amount of hemoglobin per cell (MCH; $r=-0.26$; $P=0.054$) were significantly related to age. The integrity of erythrocyte cell membranes in response to osmotic stress did not change with age ($r=-0.25$; $P=0.076$). Older birds had higher levels of heme degradation products ($r=0.34$, $p=0.0090$), indicating hemoglobin damage by interaction with reactive oxygen species. There was no correlation between age and packed cell volume ($r=-0.22$; $P=0.11$) or total hemoglobin concentration ($r=-0.066$; $P=0.63$). Overall, this study provides insights into several hematological parameters for a common veterinary species as well as a potentially novel method to be used in future studies on the relationship of aging and oxidative damage in wild and captive birds.

Research Grant: None

Student Support: Oklahoma State University CVHS 2018 Summer Research Training Program

Non-immune binding of *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi* to IgG of various species

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In recent years, there has been an increased prevalence of methicillin-resistant *Staphylococcus spp* causing canine pyoderma. Antimicrobial resistance in *S. pseudintermedius* and *S. schleiferi* is widespread and alternative methods of treatment are needed. A better understanding of how *staphylococci* evade their host's immune system may provide the necessary insight for development of new treatments that overcome this limitation. *Staphylococcus spp* employ surface proteins that bind the Fc region of immunoglobulins and negates the opsonizing effects of antibodies. The purpose of this study was to compare the relative amount of IgG bound on the surface of thirteen regionally diverse *S. pseudintermedius* and *S. schleiferi* strains and to evaluate their abilities to bind IgG of (potential) host species. IgG binding assays using flow cytometry were performed with seven strains of *S. pseudintermedius* from dogs and six strains of *S. schleiferi* from dogs and humans. Binding of canine, feline, equine, and caprine IgG and chicken IgY was measured using phycoerythrin-avidin tag and mean fluorescence intensity values were determined. Considerable variations in reactivity to immunoglobulins were seen among the strains. All *S. pseudintermedius* and *S. schleiferi* strains tested showed little binding to chicken IgY and caprine IgG, while 69.2% of the samples showed highest binding to canine IgG. Strains ED-99, 08-1661, and 06-3228 of *S. pseudintermedius* and strains H191 and C15-447 of *S. schleiferi* had the greatest ability to bind IgG of all potential host species. These results suggest the successful clonal expansion of *S. pseudintermedius* and *S. schleiferi* strains is associated with their immunoglobulin binding virulence mechanism.

Research Grant: University of Tennessee Institute of Agriculture Center of Excellence in Livestock Diseases and Human Health

Student Support: Boehringer Ingelheim Veterinary Summer Scholars Program

miR-375 controls intestinal proliferative capacity

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The intestine plays important roles in processes underlying many prominent diseases, including Crohn's disease, diabetes, and cancer. These diseases may result from a disruption of normal epithelial regeneration, which is typically a rapid 3-5 day process. Intestinal epithelial renewal is driven by intestinal stem cells (ISCs). ISC proliferation is partially regulated by microRNAs (miRs), small noncoding single stranded RNAs. Consequently, miRs are important in the development and regulation of intestinal disease. The mechanisms and factors involved in miR-mediated regulation of ISC proliferation are not well understood. miR-375, which is enriched in quiescent ISCs, is responsible for limiting cell proliferation. We have shown that loss of miR-375 leads to an increase in proliferative capacity in mouse enteroids (intestinal crypt cell cultures that form 3D "mini guts"). The mechanism by which miR-375 regulates ISC proliferation is unknown. Previous reports have indicated the JAK/STAT pathway may partially control ISC activity and have linked miR-375 to JAK regulation in other cell types. We hypothesize that miR-375 controls ISC proliferation by regulating JAK/STAT signaling. Intestinal crypts were isolated from the jejunum of wild-type (WT) and miR-375 knock-out (KO) mice and cultured with or without tofacitinib, a JAK inhibitor. Day 5 cultures were analyzed for the presence of enteroids and the number of developing buds, which indicates growth. Histological samples of jejunal tissue were analyzed to determine villus height, crypt depth, and crypt cell density. We expect that miR-375 KO enteroids will proliferate more rapidly relative to WT and that this effect will be lost upon inhibition of the JAK/STAT pathway.

Research Grant: American Diabetes Association Pathway to Stop Diabetes Award 1-16-ACE-47 (Awarded to P.S.)

Student Support: Cornell University Veterinary Investigator's Program

Dose escalation study of subcutaneous sustained-release meloxicam in mice

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Meloxicam, a non-steroidal anti-inflammatory drug, is routinely administered to rodents following minor surgical procedures. Commercially available sustained-release formulations of Meloxicam may offer alternative ways to administer long-term analgesia with less frequent animal handling to reduce stress. However, preliminary data from our group and others suggest the recommended manufacturer's dose of sustained-release Meloxicam does not exhibit typical sustained-release kinetics, and fails to provide adequate pain control for mice following minor surgical procedures. In order to determine what dose of sustained-release Meloxicam would provide therapeutic plasma levels in mice, we conducted a dose escalation study comparing plasma concentrations of Meloxicam in mice receiving Meloxicam-SR and standard Meloxicam formulations as controls. Mice were randomly assigned to 4 groups of 3 mice, each group receiving either 5, 10, 15, or 20 mg/kg of sustained-release Meloxicam by subcutaneous injections. Plasma samples were collected at 4, 8, 12, and 24 hours post injection from different mouse cohorts. An additional group of 3 mice was administered 5 and 10 mg of the standard formulation of injectable Meloxicam, and plasma samples were collected at 2 and 4 hours post injection, serving as controls. The results of our study suggest that sustained-release Meloxicam formulations would need to be administered at doses higher than the current manufacturer's recommended dose to achieve therapeutic concentrations in mice. In addition, plasma concentrations of Meloxicam were inconsistently measurable in mice administered sustained-release Meloxicam formulations.

Research Grant: Unknown

Student Support: Boehringer Ingelheim Veterinary Research Scholars Program

Tear fluid pharmacokinetics following oral prednisone administration in dogs with or without conjunctivitis

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Background. Prednisone is a corticosteroid medication with therapeutic applications ranging from anti-inflammatory (low dose) to immune-suppression (high dose). Given orally, the drug may reach the tear fluid and therefore be useful therapeutically for ocular surface diseases. We aim to determine the pharmacokinetics of prednisone in tear fluid at various oral doses, both in healthy eyes and eyes with conjunctivitis in which leaky conjunctival vessels could lead to higher drug concentrations. *Methods.* Six healthy beagle dogs were administered 4 courses (separated by washout period) of oral prednisone at dosage of 0.5, 1.0, 2.0 and 4.0 mg/kg PO once daily for 5 days. At steady state, tear and blood samples were collected at selected times and samples were analysed by liquid chromatography-mass spectrometry (LC-MS). *Results.* A total of 468 tear samples and 72 blood samples were successfully collected; LC-MS analysis is pending. *Conclusion and relevance.* In-depth knowledge of prednisone pharmacokinetics on the ocular surface will allow clinicians to determine the benefits and risks of using this drug in patients with various ocular diseases.

Research Grant: None.

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The Evaluation of microcirculatory perfusion in healthy horses treated for hypotension

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Measurement of mean arterial pressure (MAP) is used to assess perfusion; however, if MAP is maintained secondary to an increase in systemic vascular resistance, tissue perfusion may actually be diminished. Inadequate perfusion can lead to tissue hypoxia, dysfunction, organ failure, and death, thus perfusion is always a major concern. This is particularly true during anesthesia when normal regulatory mechanisms, such as MAP, are altered. Therefore, direct assessment of microcirculation may be advantageous. The objective of this study was to evaluate the microcirculation using dark field microscopy (Microscan). Using this technology, microvascular perfusion indices (MPI) can be assessed. Vascular beds of the oral and rectal mucosa, and colonic serosa of healthy anesthetized horses were used to evaluate certain MPI's: total vessel density (TVD), perfused vessel density (PVD), proportion perfused vessels (PPV), and microvascular flow index (MFI). Measurements were taken while horses were normotensive, hypotensive, and after hypotension was corrected with cardiovascular support drugs dobutamine (D), vasopressin (V), phenylephrine (P), or norepinephrine (NE). The treatments (V, P, NE) were blinded and randomized. The MPIs at each site, condition, and treatment were compared. The hypothesis is that treatment of isoflurane-induced hypotension with the support drugs will improve microcirculation parameters when compared to the microcirculation during hypotension. Our results indicated that the MPI's decreased with hypotension and that each treatment improved these values to varying degrees. Overall this study provides a better understanding of the effects of hypotension and the therapeutics to improve MAP on tissue perfusion.

Research Grant: For the Love of the Horse Research Foundation, University of Georgia College of Veterinary Medicine

Student Support: Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Investigating the role of cyclin N-terminal domain-containing 1 in regulating meiotic prophase I

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Failure of accurate segregation of chromosomes during meiosis contributes significantly to fetal mortality. Chromosome pairing and segregation is highly dependent on DNA double-strand break formation and repair during prophase I, which allows for crossover and non-crossover events to occur. Each homologous chromosome pair must have at least one crossover event to allow for accurate segregation of chromosomes; however, only a small fraction of double-strand breaks result in a crossover event. Thus, prophase I is a highly regulated part of meiosis and depends on accurate spatial and temporal organization of many proteins. Investigating proteins that designate the fate of double-strand breaks is of special interest, as these proteins allow for successful progression through prophase I. The project focused on using mice to study cyclin N-terminal domain-containing 1 (CNTD1), a protein involved in cell cycle control, allowing for the progression of gametogenesis. CNTD1 knock-out male mice are infertile, as spermatogenesis is impeded in late stages of prophase I. Mice without CNTD1 gene function also express pre-crossover factors for a prolonged period of time compared to wild-type mice and fail to localize post-crossover factors at crossover sites (Holloway et al. 2014). Immunofluorescence of spread adult male mice testis and female fetal ovaries were used to investigate the localization of CNTD1, to see if there is a sexually dimorphic difference in localization of CNTD1 and crossover site designation during pachytene.

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Student Support: NIH training grant 5T35OD010941-09

Prevalence of common tick-borne diseases in White-tailed deer and coyotes in south Texas

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Understanding which wildlife hosts are involved in the enzootic cycles of tick-borne diseases (TBD) enables better surveillance and risk assessment of spill-over to humans and domestic species. Currently, there is limited data on which tick-borne pathogens (TBP) coyotes are infected with. Additionally, while White-tailed deer (WTD) have been studied, populations in south Texas have not been surveyed as extensively. The purpose of this study was to detect current infections of common TBP in coyotes and WTD in south Texas, a trans-boundary region and common site for animal migrations across the US-Mexico border. A novel real-time PCR assay, the TickPath LayerPlex test (patent pending), was used to screen whole blood samples for species from *Borreliales*, *Rickettsiales*, and *Babesia* genuses. Gel-based PCR and sequencing of resulting positive samples confirmed the pathogen species. From 122 coyote samples, 11/122 (9%) were positive for *Babesia canis vogeli* and 1/122 (0.8%) was positive for *Borrelia turicatae*. From 245 WTD samples, 4/245 were positive for *Ehrlichia chaffeensis* (1.6%) and 18/245 (7.3%) were positive for *Theileria cervi*. Positive samples for both species, except for one coyote, were collected from counties located in south Texas along the US-Mexico border. One coyote positive for *B. canis vogeli* was sampled in northern Texas. The results demonstrate the first known *B. turicatae* infection in a coyote and show that coyote and WTD can potentially serve as sentinels for several zoonotic TBD and TBD that affect domestic animals.

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Student Support: NIH T35 Training Grant T35OD010991

Association of canine malignancy with complete blood count and serum biochemistry abnormalities

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With the difficulty of identifying malignancies in veterinary medicine, additional diagnostic signs are very helpful. Several hematologic and serum biochemical abnormalities have been associated with specific malignancies, such as hemophagocytic histiocytic sarcoma in dogs, which shows a pattern of hypoalbuminemia and hypocholesterolemia. The purpose of this study is to find additional malignancies that are likely to have their own characteristic patterns. This can lead to early recognition and a more complete differential diagnosis list in patients. A combined retrospective and prospective study was performed. Patients seen from August 2013 to June 2018 were reviewed for their initial pretreatment complete blood counts and biochemistry profiles. At least 20 patients for each neoplasm: histiocytic sarcoma, mast cell tumor, apocrine gland anal sac adenocarcinoma, and osteosarcoma were required for inclusion in the study. We anticipate that multiple of these neoplasms will have characteristic profiles distinguishing them from normal controls. Additionally, we predict that one or more of the neoplastic processes will be predictable with the development of algorithms based on the hematologic and biochemical profiles.

Research Grant: None

Student Support: Kenneth F. Burns Fellowship

Murine intestinal enteroid cultures and detection of norovirus infection

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Norovirus, a member of the family *Caliciviridae*, is a major cause of acute gastroenteritis worldwide and affects a variety of hosts. In mice, the virus often causes no symptoms, whereas humans exhibit mild to severe gastroenteritis including diarrhea that is often accompanied by vomiting. Studies in mice indicate that dendritic and macrophage cells may be targets of the virus, although the extent of target cell diversity in the gut is still under investigation. Gastrointestinal research in humans is challenging, making the study of murine norovirus infection in mice an attractive model system. Murine intestinal enteroid cultures are composed of multiple intestinal epithelial cell types derived from mouse tissue and have strong physiological relevance to living intestine. The goal of this study was to determine whether murine enteroids, or “mini-guts,” could serve as a 3D *in-vitro* system to examine murine norovirus tropism during infection. These cell cultures have not yet been evaluated for the support of murine norovirus replication, and if successful, could provide insight into how to optimize such cultures for the propagation of human norovirus pathogens. The model could then be used to define and characterize intestinal target cells using immunohistochemistry and qPCR techniques, as well as facilitate the development of antiviral or treatment protocols, none of which are currently available.

Research Grant: NIH/NIAID

Student Support: NIH/NCI Summer Research Training Award

Effects of a manipulated gut microbiota on lung histopathology in a murine model of allergen mediated asthma

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Asthma is one of many chronic inflammatory-mediated disorders increasing in prevalence in industrialized nations over the last several decades. Recent evidence supports an underlying effect of the microbiota, suggesting that shifts in the dominance of different taxa of gut microbes alters immune function and susceptibility to various atopic diseases. In this experiment, we are investigating whether mice which have inoculated with either an ‘at risk’ increased-allergy associated human microbiota will show exacerbated lung histopathology following exposure to an enteric pathogen and repeated challenge with an allergen compared to those exposed to a ‘protective’ microbiota. 36 C57BL/6 mice were divided into 4 groups. Half with were exposed to feces taken from an infant who went on to develop wheezing or eczema and was had high levels of *Shigella*/*Escherichia* and low levels of *Bacteroides*. Half were exposed to feces taken from an infant who did not show signs of wheezing or eczema and had low levels of *Shigella*/*Escherichia* and high levels of *Bacteroides*. Later, mice in each group were repeatedly sensitized intranasally with either house dust mite allergen (HDM) or the sham phosphate buffered saline. Prior to sacrifice, mice were anaesthetized and tested for lung function. Left lung lobes were fixed in formalin, sectioned transversely, embedded in paraffin, and stained for assessment of inflammation, mucus cell metaplasia, and number of eosinophils. It is expected that mice exposed to the at risk microbiota will have worse histopathology following allergen sensitization then those exposed to the protective one.

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Student Support: NIH T35T35OD016477, Boehringer Ingelheim

Evaluation of olfactory learning in mice with perinatal differentiation defects in the olfactory bulb

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Olfactory learning is critical for survival in rodents. It is a continuous process throughout life, and during early perinatal development, is regulated by neuronal stem cells (NSCs) in subependymal zone (SEZ). NSCs give rise to neuroblasts that migrate to the olfactory bulb (OB) where they differentiate into interneurons. Remarkably, this developmental process continues during adulthood whereby interneurons are continuously added to the OB circuitry and are critical for olfactory learning. In addition, NSCs also give rise to glial cells, which are critical for development and maintenance of neuronal tissues including the OBs. Our laboratory has discovered that expression of the epidermal growth factor receptor (EGFR) drives the switch from neurogenesis to gliogenesis. Mice with conditional deletion of EGFR (EGFR-cKO) exhibit severe defects in the OB during the first two weeks postnatal, including massive inflammation accompanied with OB-specific hydrocephalus, which remarkably self-repairs by 3 weeks postnatal. The long-term effect on olfactory learning in EGFR-cKO mice is evaluated by quantitative assessment of basal behaviors as well as appetitive/aversive odor detection and learning. Preliminary statistical analysis showed that EGFR-cKO mice have significantly more rearing but less sniffing/digging basal behaviors. Surprisingly, there is no significant difference in odor detection and learning although a new data set will be added. Mice are sacrificed and immunohistochemical staining of the brains will be performed to detect cell types in the OB by using different cell markers. Studying the role proper postnatal development is essential for understanding olfactory learning and detection of critical odors.

Research Grant: NIH R01 NS098370 and R01 NS089795

Student Support: NC State CVM Boehringer Ingelheim Veterinary Scholars Program

Late Abstract Submission

Screening for *COL5A1* variants responsible for canine Ehlers Danlos syndrome

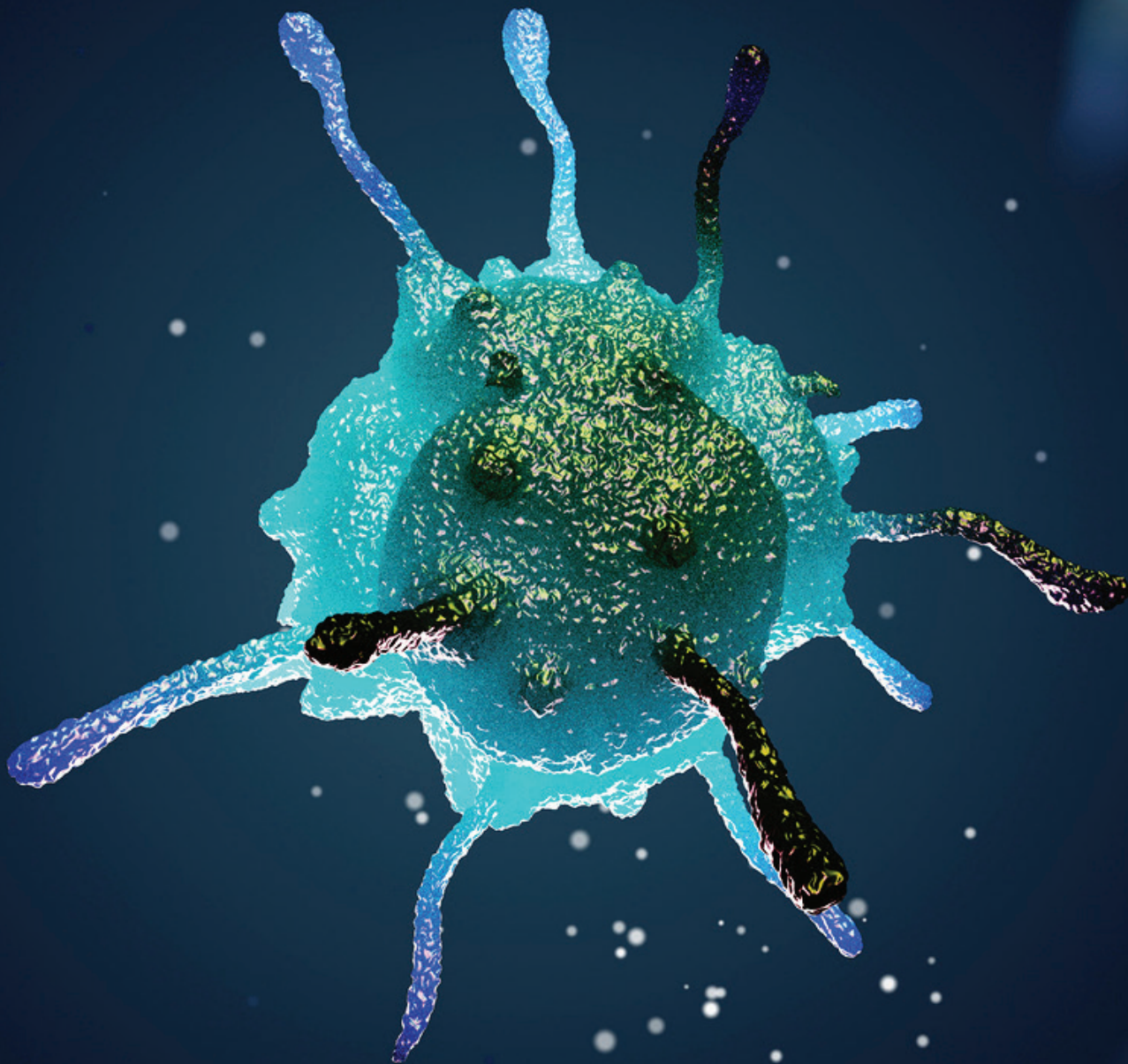
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The Ehlers-Danlos syndromes (EDS) are a heterogeneous group of hereditary connective tissue disorders characterized by joint hypermobility and skin hyper-extensibility and fragility. Our laboratory has been working to identify the molecular genetic causes for EDS by generating whole genome sequences with DNA from affected dogs. This strategy has facilitated our identification of novel causal variant alleles in *COL5A1* in three of six dogs analyzed so far, but budgetary constraints have not allowed us to generate whole genome sequences for all 27 of the affected dog samples sent to our lab from around the world. The focus of this study is to develop a new method to pre-screen DNA from dogs for *COL5A1* variants so that our limited funds for whole genome sequencing can be used to identify canine-EDS causing variants in other genes. We have devised a method for pre-screening for *COL5A1* variants based on a combination of (1) long PCR amplification of *COL5A1* exon clusters, (2) templates of pooled DNA from affected dogs, and (3) Illumina next generation sequencing. So far we have prepared a pool of 25 genomic DNA samples from unrelated dogs with EDS and we are ready to use this pool as a template for long-PCR amplification to produce 1 to 3 kb amplicons each containing from 1 to 5 of the 66 canine *COL5A1* exons.

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