2022 National Veterinary Scholars Symposium

AAVMC
American Association of Veterinary Medical Colleges

NVSS
National Veterinary Scholars Symposium

Contents

Welcome

The 2022 National Veterinary Scholars Symposium, hosted by the University of Minnesota, highlights the essential role of scientific research in veterinary medicine and provides veterinary medical students who have conducted original research through the summer scholars programs an opportunity to formally present their research in a professional environment.

"The University of Minnesota is thrilled to host the National Veterinary Scholars Symposium! The last 2 years with COVID-19 has again confirmed the importance of veterinary scientists in promoting both animal and human health. It's good to be back together in person.

We hope your summer experience has instilled a greater appreciation of research and perhaps inspired you to seek a career that unites medical and scientific skills. Take advantage of this opportunity to share your research with your peers; there's so much to learn!

Thank you to all of the students, faculty mentors, speakers, and sponsors who make this research program and the NVSS possible."

Dean Laura Molgaard, DVM

COLLEGE OF VETERINARY MEDICINE
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The 2023 Veterinary Summer Scholars Symposium will be held in Puerto Rico.
Save the date for August 3 – 5, 2023!

Thank You Sponsors

"Congratulations to all our Veterinary Scholars for their exemplary efforts this summer to further our understanding of animal and human health and welfare, and explore ways their work can transcend boundaries. We look forward to showcasing your research, sharing experiences and learning from each other at this National Veterinary Scholars Symposium, and hope that this capstone event and your summer experience inspire you for many years to come."

Monica Figueiredo, DVM, PhD, DACVIM
Executive Director, Boehringer Ingelheim Veterinary Scholars Program

Boehringer Ingelheim

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Program Schedule

Thursday, August 4	
3:00-5:00 pm	Arrivals, Registration, and Check In
5:30-7:00 pm	Welcome Reception sponsored by Boehringer Ingelheim
Friday, August 5	
7:30-8:30 am	Continental Breakfast
7:30 am-3:00 pm	Registration
8:30-9:00 am	Welcoming Remarks
9:00-10:30 am	Poster Session 1
10:45-11:45 am	Keynote Address: Dr. Vivek Kapur, *SARS CoV-2 Zoonosis*
noon-1:00 pm	Lunch
1:15-2:45 pm	A. AVMA/AVMF Early Stage Investigator Awards Program B. Mentor Panel: Careers in Private Sector (1-hour panel followed by a 30-minute meet-and-greet)
3:00-4:30 pm	Poster Session 2
4:45-6:00 pm	Breakout Sessions A. Emerging Infectious Agents: Discovery and Response B. Comparative and Translational Oncology C. Animal Models of Chronic Diseases
6:00 pm -	Dinner on your own
Saturday, August 6	
8:30-9:30 am	Continental Breakfast
8:30-9:30 am	Registration
9:00-10:30 am	Poster Session 3
10:35-11:45 am	Announcements
10:45-11:45 am	Plenary Addresses: Dr. Danika Bannasch and Dr. Molly McCue, *Applications of Veterinary Genetics*
noon-1:00 pm	Lunch
1:00-1:30 pm	Boehringer Ingelheim Awards Program
1:30-2:30 pm	Mentor Panel: Careers in Academia
2:45-4:15 pm	Poster Session 4
4:30-5:45 pm	Breakout Sessions A. Feeding a Changing Planet B. Advances in Clinical Therapeutics C. Conservation Medicine
6:30-8:30 pm	Reception and Closing Banquet

Speaker Biographies

Dr. Vivek Kapur is a professor of Microbiology and Infectious Diseases and Huck Distinguished Chair in Global Health at the Pennsylvania State University. Additionally, he is the Associate Director for Strategic Initiatives at the Huck Institutes of Life Sciences at Penn State and also serves as an adjunct professor at the Nelson Mandela African Institute of Science and Technology in Tanzania. Dr. Kapur received his veterinary training at the University of Agricultural Sciences, Bangalore; PhD from Pennsylvania State University; and Postdoctoral training from Baylor College of Medicine. He served as a professor at the University of Minnesota until he moved to his current position. Dr. Kapur's lab continues to research public health issues, pathogenic microbes, and the human-animal disease transmission interface. His particular interests are in global health and the mitigation of risk of major animal and zoonotic diseases, including tuberculosis. He has led the USDA's Johne's Disease Integrated Program and Mycobacterial Diseases in Animals consortia for more than two decades. Additionally, his group leads multiple international collaborations on animal health and zoonotic diseases in India, Tanzania, Rwanda, and Ethiopia with funding from the NIH, US Department of Defense, and the Bill & Melinda Gates Foundation.

Dr. Danika Bannasch is a veterinary geneticist who specializes in canine and equine genetics. She obtained her PhD in Molecular Biology from Princeton University and her DVM from the University of California at Davis. She has been a professor at the School of Veterinary Medicine at the University of California at Davis since 2001. She was appointed the Maxine Adler Endowed Chair in Genetics in 2013. In 2019, Dr. Bannasch was awarded the International Canine Health Award for her research accomplishments in canine genetics. Dr. Bannasch has published over a hundred peer reviewed manuscripts, and her laboratory has identified the causative variant for 16 inherited diseases in dogs and horses and collaborated on the identification of 8 more. Her research goals are to improve the lives of dogs through understanding the relationship between their genetic variation and the resulting phenotypes.

Dr. Molly McCue runs an equine-genetics laboratory and serves as the associate dean of research at the College of Veterinary Medicine at the University of Minnesota. Dr. McCue graduated from Kansas State University College of Veterinary Medicine and went on to do an internship in large animal medicine at the University of Georgia before returning to Kansas State in 2001 to pursue a master's and complete a residency in equine internal medicine. In 2007, Dr. McCue moved to Minnesota to pursue doctoral and postdoctoral research, taking a position at the College of Veterinary Medicine as an assistant professor in 2008.

Dr. McCue's research focuses on how the horse can be leveraged as a biomedical model. To do that, her lab spends about one-third of its time building the tools it needs to conduct what's called network biology (understanding a cell's functional organization), which can be applied across species. In 2014, Dr. McCue was named one of six University of Minnesota Informatics Institute Transdisciplinary Faculty Fellows. In 2019, Dr. McCue was nominated and chosen by her colleagues as the recipient of the Zoetis Award for Veterinary Research Excellence, the highest honor the college bestows on research faculty. She has received the University of Minnesota Inventor Recognition Award three times.

Award Winners

AVMA Excellence in Research Award Recipients 2022

- **AVMA Career Achievement in Canine Research Award** recognizes AVMA member's for their long-term contribution to the field of canine research. **Dr. Stanley Marks** received this award for his sustained and vast contributions, often using a collaborative One-Health approach, to understanding and treating dysphagia and enteropathies in dogs.

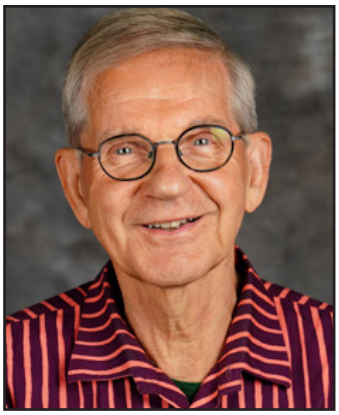
- **AVMA Clinical Research Award** recognizes a veterinarian in recognition of his/her achievements in patient-oriented research, including the study of mechanisms of disease, therapeutic interventions, clinical trials, development of new technologies, and epidemiological studies **Dr. Stephen White** was honored with this award for contributions to the understanding and practice of veterinary medicine by identifying and characterizing novel dermatologic diseases in a wide variety of species, including evaluation of efficacy of novel therapies for treating particular skin diseases.

- **AVMA Lifetime Excellence in Research Award** recognizes a veterinary researcher based on lifetime achievement in basic, applied, or clinical research. This award was bestowed upon **Dr. Yrjö Gröhn** for his use of mathematical modeling and data-driven decision making particularly as applied to agricultural animals and venues.

- **AVMF/EveryCat Health Foundation Research Award** recognizes a candidate's contribution to advancing feline health through their research. **Dr. Mike Nolan** was honored with this award for his research in translational and comparative oncology

Dr. Stanley Marks

Dr. Stephen White



Dr. Yrjö Gröhn

Dr. Mike Nolan

Boehringer Ingelheim Awards

2022 Veterinary Graduate Award

Dr. Ashley Rasys

Dr. Ashley Rasys
University of Georgia

Research title: CRISPR-Cas9 Gene Editing in Lizards through Microinjection of Unfertilized Oocytes

My future aspirations include establishing my own lab at an academic research institution affiliated with veterinary medical college, where I will pursue translational research that will benefit humans and animals alike. As I strive to achieve these goals, I will continue pioneering the lizard as a new modal organism and look forward to tackling new challenges associated with the vertebrate visual system.

2022 Veterinary Graduate Award — Honorable Mention

Rosemary Bayless
North Carolina State University

Research title: Withaferin A inhibits neutrophil adhesion, migration, and respiratory burst and promotes timely neutrophil apoptosis

2022 Veterinary Student Award

Sydney Womack

Sydney Womack
Cornell University

Research title: Multispecies proteomics reveals potential biomarkers for osteoarthritis

Right now, I am interested in pursuing a career as a clinician scientist in academia or working in veterinary biotechnology, potentially starting my own business. Although I have a wide variety of research interests and enjoy most lab activities (especially data analysis!), I am most excited by learning how to approach finding answers to new research questions.

2022 Veterinary Student Award — Honorable Mentions

Lauren Ellison
Mississippi State University

Research title: Transfection of bovine preputial keratinocytes for expression of antibody against Tritrichomonas foetus

Myranda Gorman
The University of Tennessee

Research title: Leptospira enrichment culture followed by nanopore sequencing allows better detection of Leptospira contamination and diversity in water and soil samples.

Abstracts Listed by Area of Research

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Atlantic Veterinary College	Forbes	Brianna	140
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Atlantic Veterinary College	Kauffman	Mary Quinn	226
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Atlantic Veterinary College	Ousley	Carey	359
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Auburn University CVM	Jaros	Megan	212
Auburn University CVM	Meier	Jessica	314
Auburn University CVM	Rossow	Quintan	420
Auburn University CVM	Speights	Kaitlyn	473
Colorado State University CVM	Baughman	Jennifer	25
Colorado State University CVM	Bruening	Anneliese	55
Colorado State University CVM	Droeger	Alice	107
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Colorado State University CVM	Gallagher	Brooke	151
Colorado State University CVM	Greenberg	Sonya	168
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Colorado State University CVM	Leverett	Jack	258
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Colorado State University CVM	Lugo	Emily	275
Colorado State University CVM	Martin	Brittany	295
Colorado State University CVM	McKee	Hannah	310

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Colorado State University CVM	Michalko	Bridget	319
Colorado State University CVM	Mulligan	Lisa	335
Colorado State University CVM	Nelson	Alyssa	343
Colorado State University CVM	Orozco	Paloma	354
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Colorado State University CVM	Rockow	Meagan	412
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Colorado State University CVM	Snow	Aimee	469
Colorado State University CVM	Snyder	Taylor	470
Colorado State University CVM	Sparrer	McKenzie	472
Colorado State University CVM	Swiderski	Maya	486
Colorado State University CVM	Thometz	Mary	495
Colorado State University CVM	Uzan	Olivia	511
Colorado State University CVM	Wilford	Megan	533
Cornell University CVM	Acuna Gutierrez	Sergio	1
Cornell University CVM	Anil	Gayatri	10
Cornell University CVM	Bakhle	Kimaya	18
Cornell University CVM	Cockey	James	75
Cornell University CVM	Fahey	Megan	123
Cornell University CVM	Giudici	Alysa	159
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Cornell University CVM	Hommer	Alexandra	199
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Cornell University CVM	Koch-Laskowski	Kieran	239
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Cornell University CVM	Li	Lynna	261
Cornell University CVM	Loehr	Amanda	269
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Cornell University CVM	Maymi	Viviana	299
Cornell University CVM	Morse	Benjamin	332
Cornell University CVM	Reid	Abigail	399
Cornell University CVM	Ríos-Guzmán	Hery	409
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Cornell University CVM	Schayes	Julia	431
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Cornell University CVM	Varnadoe	Ashley	515
Cornell University CVM	Womack	Sydney	540

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Cummings SVM at Tufts University	Chen	Yuwen	73
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Iowa State University CVM	Bos	Abigail	43
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Iowa State University CVM	Frese	Rylie	144
Iowa State University CVM	Gainer	Sarah	149
Iowa State University CVM	Jorgensen	Hannah	220
Iowa State University CVM	Lopez	Lizeth	270
Iowa State University CVM	Mathias	Claire	297
Iowa State University CVM	McMahan	Krysta	312
Iowa State University CVM	Payne	Jessica	369
Iowa State University CVM	Peterson	Heather	375
Iowa State University CVM	Pyle	Shannon	383
Iowa State University CVM	Rasmussen	Macy	391
Iowa State University CVM	Saboda	Savannah	422
Iowa State University CVM	Scheunemann	Kaitlyn	432
Iowa State University CVM	Slocum-Coffey	Julia	459
Iowa State University CVM	Sterzinger	Nicole	478
Iowa State University CVM	Tapia	Meghan	490
Justs-Liebig-Universität Giessen	Weitkamp	Lennart	525
Kansas State University CVM	Brown	Rachel	52
Kansas State University CVM	Calo	Grace	63
Kansas State University CVM	Cameron-Harp	Kelly	64
Kansas State University CVM	Farleigh	Douglas	128
Kansas State University CVM	Fritz	Bailey	146
Kansas State University CVM	Hamilton	Megan	176
Kansas State University CVM	Jensen	Makenna	214
Kansas State University CVM	Keyser	Abigail	230
Kansas State University CVM	Kopp	Dannell	243

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Kansas State University CVM	Mayhue	Erin	298
Kansas State University CVM	Peng	Jingwen	373
Kansas State University CVM	Robbins	Marilee	410
Kansas State University CVM	Rojas	Catherine	415
Kansas State University CVM	Rollerson-Clark	Franchesca	417
Kansas State University CVM	Timmerman	Sarah	497
Kansas State University CVM	Uribe	Olivia	510
Kansas State University CVM	VanDonge	Kortnee	514
Kansas State University CVM	Wen	Yi	526
Kansas State University CVM	Wilson	Grace	536
Lincoln Memorial University CVM	Fellows	Tara	132
Lincoln Memorial University CVM	Lynch	Savannah	281
Lincoln Memorial University CVM	Medlin	Kayla	313
Lincoln Memorial University CVM	Trent	Domenica	501
Lincoln Memorial University CVM	Turrell	Nathan	509
Long Island University CVM	Shahraki	Roxanna	444
Louisiana State University SVM	Akers	Nicole	7
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Louisiana State University SVM	Tuminello	John	506
Louisiana State University SVM	Windham	Jennifer	537
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Michigan State University CVM	Bahr	Khelsea	15
Michigan State University CVM	Bajric	Shayla	16
Michigan State University CVM	Boger	Brooke	40
Michigan State University CVM	Catala	Miguel	69
Michigan State University CVM	Daniel	Josephine	92
Michigan State University CVM	Davis	Nicole	95
Michigan State University CVM	DeQuattro	Cheyenne	98
Michigan State University CVM	Ferchaw	Hunter	134
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Michigan State University CVM	Hawley	Robyn	185
Michigan State University CVM	Lacuesta	Cyrus Victor	251
Michigan State University CVM	Lindsey	Kylee	267
Michigan State University CVM	Lyons	Courtlandt	282
Michigan State University CVM	Maresca-Fichter	Hailey	291
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Michigan State University CVM	Simmons	Kathryn	452
Michigan State University CVM	Smith	Abigail	460
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Michigan State University CVM	Van Hoof	Cassie	513
Midwestern University CVM	Cugliari	Marlena	89
Midwestern University CVM	Evans	Karly	121
Midwestern University CVM	Feliciano Nieves	Kathia	130
Midwestern University CVM	Felix	Mia	131
Midwestern University CVM	Hurley	Lauren	203
Midwestern University CVM	McCauley	Kayla	302
Midwestern University CVM	Myers	Mikaela	339
Midwestern University CVM	Napoles	Emily	340
Midwestern University CVM	Riar	Navreen	402
Midwestern University CVM	Sheffer	Sara	446
Midwestern University CVM	Tan	Stephanie	489
Midwestern University CVM	Vitello	Emma	520
Mississippi State University CVM	Barber	Jessica	19
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Mississippi State University CVM	Bordages	Jenna	41
Mississippi State University CVM	Brines	Hayden	47
Mississippi State University CVM	Burke	Caitlyn	58
Mississippi State University CVM	Cervantes Linares	Dania	71
Mississippi State University CVM	DiFiore	Julia	101
Mississippi State University CVM	DiNicola	Catherine	102
Mississippi State University CVM	Harjes	Matthew	179
Mississippi State University CVM	Holsinger	Martin	198
Mississippi State University CVM	Ladner	Shelby	252
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Mississippi State University CVM	Mitsch	Matthew	327
Mississippi State University CVM	Mosby	Nicholas	333
Mississippi State University CVM	Nicaise	Ashleigh	346
Mississippi State University CVM	Rapp	Kathryn	390
Mississippi State University CVM	Renfroe	Hannah	400
Mississippi State University CVM	Sherman	Emily	448
Mississippi State University CVM	Swistek	Sabrina	488
Mississippi State University CVM	Tate	Julia	491
Mississippi State University CVM	Wheeler	Nicholas	529
Mississippi State University CVM	Wonnacott	Caitlin	543
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North Carolina State University CVM	Amoriello	Carrisa	9
North Carolina State University CVM	Benedict	William	31
North Carolina State University CVM	Blawas	Megan	39
North Carolina State University CVM	Carrero	Jamirelis	66
North Carolina State University CVM	Cave	Ashley	70
North Carolina State University CVM	Coleman	Shanti	77
North Carolina State University CVM	Edwards	Madeleine	114
North Carolina State University CVM	Elliot	Baxter	115
North Carolina State University CVM	Falto Zeno	Carlos	125
North Carolina State University CVM	Ferraro	Emma	135
North Carolina State University CVM	Heniff	Ashlyn	190
North Carolina State University CVM	Johnson	Haley	215
North Carolina State University CVM	Ludwig	Claire	274
North Carolina State University CVM	Lunking	Vienna	277
North Carolina State University CVM	Lutz	Halle	279
North Carolina State University CVM	Manickam	Sayee Shruthi	288
North Carolina State University CVM	Fabiola	Fabiola	293
North Carolina State University CVM	McIver	Jasmine	309
North Carolina State University CVM	Moore	Jasmine	330

Vet School	Last Name	First Name	Poster #
North Carolina State University CVM	Moore	Logan	331
North Carolina State University CVM	O'Donnell	Kerry	350
North Carolina State University CVM	Olivier	Amanda	352
North Carolina State University CVM	Roberts	Darby	411
North Carolina State University CVM	Sanchez	Kiomara	425
North Carolina State University CVM	Simon	Katherine	453
North Carolina State University CVM	Simpson	Jacob	454
North Carolina State University CVM	Souther	Alexis	471
North Carolina State University CVM	Velez Contreras	Raisa	517
North Carolina State University CVM	Vester	Caitlyn	519
North Carolina State University CVM	Wubbenhorst	Hannah	547
Ohio State University CVM	Behmer	Virginia	28
Ohio State University CVM	Bryant	Ellen	57
Ohio State University CVM	Graham	Brittney	164
Ohio State University CVM	Hegde	Shridula	187
Ohio State University CVM	Hernandez Cuevas	Juan	192
Ohio State University CVM	Keefer	Katelyn	227
Ohio State University CVM	Kimberly	Emma	233
Ohio State University CVM	Li	Becky	260
Ohio State University CVM	Mamo	Esther	286
Ohio State University CVM	McGlynn	Andrew	308
Ohio State University CVM	McKenna	Brandon	311
Ohio State University CVM	Midkiff	Amanda	322
Ohio State University CVM	Satern	Faith	428
Ohio State University CVM	Schrandt	Annaliese	437
Ohio State University CVM	Seabeck	Spencer	440
Ohio State University CVM	Shields	Morgan	449
Ohio State University CVM	Stewart	Cameron	480
Ohio State University CVM	Stone	Charles	481
Ohio State University CVM	Strader	Summer	482
Ohio State University CVM	Trimble	Luke	502
Ohio State University CVM	Weaver	Hannah	524
Ohio State University CVM	Wetzel	Lisa	528
Oklahoma State University CVM	Burton	Kristyn	60
Oklahoma State University CVM	Busby	Morgan	61
Oklahoma State University CVM	Conchiglia	Allison	80
Oklahoma State University CVM	Furman	Mahala	147
Oklahoma State University CVM	Gaston	Mary	155
Oklahoma State University CVM	Hyatt	Julia	205
Oklahoma State University CVM	Intihar	Ashley	207
Oklahoma State University CVM	Janak	Lauren	211

Vet School	Last Name	First Name	Poster #
Oklahoma State University CVM	Johnston	Olivia	217
Oklahoma State University CVM	Komlodi	Tanner	242
Oklahoma State University CVM	Maker	Rebekah	285
Oklahoma State University CVM	Mills	Taron	324
Oklahoma State University CVM	Reed	Sarah	396
Oklahoma State University CVM	Rolo	Hannah	418
Oklahoma State University CVM	Snook	Justin	468
Oklahoma State University CVM	Swinsky	Catherine	487
Oklahoma State University CVM	Tryzbiak	Madison	504
Oklahoma State University CVM	Yeater	Jessica	550
Oniris Nantes	Meyer	Lisa	318
Ontario Veterinary College	Kumar	Ayush	246
Ontario Veterinary College	Pedersen	Julie	370
Oregon State University CVM	Dash	Alexandra	93
Oregon State University CVM	Michlanski	Miranda	321
Oregon State University CVM	Steeneck	Amy	476
Purdue University CVM	Johnson	Victoria	216
Purdue University CVM	Jones	Allyson	219
Purdue University CVM	Lenters	Brooke	257
Purdue University CVM	Lewis	Aaron	259
Purdue University CVM	Nowak	Mary	349
Purdue University CVM	Ramon	Marissa	388
Purdue University CVM	Schmidt	Krysten	434
Purdue University CVM	Wolfert	Kathryn	539
Purdue University CVM	Yanez Diaz	Maria	548
Ross University SVM	Feng	Jiashi	133
Ross University SVM	Hall	Alexis	174
Royal Veterinary College	Thomas	Sophie	494
St Georges University SVM	Graham	Rowan	165
St Georges University SVM	Jones	Adrian	218
St Georges University SVM	Luscinski	Jillian	278
St Georges University SVM	Schafsteck	Emily	430
St Georges University SVM	Smith	Nicolette	463
St Georges University SVM	Verma	Niharika	518
Stephens College (Undergrad)	Helzer	Anya	189
Texas A&M University CVM	Adams	Sasha	3
Texas A&M University CVM	Bettencourt	Alexandra	33
Texas A&M University CVM	Boyd	Haley	45
Texas A&M University CVM	Crow	Keegan	88
Texas A&M University CVM	Fehrenbach	Shannon	129
Texas A&M University CVM	Garcia	Cora	152

Vet School	Last Name	First Name	Poster #
Texas A&M University CVM	Gomez	Jordan	161
Texas A&M University CVM	Gore	Jillian	162
Texas A&M University CVM	Hartis	Coleman	183
Texas A&M University CVM	Knue	Joseph	237
Texas A&M University CVM	Kutrybala	Ian	248
Texas A&M University CVM	McCord	Morgan	303
Texas A&M University CVM	Meneses	Xandra Christine	316
Texas A&M University CVM	Ridlon	Ashley	407
Texas A&M University CVM	Wilson	Bailey	535
Texas A&M University CVM	Wong	Cailin	541
Texas Tech University School of Veterinary Medicine	Brown	Audrey	51
Texas Tech University School of Veterinary Medicine	Foster	Brooke	142
Texas Tech University School of Veterinary Medicine	Waugh	Lyric	522
Tuskegee University CVM	Bradshaw	Christina	46
Tuskegee University CVM	Duhart	Lauryn	110
Tuskegee University CVM	Griggs	Evan	171
Tuskegee University CVM	Harden	Mark	178
Tuskegee University CVM	Hunter	Pauline	202
Tuskegee University CVM	Mason	Jamia	296
Tuskegee University CVM	McGirt	Shakara	307
Tuskegee University CVM	Palmer	Kamaria	361
Tuskegee University CVM	Reeves	Jaelyn	397
Tuskegee University CVM	Wilder	Tyson	532
Universität Leipzig	Peters	Tilman	374
University of Bristol	Raw	Zoe	393
University of California - Davis SVM	Ad	Yael	2
University of California - Davis SVM	Bechtold	Lalita	26
University of California - Davis SVM	Conner	Kristin	81
University of California - Davis SVM	Cook	Shannon	82
University of California - Davis SVM	Dombroski	Catherine	106
University of California - Davis SVM	Engs	Natalie	118
University of California - Davis SVM	Garrison	Mary	154
University of California - Davis SVM	Gill	Sarah	157
University of California - Davis SVM	Gretler	Sophie	170
University of California - Davis SVM	Han	Stephanie	177
University of California - Davis SVM	Kuijpers	Fianne	244
University of California - Davis SVM	Li	Tianjiao	262
University of California - Davis SVM	Luker	Madison	276
University of California - Davis SVM	Mandel	Avery	287
University of California - Davis SVM	McCuskey IV	Samuel	305
University of California - Davis SVM	Myers	Danielle	338

Vet School	Last Name	First Name	Poster #
University of California - Davis SVM	Oertle	Danielle	351
University of California - Davis SVM	Pacumio	Lisa	360
University of California - Davis SVM	Poth	Meghan	380
University of California - Davis SVM	Pritchard	Celeste	381
University of California - Davis SVM	Ramarapu	Raneesh	387
University of California - Davis SVM	Razmara	Aryana	395
University of California - Davis SVM	Rickerl	Kaitlin	405
University of California - Davis SVM	Smith	Sarah	465
University of California - Davis SVM	Tueshaus	Tisa	505
University of California - Davis SVM	Wilcox	Callie	531
University of Edinburgh	Towell	Isabelle	500
University of Florida CVM	Cohen	Molly	76
University of Florida CVM	Coll-Roman	Lisette	78
University of Florida CVM	Cottingham	Sydney	84
University of Florida CVM	Daly	Edward	90
University of Florida CVM	Pathak	Nirali	366
University of Georgia CVM	Baker	Michaela	17
University of Georgia CVM	Basco	Adelaide	22
University of Georgia CVM	Batson	Samuel	23
University of Georgia CVM	Chiu	Veronica	74
University of Georgia CVM	Devorak	Anne	99
University of Georgia CVM	Drop	Samantha	108
University of Georgia CVM	Dunham	Cierra	111
University of Georgia CVM	Hatch	Abigail	184
University of Georgia CVM	Holmes	Jessica	197
University of Georgia CVM	Jackson	Erin	210
University of Georgia CVM	Kalphat-Losego	Kathleen	223
University of Georgia CVM	Keough	Kate	229
University of Georgia CVM	Lemons	Margaret	256
University of Georgia CVM	Nathan	Mei	341
University of Georgia CVM	Orzech	Sierra	356
University of Georgia CVM	Parker	Merrianna	362
University of Georgia CVM	Patel	Niki	365
University of Georgia CVM	Rubio	Samantha	421
University of Georgia CVM	Schiff	Erica	433
University of Georgia CVM	Sheridan	Taylor	447
University of Georgia CVM	Simpson	Zoe	455
University of Georgia CVM	Slade	Kaylee	458
University of Georgia CVM	Smith	Regan	464
University of Georgia CVM	Stange	Emma	474
University of Georgia CVM	Thorbrogger	Chloé	496

Vet School	Last Name	First Name	Poster #
University of Georgia CVM	Turn	Jeffrey	507
University of Georgia CVM	Wint	Sara	538
University of Ibadan	Adesola	Ridwan	4
University of Ibadan	Ajibade	Favour	6
University of Ibadan	Olowu	Babatunde	353
University of Illinois CVM	Carswell	Bethany	67
University of Illinois CVM	Graham	Sarah	166
University of Illinois CVM	Hearn	Aimee-Joy	186
University of Illinois CVM	Jackson	Erica	209
University of Illinois CVM	Kirkland	Simone	234
University of Illinois CVM	Miller	Taylor	323
University of Illinois CVM	Peterson	Mark	376
University of Illinois CVM	Pico	Marisa	378
University of Illinois CVM	Rodriguez	Melissa	413
University of Liverpool	Richardson	Amy	404
University of Minnesota CVM	Ahmed	Danielle	5
University of Minnesota CVM	Becker	Zoe	27
University of Minnesota CVM	Blanco	Cristina	37
University of Minnesota CVM	Callaghan	Caitlyn	62
University of Minnesota CVM	Dobrowski	Brittany	105
University of Minnesota CVM	Falck	Alaina	124
University of Minnesota CVM	Helman	Aharon	188
University of Minnesota CVM	Kobluk	Landon	238
University of Minnesota CVM	Labe	Courtney	250
University of Minnesota CVM	Likar	Samantha	265
University of Minnesota CVM	liu	qian	268
University of Minnesota CVM	Pendleton	Diana	372
University of Minnesota CVM	Rice	Anna	403
University of Minnesota CVM	Riley	Meghan	408
University of Minnesota CVM	Schmieley	Rebecca	435
University of Minnesota CVM	Skarda	Ashley	457
University of Minnesota CVM	Smith	Whitney	467
University of Minnesota CVM	Stark	Sarah	475
University of Minnesota CVM	Velazquez	Jossette	516
University of Minnesota CVM	Wood	Elinor	544
University of Minnesota CVM	Young	Jared	551
University of Missouri CVM	Browne	Marshall	54
University of Missouri CVM	Bruer	Caroline	56
University of Missouri CVM	Burke	Morgan	59
University of Missouri CVM	Cowen-Matteson	Jainee	85
University of Missouri CVM	D'Angelo	Davina	91

Vet School	Last Name	First Name	Poster #
University of Missouri CVM	Ellis	Tenleigh	116
University of Missouri CVM	Fang	Kexin	127
University of Missouri CVM	Fletcher	Cassandra	138
University of Missouri CVM	Ford	Tamara	141
University of Missouri CVM	Graham	Tucker	167
University of Missouri CVM	Hoffman	Emily	196
University of Missouri CVM	Isensee	Paige	208
University of Missouri CVM	Kemmerly	Christine	228
University of Missouri CVM	Kolbe	Taylor	241
University of Missouri CVM	Kujiraoka	Amanda	245
University of Missouri CVM	Lopez	Stephanie	271
University of Missouri CVM	McAllister-Day	Ali	300
University of Missouri CVM	Murray	Morgan	336
University of Missouri CVM	Nguyentran	Sarah	345
University of Missouri CVM	Osterland	Alexandra	357
University of Missouri CVM	Porter	Madison	379
University of Missouri CVM	Seilhamer	Nikki	442
University of Missouri CVM	Smith	Tiernan	466
University of Missouri CVM	Sturgeon	Ashley	483
University of Missouri CVM	Topka	Jessica	498
University of Missouri CVM	Williams	Estela	534
University of Missouri CVM	Wray	John	545
University of Pennsylvania SVM	Bauer	Natalie	24
University of Pennsylvania SVM	Benavides	Estefania	30
University of Pennsylvania SVM	Brisman	Rebecca	48
University of Pennsylvania SVM	DeMers	George	96
University of Pennsylvania SVM	DiStefano	Jessica	103
University of Pennsylvania SVM	Dunlap	Andrew	112
University of Pennsylvania SVM	Even	Kayla	122
University of Pennsylvania SVM	Friedman	Elisse	145
University of Pennsylvania SVM	Gregorio	Elizabeth	169
University of Pennsylvania SVM	Kalkus	Antonina	222
University of Pennsylvania SVM	Marciano	Katherine	290
University of Pennsylvania SVM	Pattada	Nimisha	367
University of Pennsylvania SVM	Pickford	Mackenzie	377
University of Pennsylvania SVM	Quincey	Corisa	384
University of Pennsylvania SVM	Reynoso	Juliana	401
University of Pennsylvania SVM	Seiberlich	Melissa	441
University of Pennsylvania SVM	Sila	Stephanie	450
University of Pennsylvania SVM	Smith	Anna	461
University of Pennsylvania SVM	Tevere	Rachel	493

Vet School	Last Name	First Name	Poster #
University of Pennsylvania SVM	Wu	Yucheng	546
University of Tennessee CVM	Broadway	Katelyn	50
University of Tennessee CVM	Demers	Taylor	97
University of Tennessee CVM	Gorman	Myranda	163
University of Tennessee CVM	Schonvisky	Kayla	436
University of the Oriental Republic of Uruguay	Salomone	Federica	424
University of Veterinary Medicine Hanover, Foundation (Germany)	Truyen	Lotta	503
University of Wisconsin SVM	Aviles	Matthew	13
University of Wisconsin SVM	Biancalana	Andrea	34
University of Wisconsin SVM	Corona	Amelia	83
University of Wisconsin SVM	Illgen	Rachel	206
University of Wisconsin SVM	Kim	Lisa	232
University of Wisconsin SVM	Li	Yiyao	263
University of Wisconsin SVM	Lim	Keegan	266
University of Wisconsin SVM	Lynch	Brianna	280
University of Wisconsin SVM	Marino	Nicholas	292
University of Wisconsin SVM	Mueller	Erika	334
University of Wisconsin SVM	Murray	Samuel	337
University of Wisconsin SVM	Rastas	Jake	392
University of Wisconsin SVM	Sandblom	Alyssa	426
University of Wisconsin SVM	Savage-Gibson	Marissa	429
University of Wisconsin SVM	Stevenson	Christina	479
University of Wisconsin SVM	Wichman	Kailey	530
University of Wisconsin SVM	Zheng	Yinan	555
University of Wisconsin SVM	Zutz	Madeline	556
Utrecht University	Marchand	Josephine	289
Utrecht University	van Bentem	Nick	512
Utrecht University	Zeeman	Fay	554
VetAgro Sup (National Veterinary School of Lyon)	Dewever	Emilie	100
Virginia-Maryland Regional CVM	Britton	Eric	49
Virginia-Maryland Regional CVM	Echols	Olivia	113
Virginia-Maryland Regional CVM	Foley	Anna	139
Virginia-Maryland Regional CVM	Gingrich	Katherine	158
Virginia-Maryland Regional CVM	Hixson	Haleigh	195
Virginia-Maryland Regional CVM	Kirkpatrick	Zachary	235
Virginia-Maryland Regional CVM	Markey	Corrin	294
Virginia-Maryland Regional CVM	Rojas	Kayla	416
Virginia-Maryland Regional CVM	Santos	Matthew	427
Virginia-Maryland Regional CVM	Smith	Mishana	462
Washington State University CVM	Fisher	Carolyn	137

Vet School	Last Name	First Name	Poster #
Washington State University CVM	Hinnant	Holly	194
Washington State University CVM	Hudgins	Amy	201
Washington State University CVM	Skarbek	Agata	456
Washington State University CVM	Stegelmeier	Neils	477
Western College of Veterinary Medicine	Brown	Vanessa	53
Western College of Veterinary Medicine	Lai	Evanna	253
Western College of Veterinary Medicine	Minkova	Stephanie	325
Western College of Veterinary Medicine	Turnbull	Katie	508
Western University of Health Sciences	Antoszewski	Aleksandra	11
Western University of Health Sciences	Bernas	Cassandra	32
Western University of Health Sciences	Castelo	Emely	68
Western University of Health Sciences	Do	Tram	104
Western University of Health Sciences	Escalante	Leobardo	120
Western University of Health Sciences	Garcia	Kathryn	153
Western University of Health Sciences	Gilbert	Natalia	156
Western University of Health Sciences	Harrison	Scarlett	181
Western University of Health Sciences	Hernandez	Jennifer	191
Western University of Health Sciences	Junghans	Kristina	221
Western University of Health Sciences	Kantserova	Anastassiya	225
Western University of Health Sciences	Knieriemen	Josie	236
Western University of Health Sciences	Kumar	Rika	247
Western University of Health Sciences	Kyan	Austin	249
Western University of Health Sciences	McCorkell	Michelle	304
Western University of Health Sciences	Nguyen	Jeffrey	344
Western University of Health Sciences	Salmeron	Yessenia	423
Western University of Health Sciences	Touze	Angelica	499
Western University of Health Sciences	Weakley	Mina	523
Western University of Health Sciences	Wong	Theresa	542
Western University of Health Sciences	Ybarra	Alexis	549

Complete Abstracts

Effect of subunit composition on channel activity of voltage-gated calcium channel $Ca_v2.3$

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Acrosomal exocytosis (AE) is a process crucial for successful fertilization of oocytes, involving the fusion of sperm acrosomal and plasma membranes and release of the acrosome contents. This process is strongly dependent on Ca^{2+} influx, where CatSper channels are currently thought to be the only mediators of Ca^{2+} flux required for AE. New findings, however, identified that the voltage gated Ca^{2+} channel $Ca_v2.3$ also plays a role in Ca^{2+} signaling leading to AE. $Ca_v2.3$ are multi-protein complexes ($\alpha1$, $\alpha2\delta$, and β subunits) which are highly regulated by membrane lipids-the mechanisms of which are still unclear. This study aims to investigate the influence of $Ca_v2.3$ subunit composition on channel activity through a reductionist approach. The main approach of this project involves transfection of CHO-K1 (Chinese Hamster Ovary) and HEK293 (Human embryonic Kidney) cell lines with only $\alpha1E$, the pore-forming subunit of $Ca_v2.3$, fused to a Ca^{2+} indicator (GCaMP) and/or an mCherry fluorescence protein. Localization and intracellular Ca^{2+} levels were evaluated using confocal microscopy. We then assessed whether co-transfection with additional subunits (β or $\alpha2\delta$) led to a change in localization or a change in relative calcium levels. Results and further testing will be explored during the presentation. Findings will provide more context on how each subunit plays a role in channel activity and where lipids may modulate structures.

Research Grant: NIH 5R01HD093827-04
Student Support: Cornell University College of Veterinary Medicine

Assessment of a point of care assay to determine protective vaccinal antibody titers for canine viral diseases

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Canine vaccination guidelines recommend dogs get vaccinated for canine distemper virus (CDV), canine parvovirus (CPV), and canine adenovirus (CAV) every 3 years. An alternative is to monitor antibody titers, and to revaccinate when titers fall below a protective threshold. In this study, a point-of-care (POC) assay was compared to the gold standard antibody titers to assess need for revaccination. Sixty-nine healthy dogs due for the DAP vaccine were enrolled. Dogs had a median weight of 22.5 kg (range 1.9-47.1kg) and a median age of 8 years (range 3-8yr). The gold standard antibody measurements revealed protective titers for CDV in 58, CPV in 68, and CAV in 68 of 69 dogs. The POC assay results indicated protective titers to CDV in 67, CPV in 68, and CAV in 69 of 69 dogs. The sensitivity of the POC assay for protective levels of CDV antibodies was 100% (CI 95%: 93.79-100%). However, 9 dogs were considered protected by the POC assay and not by the gold standard test with a specificity of 18.18% (CI 95%: 3.23-47.70%). The sensitivity for the POC assay to detect protective CPV titers was 98.53% (CI 95%: 92.13-99.92%). One dog was falsely assessed as protected by the POC assay as compared to the gold standard. The sensitivity of the POC assay to detect protective antibodies titers against CAV was 100% (CI 95%: 94.65-100%). This POC assay has high sensitivity for the detection of protective antibody titers; however, some dogs were falsely categorized as protected, especially for CDV. Utilizing only the high positive results on the POC assay may improve specificity of this assay. The specificities for protective antibodies against CAV and CPV were unable to be determined due to low numbers of unprotected dogs in the study.

Research Grant: Endowment in the SVM Office of Research and Graduate Education CCAH Allocation#: 2021-29-F
Student Support: None

Don't lose your head: screening black flies using a probe-based qPCR for detection of zoonotic *Onchocerca lupi*

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Onchocerca lupi is a zoonotic vector-borne nematode that infects companion animals, wild carnivores, and humans in the Old World and North America, where is endemic to the southwestern United States. Molecular evidence suggests that black flies (Diptera; Simuliidae) may serve as intermediate hosts for *O. lupi*, but this remains to be unequivocally confirmed. The aim of our project is to assess if different black fly species may act as vectors of *O. lupi* in an endemic area of the southwestern United States using a species-specific probe-based qPCR assay. Female *Simulium* black flies (n = 26,651) of 5 species were collected by the local vector control agency across Bernalillo County, New Mexico. There were 21 and 19 collection sites in 2018 and 2019, respectively. By targeting the black fly heads, we expect to detect DNA of *O. lupi* infective third-stage larvae present in the insect's mouthparts, thus confirming the vector suitability of one or more simuliid species. Black fly heads were pooled by species, collection date, and site. DNA extraction was performed using an automated system. After protocol optimization, 1:10 DNA lysates dilutions were used for qPCR analysis. To date, 359 pools comprising a total of 1,820 black fly heads have been screened by qPCR (2018: 197 pools with 387 flies; 2019: 162 pools with 1,433 flies). While no *O. lupi* DNA has been detected yet, processing of additional samples is still ongoing. This research will provide evidence as to whether black flies of one or more species can transmit *O. lupi*. Confirming the vector(s) of *O. lupi* will guide epidemiological surveillance studies, as well as management and control strategies for this zoonotic parasite in endemic areas in the United States and abroad.

Research Grant: Verocai Parasitology Lab, Department of Veterinary Pathobiology
Student Support: NIH T35OD010991-17, Texas A&M School of Veterinary Medicine & Biomedical Sciences

Characterization of antibiotic-resistance genes between species of commensal bacteria in Tanzania

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Antimicrobial resistance (AMR) is a significant burden for public health. Many AMR genes of clinical concern are harbored on plasmids that can move between bacteria through a process called conjugation. We hypothesized that if the horizontal transfer of antibiotic resistance genes between species of *Enterobacteriaceae* is common, the variance in the distribution of these genes and associated plasmid markers should be similar within and between species. A total of 1,262 Whole-genome sequences (WGS) were generated for randomly selected bacterial isolates collected at 14 Tanzanian households and proximal areas (human, animal, water, and wildlife sources). CLC Premier was used to compare strains from the Tanzania collection and other genomes available through GenBank. *Escherichia coli* was most abundant (n = 776) of which 45.7% harbored at least one resistance gene, followed by *Enterobacter cloacae* (n = 176, 25%) and *Klebsiella pneumoniae* (n = 111, 15.8%). Other species (n = 159, 13.6%) composed the remainder of the data. *E. coli* had the richest diversity of genes (n = 104) while the other groups were roughly equal (49-54 genes). The most common resistance genes for *E. coli* included aminoglycosides and sulfonamide resistance, which was different from *E. cloacae* (tetracycline), *K. pneumoniae* (quinolone) and other organisms (tetracycline and sulfonamides). The distribution of resistant strains ranged from a high of 28% from milk container swabs and 18.8% from chickens, to 11.4% for people and cattle, with remaining hosts having ≤ 8.3% resistant bacteria (5.5% for sheep and goats; 3% for isolates from open water sources).These findings help to increase our understanding on the significance of the commensal horizontal gene pool.

Research Grant: Nil
Student Support: Student Support: Washington State University Summer Research Fellowship

Screening natural products derived from extremophiles for antiparasitic activity

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Apicomplexans are a phylum of parasites that include Toxoplasma and Cryptosporidium. They cause disease in several mammalian species and devastating long-term effects in immunocompromised patients. *Toxoplasma gondii (T. gondii)* is known to cause birth defects and abortion in several animal species, while *Cryptosporidium parvum (C. parvum)* is a common cause of juvenile diarrhea worldwide and the most important cause of calf scours in ruminants. Current treatments for *T. gondii* infections are not efficacious against the bradyzoite form of the parasite. The discovery of compounds with activity against *T. gondii* is important for both the veterinary and medical communities. Unlike synthetic compounds, natural products have been optimized by millions of years of evolution and may target multiple molecular sites. In the interest of finding compounds with antiparasitic activity, crude extracts from fungi isolated from the Soudan Mine in Minnesota and from Antarctica are being tested against a *T. gondii* infection model. Human foreskin fibroblasts are infected with *T. gondii*, followed by treatment with natural compounds. Luciferase expression is used as an indicator for parasite growth. A similar experiment is screening for activity against *C. parvum*. Initial results suggest several compounds with activity against apicomplexan parasites. Future experiments should determine compound cytotoxicity to host cells, the half maximal effective concentration, and the molecular target. Suitable compounds would then enter pre-clinical animal trials.

Research Grant: None
Student Support: Boehringer Ingelheim and the College of Veterinary Medicine

Rapid barn-side detection of swine pathogens

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Porcine Reproductive and Respiratory Syndrome virus (PRRSV) causes over $660 million in losses to North American farmers. Similarly, the recent emergence of African Swine Fever Virus (ASF) in Asia increases the potential that the virus may spread to the US from other countries. ASF is endemic in Nigeria. PRRS is endemic in the US. PRRSV infection is detected in the US using PCR and serology. PRRSV has not previously been reported in Nigeria. This project will develop barn-side assays for the detection of PRRSV and ASFV in Nigeria. A PCR amplicon of the synthetic p30 gene of ASFV was cloned into the BamH1/EcoR1 site of the pRen2 plasmid and expressed in Cos-1 cells as a fusion protein with Renilla luciferase. Nucleic acid (NA) was extracted from lung homogenates of PRRS-infected pigs using a manual magnetic rack system and compared to automated KingFisher MagMax NA extraction. Real-time PCR was used to detect PRRSV in NA extracts. A luciferase assay (LIPS) using p30 protein will be validated and optimized for rapid detection of antibodies to ASF. An isothermal transcription-mediated amplification (TMA) assay will be developed and validated to detect PRRSV in magnetic-rack-isolated NA samples. Preliminary data showed that manual NA extraction system produced comparable results to the MagMax System. On returning to Nigeria, the LIPS assay developed and validated with monoclonal antibody to ASFV in the United States will be used to test samples collected from pigs. Similarly, the TMA developed in the United States will be used to screen serum samples for PRRSV in Nigeria. Thus, this project will provide rapid barn-side assays that can be used to monitor ASFV and PRRSV in resource limited countries.

Research Grant: None
Student Support: University of Missouri, College of Veterinary Medicine, Office of Research

Effects of pro-motility drugs on liquid phase gastric emptying in dogs with clonidine-delayed gastric emptying

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Delayed gastric emptying (GE) is a disorder that affects dogs, humans, and other species. In dogs, delayed GE is a common sequela of abdominal inflammation and abdominal surgery. Affected dogs display nausea, vomiting and/or regurgitation, inappetence, and abdominal discomfort. Radionuclide scintigraphy is the current standard for evaluating GE; however, it is not readily available outside of academic centers. When given orally, acetaminophen is rapidly absorbed in the proximal duodenum of dogs and has been established experimentally as a marker for liquid phase gastric emptying and may be a useful marker for evaluation of GE in the clinical setting. The goal of this study was to evaluate acetaminophen as a marker for GE of liquids in healthy dogs that were given clonidine, an $\alpha 2$-adrenergic agonist that delays GE, and measure the effects of the gastric prokinetics metoclopramide and azithromycin in this model using a randomized, cross-over design. Plasma acetaminophen levels were measured using reverse-phase high performance liquid chromatography. We anticipate that clonidine will cause a delay in GE that will be either completely or partially corrected by metoclopramide and azithromycin.

Research Grant: LSU Foundation funds to Frederic Gaschen
Student Support: Blue Buffalo and the Arkansas Veterinary Medical Foundation

Cytochemical Characterization of Hamster Neutrophils

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While the hamster is a widely utilized animal model for numerous diseases, little work has been done to characterize hamster neutrophils. There has been controversy over the name of the hamster primary polymorphonuclear cell (PMN) between neutrophil, heterophil, or pseudo-heterophil based on the faint eosin staining of the primary granules which is similar to human neutrophil granules. Often neutrophils and true heterophils differ in cytochemical staining which correlates to the biochemical content of granules. Hamsters have been assumed, as rodents, to have the same neutrophil granule cytochemical contents as mice and rats; however, those two species differ in alkaline phosphatase and defensin content. The neutrophil granule morphology and content are important to: 1) identify neutrophils from other granulocytes, 2) determine if cell staining morphology correlates to granule contents seen in neutrophils or heterophils (MPO often decreased to absent), and 3) determine if hamster neutrophil granule content differs from other species. Hamster blood smears were made with no anti-coagulant or EDTA, with dog EDTA blood smears as quality controls. Neutrophils were positive for myeloperoxidase (MPO), Sudan black B, naphthol AS-D chloroacetate esterase, and periodic acid-Schiff (PAS); positive for acid phosphatase and tartrate sensitive (negative staining); and negative for alpha-naphthyl acetate esterase and Luna's eosinophil granule stain. Alkaline phosphatase reagents were not available. EDTA and the age of the blood smear, even within acceptable recommendations, diminished staining for some reactions. Hamster neutrophil cytochemical staining is consistent with mammalian neutrophil reactions.

Research Grant: Dr. Roger and Marilyn Mahr Professorship in One Health
Student Support: NIH T35 Training Grant 5T35OD027967-03

Food animal welfare and antimicrobial use on Floreana Island, Galapagos, Ecuador

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The Galapagos archipelago has been identified as an area of interest regarding antimicrobial resistance (AMR) due to unique contributing factors such as large transient populations, access to over-the-counter antibiotics, and the presence of animal agriculture. Previous studies suggest that animal welfare and management practices can influence AMR risk on-farm. However, no research to date has evaluated welfare and management practices in the context of antimicrobial use on farms in the Galapagos Islands. To bridge this gap, a survey on animal welfare and antimicrobial use practices was conducted on eight multi-species farms on Floreana Island. The survey was delivered orally in Spanish and assessed five competencies: access to feed and water, shelter, treatment of disease, behavior, and appropriate euthanasia. Descriptive analysis is in progress. The present abstract will discuss relevant findings from the welfare portion of the survey, aiming to identify areas of farm excellence and areas in which opportunities for improvement exist. These results will be shared with local stakeholders and producers to develop on-farm training tools and resources for optimizing welfare. Future research will include expanding producer enrollment to additional islands to identify further opportunities to improve food animal welfare and enhance understanding of AMR in the Galapagos Islands.

Research Grant: NC State CVM Global Health Seed Grant
Student Support: NC State CVM Global Health Seed Grant

Development of a real-time polymerase chain reaction assay to detect *Salmonella* Dublin

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Salmonella enterica subspecies *enterica* serovar Dublin (*S*. Dublin) is host-adapted in cattle and can cause respiratory disease and severe systemic infections in calves. In addition, *S*. Dublin infections are highly virulent in humans and often multidrug resistant, and since recovered carriers may continue to shed the bacteria in milk, monitoring *S*. Dublin is an important public health concern. *S*. Dublin is difficult to identify using bacterial culture techniques because it is often outcompeted by other bacteria present in environmental samples. Therefore, this study seeks to develop a real-time polymerase chain reaction (rtPCR) assay specific to *S*. Dublin to identify the presence of the pathogen directly from clinical and environmental samples. Genomic regions unique to *S*. Dublin but with varying numbers of mismatches to all other *Salmonella* serovars were identified using genome sequences from the National Center for Biotechnology Information Pathogen Detection database. The most promising genomic region was then aligned against sequences of other *Salmonella* serovars, and a sub-region containing a single nucleotide polymorphism unique to *S*. Dublin was identified. Based on this sub-region, candidate primers and probes for the rtPCR are being designed and benchmarked against two previously published assays. This evaluation will be performed using environmental and clinical samples which have been cultured, serotyped, and confirmed via whole genome sequencing as part of an ongoing project on *S*. Dublin dairy farm reservoirs and management. Development of a specific molecular detection assay for *S*. Dublin will be helpful for monitoring farms and for public health and disease ecology studies.

Research Grant: USDA NIFA award (Cummings)
Student Support: Boehringer Ingelheim and the Cornell University College of Veterinary Medicine

Influence of Tibial Osteotomies on Rotational Instability of the Canine Stifle

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Cranial Cruciate Ligament Deficiency (CCLD) is the most common cause of lameness as well as degenerative joint disease in canine stifles, specifically large breed dogs. CCLD causes pain and future arthritis due to the cranial shift and thrust of the tibia and an internal rotational instability of the stifle (RIS) by the tibia in relation to the femur. One of the two tibial osteotomies, the tibial tuberosity advancement (TTA) and the tibial plateau leveling osteotomy (TPLO), are often chosen by surgeons to correct these conformational changes by altering the biomechanics of how the weight of the femur impacts the tibial plateau within the stifle joint. Another technique we will be testing will be the lateral fabellar suture (LFS) following either TTA or TPLO. Our study determined the RIS in stifles with an intact ligament, CCLD, after a TTA or TPLO, and with an LFS procedure. To test the RIS, we created a 3D printed device that was used in a previous RIS study to produce a torque on *ex vivo* stifle joints that will determine a numerical value of the level of rotation at each given point in the study. Our hypothesis is that once there is CCLD in the stifle joint, there will be an increased amount of RIS in comparison to the intact cranial cruciate ligament that would require the LFS procedure along with a TTA or TPLO. Our data determined that there were no substantial changes in the RIS following either TTA or TPLO until the LFS procedure was performed, concluding that the LFS procedure will provide the most improvement on internal rotation in the canine stifle. This evidence can improve evaluations as well as give surgeons options for additional recommended procedures with tibial osteotomies to control the unwanted RIS.

Research Grant: Partially funded by Morris Animal Foundation
Student Support: WesternU CVM Veterinary Summer Research Program (VSRP) with Boehringer Ingelheim

The effects of chew toys on cognition in working dogs

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Cognitive welfare is that which enhances the wellbeing of an animal through enhancing their cognitive abilities. Working dogs rely on their cognitive abilities to perform their jobs, such as remembering trained odors and navigation of complex environments. Research in humans and rats suggests that chewing is a form of stress relief which enhances cognitive abilities; however, no research has been conducted to show the effects of chewing on canine cognition, specifically. This project aimed to examine the effects of chewing directly following a working memory and problem-solving task on dogs' short and long-term memory, respectfully. It also intended to compare the degree of chewing to the level of the dog's anxiety. Using a sample of the working dogs from Auburn University's Canine Performance Sciences, data was collected on each dog for baseline anxiety levels and performance on a spatial working memory test and a maze learning and memory test. Generalized linear models were used to assess the relationship between dogs' interaction with a chew toy during cognitive testing and their short and long-term memory retention. The preliminary results are supportive of our hypothesis showing that the faster a dog begins to chew on their bone following a task, the better long-term memory they have for that task, but this is only true for the low anxiety dogs. These findings may help key stakeholders, such as kennel managers and animal care professionals, draw conclusions about the utility of chew toys as cognitive enrichment for their working dogs.

Research Grant: ASPCA Seed Grant
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Metagenomic shotgun sequencing of digital dermatitis lesions to characterize microbial pathogenicity

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Cattle lameness is a large source of economic loss in the cattle industry. One of the most common causes of lameness in cattle is digital dermatitis (DD). Clinical signs for DD include hindlimb lameness and ulcerative lesions on the coronary band of mostly hind limbs in cattle. Different stages of DD are associated with distinct lesions, among which M2 (active ulcerative, > 2cm in diameter) and M4 (chronic stage) result in lameness and transmission. DD is a multifactorial disease with a strong bacteriological component. Microbiome variation in different stages of DD lesions implies that these microbes function in DD pathogenesis. *Treponema spp*. are always present in DD lesions and have displayed multiple antimicrobial resistances (AMR) relevant to human medicine. Little is known about the genetic makeup of the microbial species found in DD lesions. We hypothesize there are significant changes in the microflora of M2 or M4 lesions from M0 lesions (healthy tissue), and that microbes detected in chronic lesions are associated with AMR genes. In a field trial, lesions from 20 cows affected by different stages of DD were biopsied and submitted for metagenomic shotgun sequencing (MSS). During a follow up trial, 20 lightweight HF steers were biopsied to complete MSS. We expect that unhealthy tissue will have a significantly different microbiome compared to M0 lesions. Since DD is a common cause of lameness in cattle, research is critical to improving hoof health, production, and welfare. Finally, by understanding mechanisms of spirochete virulence in DD, this study could provide insight on how these microbes affect human health and the pathogenesis of human disease such as syphilis, borreliosis, and Framboise.

Research Grant: Unknown
Student Support: Boehringer Ingelheim Veterinary Scholars Program BIVSP-UW-Madison

Predicting protection from Equine Herpesvirus-1 Myeloencephalopathy using antibody sub-isotype responses

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Equine herpesvirus-1 (EHV-1) myeloencephalopathy (EHM) is a clinically important manifestation of EHV-1 infection, causing devastating consequences economically and emotionally. Unfortunately, while there are numerous vaccines available for EHV-1, there are currently none that are labeled to protect from EHM. Although EHM is relatively rare and occurs in approximately 10% of infected horses, previous studies have shown that the risk is higher in older (> 18 years) horses. Recent data indicate that protection of horses from EHM is associated with increased Immunoglobulin G sub-isotype 4/7 (IgG4/7) and decreased immunoglobulin G sub-isotype 3/5 (IgG3/5) in serum prior to challenge infection. Further data show a correlation between older age and an increase in serum IgG3/5. Using pre-existing data along with data from a recent clinical study that included EHV-1 infected horses with and without clinical EHM, this project aims to clarify the validity of using serum concentrations of IgG3/5 and IgG4/7 to predict protection from EHM. Preliminary data shows that horses who developed EHM exhibited higher ratios of IgG3/5 to IgG4/7 both pre- and post-challenge. Together with other markers of cellular/humoral immunity this data elucidates our current understanding of the immune responses associated with the development of EHM. This information is critical for the development of future vaccines targeted to protect horses of all ages from EHM.

Research Grant: Unknown
Student Support: Boehringer Ingelheim and the Graduate School at Michigan State University

A search for unique genomic signatures among elk isolates of *Mycobacterium tuberculosis* variant bovis

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Tuberculosis is a disease of multiple host species, caused by members of the *Mycobacterium tuberculosis Complex*. Tuberculosis remains as one of the leading causes of death among people (caused by *Mycobacterium tuberculosis* variant *tuberculosis*) and devastating economic losses to animal agriculture(*Mycobacterium bovis* or MBO). The purpose of this research project is to index genomic signatures among elk isolates of MBO and provide an understanding of their divergence from other strains infecting animals and humans. In previous work using MassArray based SNP typing it was shown that all elk isolates from different geographic locations in the US, clustered separately into a unique clade. That study was based on a *priori* knowledge of genomic SNPs that was biased since those unique to these isolates based on de novo analysis were likely missed. Thus, we posit that genome wide analysis for unique single nucleotide polymorphisms and/or insertion-deletion events is expected to provide strong scientific foundations to understand host adaptation, host range, and zoonotic potential. Whole genomic DNA from MBO isolates (from elk) were prepared and PCR analyzed for MBO-specific polymorphisms in *oxyR* and *pncA*. MBO genomic DNA from elk obtained from USDA were amplified and genomes sequenced with Illumina (NovaSeq) technology. Genomes are assembled *de novo* and compared against a library of MBO draft genomes available from NVSL and the PARTIC databases to identify unique changes in the lineages and define the evolutionary trajectory of elk isolates. Genome analysis of this bacterium will establish within host evolution and provide a repertoire of targets that can be used in diagnostics and/or as targets for subunit vaccine development.

Research Grant: Sreevatsan lab is funded by USDA and Ag Experiment Station
Student Support: NIH grant R25HL103156

Comparative genomic analysis of methicillin resistant *Staphylococcus aureus* isolates from animals and humans

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Staphylococcus aureus is a gram-positive bacterium that colonizes the skin and nasal tract of animals and humans. Methicillin-resistant *S. aureus* (MRSA) causes a range of conditions from skin infections to invasive disease including bacteremia, endocarditis, and necrotizing pneumonia. MRSA was first identified in a hospital (HA-MRSA) and has since been isolated from communities (CA-MRSA), companion animals, and livestock (LA-MRSA). Resistance to β-lactam antibiotics poses a significant public health risk as these antimicrobial agents are largely used in the treatment of infections. We hypothesize that MRSA isolates from companion dogs share ancestry and cluster with HA- or CA-MRSA of human origin and a distant relatedness with LA-MRSA. MRSA isolates from animals are genome sequenced to define lineages of these isolates from diverse hosts and define the extent of strain sharing across animals and humans. Comparative genomics will include genomes of methicillin-susceptible *S. aureus* isolates from animals and humans. The isolates are recovered on brain heart infusion plates and reconfirmed using MALDI-TOF, coagulase testing, and an antibiogram generated using disk diffusion with cefoxitin. Bacterial DNA is then extracted for genomic sequencing from which phylogenetic analysis is performed on genome-wide SNPs of animal and human strains to determine the evolutionary relatedness of MRSA strains from the dogs, milk samples, and humans. The staphylococcal cassette chromosome mec (SCCmec) type is determined using a multiplex PCR assay to evaluate the strain types of the isolates. This study is expected to develop an understanding of interspecies transmission pathways and provide a baseline for prospective studies in the future.

Research Grant: Studies in Sreevatsan lab are funded by USDA, MI-DNR, MAAA, and some start-up funds.
Student Support: Student Funding provided by NIH Grant 5T35OD016477-20 to Michigan State University.

Histopathological and immunohistochemical comparison of pemphigus foliaceus and epidermal collarettes in dogs

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Canine pemphigus foliaceus (cPF) is an autoimmune blistering skin disease characterized by autoantibodies targeting the adhesion molecule Desmocollin 1 (DSC1), resulting in a loss of keratinocyte adhesion and development of acantholytic keratinocytes in in the superficial epidermis. Epidermal collarettes (EC), a form of canine superficial pyoderma, can clinically and histologically mimic skin lesions of cPF; however, there have not been in-depth comparative studies between these two entities. Our aim was to evaluate DSC1 expression and antibody (IgA, IgG, and IgE) deposition in the skin lesions of dogs with cPF and EC hypothesizing there would be a difference in staining patterns for IgA, IgG, IgE, and DSC1 between skin lesions of cPF and EC. Skin samples from six dogs with cPF and six dogs with EC were stained with anti-canine IgA, IgG, IgE, and anti-DSC1 using an immunohistochemical (IHC) labeling method. IHC and HE stained sections were assessed for IHC staining patterns and histopathologic findings, including inflammation (dermis, epidermis, perivascular structures, and periadnexa), epidermal hyperplasia, re-cornification, hyperkeratosis, external root sheath involvement and acantholytic keratinocytes. We expect cPF cases to have disrupted DSC1 staining in the superficial epidermis as DSC1 is the major autoantigen targeted by autoantibodies in cPF. Positive staining for IgG around epidermal keratinocytes is expected in early cPF lesions undergoing active acantholysis, while IgA and IgE are not expected to be detected in cPF skin lesions but may appear in EC sections.

Research Grant: None
Student Support: Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

A microRNA-based breast cancer screening approach

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Breast cancer is the most common cancer diagnosed in women, with early detection being crucial to improve treatment options and survival rate. Mammography is a useful screening tool; however, it only detects existing lesions. Therefore, there is a critical need for additional screening methods. MicroRNAs (miRNAs), which are ~20 nucleotide modulators of gene expression, are shown to be aberrantly expressed in patient biofluids during periods of disease. Therefore, miRNAs are implicated as stable biomarkers that indicate an individual's predisposition to develop breast cancer. To identify miRNAs that may predict breast cancer risk, we took advantage of the natural variation in mammary cancer incidence across mammals. Next-generation small RNA sequencing (small RNAseq) identified a panel of 21 microRNAs that were differentially expressed in mammary cell cultures (MDECs) from mammals with high natural incidence of mammary cancer (humans, dogs, and rats) and low natural incidence of mammary cancer (horses, cows, and pigs). To identify the physiological relevance of these miRNAs, we compared miRNA levels in conditioned media (CM) of MDECs derived from high and low natural incidence species using RT-PCR. Our results showed differences in secreted miRNA expression in CM samples from high and low natural incidence species. These findings will guide miRNA analysis of human biofluids using the nCounter Nanostring platform to assess risk-associated miRNA levels in age-matched healthy and breast cancer patients that may be predictive of breast cancer risk.

Research Grant: Carol M. Baldwin Breast Cancer Research Award
Student Support: Boehringer Ingelheim and Cornell University College of Veterinary Medicine

Clinical utility of standing fecal flotation in hookworm identification in fecal aliquots from shelter canines

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Ancylostoma/Uncinaria spp (hookworms) are of the most common canine intestinal parasites that are easily transmitted and carry zoonotic potential. Centrifugal Fecal Flotation is preferred for fecal detection but due to practicality, speed, and low economic cost, shelters and veterinary hospitals frequently use the Standing Fecal Flotation. These methods vary in flotation media, centrifugation, and fecal mass. Fecal loop tools are often employed for fecal collection, and generally collect 0.5 or 0.25 grams(g). Variations from the preferred method were the focus of this study, in which the variability in eggs per gram (EPG) of hookworm ova and sensitivity of hookworm detection within varying fecal quantities were investigated. Thirty-one voided samples were collected from apparently healthy canines in shelters. Canines not yet treated for intestinal parasites, or those that had been treated within the previous three days or two weeks, were included. For each sample, aliquots of 2, 1, 0.5, 0.25 g were evaluated for hookworm ova. Average EPG were calculated using the 2 g aliquot based on the presence of hookworms. Results were recorded with Excel and analyzed with SAS Studio 9.4.1. From the 360 fecal floats, 331 were positive. Overall, there was no statistically significant difference in fecal gram size for hookworm ova identification. However, when each aliquot was compared to the 2 g sample standard, statistically significant differences were revealed. Each size aliquot provided a high probability of identifying hookworm ova, contributing to the on-going battle against hookworm infections.

Research Grant: Boehringer-Ingelheim
Student Support: Mississippi State University Office of Research and Graduate Studies

Validation of a revised measure of veterinary general decision-making preference

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The Veterinary General Decision-Making Preference Scale (VGDMPS) is a measure of pet owner preference for autonomy when making medical decisions for their pets. It is adapted from the Autonomy Preference Index (API) used in human medical decision-making. Previous attempts to validate the VGDMPS found that clients reacted negatively to the wording of items. The objective of this research was to revise the Veterinary General Decision-Making Preference Scale to make it acceptable to clients and validate it in a non-clinical population. We revised the VGDMPS and assessed face and content validity through cognitive interviews with 5 small animal veterinarians and 11 clients at a veterinary school-based community practice, pausing in the middle of data collection to make further revisions to the instrument. Once the Revised Veterinary General Decision-Making Preference Scale (RVGDMPS) reached acceptable levels of acceptability, clarity, and completeness, we administered the instrument to an online sample of pet owners (n = 229) and administered a follow-up survey to a subsample of pet owners (n = 100). The scale was unidimensional and reached acceptable levels of internal consistency (Cronbach's alpha = .81). Item Response Theory analysis determined that the instrument discrimination parameters for all items are high or very high and that response categories allow for adequate differentiation among differing levels of preference for autonomy. Test-retest reliability was acceptable (ICC = .64, $P < .011$). The validation of the RVGDMPS provides us with a basis for further veterinary communication research that can be used to examine the effect of client preferences on client satisfaction, adherence, and improved pet health.

Research Grant: Research Grant: None
Student Support: Student Support: Boehringer-Ingelheim

Examination of a small ORF predicted to encode a novel transmembrane protein of ASBV using CHSE-214 cells

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Aquaculture is a large part of food production. The increase in aquaculture production will be accompanied by growing disease risk in fish and a rising risk of emerging pathogens that can quickly adapt to new hosts to pose zoonotic risks to terrestrial animals and humans. A small ORF in the salmonid nidovirus (Atlantic salmon bafinivirus - ASBV) encodes a novel integral membrane protein that could be targeted by antivirals. Therefore, we intend to functionally characterize the ASBV ORF E after inducibly expressing it in a fish cell line. In my summer project, I cloned the flag-tagged ASBV E ORF sequence into the pSLIK neo plasmid (with resistance to neomycin) and expressed in Chinook salmon embryo cells (CHSE-214) following lentivirus transduction. Neomycin resistant CHSE-214 cells were treated with doxycycline to inducibly express the ASBV E protein before harvesting total protein for immunoblotting to detect the expression of ORF E. To determine the localization of inducibly expressed ORF E protein, confocal microscopy will be used to examine the distribution of ORF E at different time points after expression. The findings of this study will provide fundamental knowledge on the expression pattern of ASBV ORF E protein.

Research Grant: none
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Multiplexing avian diagnostic testing for avian influenza virus (AIV) and Newcastle disease virus (NDV)

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Virulent Newcastle disease (NDV) and Avian Influenza (AIV) are important viral respiratory diseases that produce similar clinical manifestations and cause huge economic losses to the poultry industry worldwide. Current NAHLN approved PCR protocols for the detection of NDV and AIV are singleplex real-time reverse transcription polymerase chain reaction (rRT-PCR) assays. The current limitations of these tests are that neither exogenous nor endogenous inhibitory controls (IC) are included, they require specific separate thermocycling conditions for each target, both assays are to be independently run causing further delay to turnaround times, and there is an increased cost of testing by virtue of singleplex assay design and increased consumption of reagents and consumables. The primary goal of this project is to develop multiplex rRT-PCR assays that allow simultaneous, rapid and accurate detection and differentiation of both NDV matrix and AIV matrix targets with exogenous extraction control, all in one tube. Also, we propose to develop a multiplex assay for NDV matrix and vNDV F-gene. These new assays will be developed and validated with clinical samples and compared to approved assays in an effort to provide robust, rapid, cost effective and accurate testing for diseases under NAHLN scope and other economically important diseases to poultry producers. These tests may also prove useful in testing wildlife cases for AIV and NDV when outbreaks occur.

Research Grant: USDA-NAHLN Farm Bill Grant 2021
Student Support: UGA-Chanin Foundation scholarship

Cytoplasmic proximity biotinylation and localization of mitochondrial proteins within *Toxoplasma gondii*

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Toxoplasma gondii is an apicomplexan parasite that infects a third of the human population and causes toxoplasmosis in immunosuppressed individuals. A characteristic of apicomplexans is the presence of a non-photosynthetic plastid, the apicoplast, which is involved in essential metabolic pathways such as heme synthesis. Many pathways involve a close interaction between their single mitochondrion and the apicoplast and we aim to visualize this inter-organellar interaction through proximity biotinylation (PB) with a previously generated strain expressing the biotin ligase TurboID at the mitochondrial surface. An important step to perform PB experiments is the generation of a spatial reference, which we will attempt to do via endogenously tagging Fbox014, a non-essential cytoplasmic protein, with TurboID. This will allow us to perform PB with a cytoplasmic control, generating a tool that can be used in future experiments. In a second project, we aim to tag a sirtuin protein of unknown localization. This protein has a well-defined human homolog (SIRT4) which has been localized to the mitochondria and regulates a diverse array of biochemical pathways but is understudied in apicomplexans. We will utilize a high-affinity tagging approach consisting of spaghetti monster-HA, a technique previously used to characterize the localization of lowly expressed proteins in *T. gondii.* We will assess the visualization of this sirtuin via immunofluorescence assays. As there are no reliably successful treatments against apicomplexans, our projects will help us to identify possible targets for future therapy options, while expanding our knowledge about sirtuins in *T. gondii.*

Research Grant: Research Grant: None
Student Support: Student Support: NIH Office of Research Infrastructure Programs, Grant Number 5T35OD 010433-14

Detecting SARS-CoV-2 in White-Tailed Deer (*Odocoileus virginianus*) Lymph Nodes using Real-Time RT-PCR

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SARS-CoV-2 is the causative agent of the COVID-19 pandemic. It has subsequently spilled over from people into multiple animal species due to the broad host range of this coronavirus along with its widespread distribution. Established infections in wild species could lead to accelerated evolution of the virus as well as novel viral variants with potential capacity to escape acquired or vaccine derived immunity in people. Sampled populations of white-tailed deer in the United States have high PCR positivity rate for SARS-CoV-2 (25-35%). This indicates deer could be established as an expansive reservoir host with the risk of infecting other wild animals and possible reemergence back into humans. To further investigate the extent of SARS-CoV-2 infection in deer we will determine the prevalence in Pennsylvania deer over time by testing lymph node samples collected between 2019-2021 for the presence of SARS-CoV-2. Viral RNA will be extracted and then identified through RT-qPCR. Having temporal samples available for genetic material detection can help provide valuable insight into the course of outbreak among the deer population. To effectively prepare for potential risks associated with spillover infections, it is imperative to recognize the amount of infection in deer as well as the variant types. Genome sequencing could then compare virus strains to determine shared lineages with humans and facilitate better understanding of the evolution of SARS-CoV-2.

Research Grant: University Research Foundation
Student Support: NIH T35 OD010919, Boehringer-Ingelheim, and the University of Pennsylvania

ACVECC-VetCOT Retrospective Analysis of Traumatic Brain Injury (TBI) in Feline Trauma Patients

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Traumatic brain injury (TBI) is an understudied topic in veterinary medicine. Despite TBI being a leading cause of traumatic death in companion animals, the epidemiology is still poorly understood, especially in felines. This retrospective study of 5742 cats from April 2017 to December 2021 used data from the American College of Veterinary Emergency and Critical Care (ACVECC) Veterinary Committee on Trauma (VetCOT) registry to help further characterize the feline TBI population. Descriptive statistics were calculated for relevant data points. TBI was defined as a modified Glasgow Coma Score (mGCS) of less than 18. Feline trauma patients with TBI had a significantly lower chance of surviving compared to those without TBI (47.3%, 93.7%, *P* value < 0.00001). Intact felines were also more likely to have TBI when compared to altered felines (32.7%, 21.0%, *P* value < 0.00001). TBI was most commonly caused by both blunt and penetrating trauma (36.9% both blunt and penetrating, 28.2% blunt, 10.5% penetrating). When evaluated based on severity of initial injury (mild, moderate, severe) as determined by the Animal Trauma Triage (ATT) score, TBI injuries occurred most frequently in severely injured patients (ATT > 5/18) (41.7%, 3.7%, *P* < 0.00001). Initial descriptive statistics will be followed by additional comparisons - including data on diagnostic and interventions - as well as logistic regression. This study will help create a better understanding of TBI in cats, paving the way for improved diagnostics, interventions, and outcomes of trauma in veterinary patients overall. Additionally, this study helps to develop a translational model of TBI, leading to informed and impactful knowledge-sharing across human and veterinary medicine.

Research Grant: None
Student Support: Mumford Feline Foundation

Salmonella Typhimurium modifies transcription of amino acid (AA) transporters to overcome growth inhibition

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Salmonella Typhimurium (S.Tm) is a zoonotic pathogen that replicates to high burdens in the large intestine causing gastroenteritis in animals and humans. During infection, S.Tm invades intestinal epithelial cells using two type 3 secretion systems (T3SS) resulting in an inflammatory response by the host. During homeostasis, the establishment of S.Tm in the intestine is prevented by short chain fatty acids (SCFA) generated by host microbes. SCFA lead to growth inhibition of S.Tm through acidification of the cytosol. Data show that S.Tm can overcome this growth inhibition through deacidification of the cytosol using proton fueled AA decarboxylase enzymes. The origin of AA that fuel these reactions in the large intestine are unknown and conflict with the fact that AA absorption mainly occurs in the small intestine. We demonstrate that S.Tm invasion of intestinal epithelial cells leads to malabsorption of AA in the small intestine by decreasing the expression of AA transporters responsible for the influx from the lumen of the small intestine. The increased availability of AA in the large intestine, enables S.Tm to overcome initial growth inhibition by the microbiome. To show this mice were infected with wild type S.Tm, a T3SS deletion mutant or mock infected through oral inoculation. Groups of mice were sacrificed for 4 consecutive days to isolate infected intestinal tissue. Ileal tissue was processed and enterocytes isolated for subsequent RNA extraction. Abundace of mRNA transcripts for genes of interest encoding relevant transporters were assessed by qRT-PCR. A more complete understanding of how S.Tm overcomes growth inhibition through malabsorption will reveal treatment methods that don't dependent on antimicrobials.

Research Grant: unknown
Student Support: Boehringer Ingelheim

Effects of nutrition on the urobiome of 15 clinically healthy dogs

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The urine of humans and other animals contains diverse populations of bacteria, collectively known as the urobiome. These microbial populations play a significant role in influencing the health and disease of the urinary system. The aim of this project was to use a dog model to discover how nutritional components shape the urobiome. Fifteen clinically healthy dogs were prospectively recruited. Diet histories were collected from all dogs, and the major nutrients (protein, fiber, and fat) were determined on a g/100kcal basis. Urine samples were collected via cystocentesis. Microbial DNA was extracted from each urine sample, followed by amplicon sequencing of the V4 region from the bacterial 16S rRNA gene. Data was analyzed to determine how variation in major nutrients and dietary sources contributed to the diversity of the urobiome. Protein, fiber, and fat content had no statistically significant effect on alpha or beta diversity. However, beta diversity differed (PERMANOVA; $P = 0.002$) between dogs fed one particular commercial diet brand compared to dogs consuming other brands, characterized by lower alpha diversity ($P = 0.03$) and lower relative abundances of *Bacillus halodurans* ($P = 0.009$) and *Staphylococcus.* ($P = 0.03$). Beta diversity also differed ($P = 0.02$) between dogs fed two or less diet or treat sources compared to those receiving more. Overlap was observed between dogs consuming the commercial brand associated with reduced urobiome diversity and fewer dietary sources. Therefore, we cannot determine which was the driving force for diversity. Overall, results suggest that nutrition impacts the canine urobiome.

Research Grant: University of Minnesota College of Veterinary Medicine Resident and Graduate Student Research Grant. Partial support for Dr. Emily Coffey is provided by a National Institutes of Health T32
Student Support: NIH, Office of Director, Award Number T35OD011118

A review of animal cruelty legislation across states in the United States of America

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Every state in the US has laws against animal cruelty, abuse, and neglect. However, the regulations vary between states. There are major differences in definitions, penalties and even the type of events that are illegal. For example, animal hoarding is specifically addressed in some states but not in others. Other differences include who is required to report and who investigates suspected acts of cruelty. With the demonstrated link between animal cruelty and other forms of domestic abuse, some states have mandated cross-reporting of human and animal abuse. Humane investigators may be civilian or sworn officers depending on the state. To date, no review of animal cruelty regulations across all of the states has been conducted. This project aims to fill this gap by assessing the landscape of animal cruelty classification, investigation, and reporting regulations across the US. This will be conducted using a systematic review of the revised and administrative codes for each state. Specific factors will be evaluated, including: the presence and role of humane societies, which professionals investigate animal cruelty complaints, the training and employer of said professionals, cross-reporting regulations, liability and immunity clauses for reporters of suspected animal cruelty, and the classification of specific types of animal cruelty. These data will be described and compared to identify trends across states or regions. Information from this study can shed light on how animals are treated, classified, and protected in each state. These findings potentially reflect legal and cultural impacts to interventions aimed at decreasing animal cruelty and other violent crimes associated with animal cruelty.

Research Grant: PetSmart Charities Summer Research Fund
Student Support: Ohio State University Veterinary Summer Research Scholars

Racehorse Safety in Louisiana (2009-2022)

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Horse racing has grown in popularity over the years, but like other equestrian events injuries occur regularly. The Louisiana Racing Commission (LRC) keeps records of fatal injuries in horses at Louisiana racetracks. Since 2009, the Louisiana Animal Disease Diagnostic Laboratory (LADDL) has performed necropsies on dead or euthanized horses at the Fair Grounds Race Course (FGRC) in New Orleans as part of the National Thoroughbred Racing Association Safety and Integrity Alliance, created to improve the safety of racehorses. The results of the necropsies are communicated to the Alliance; however, a full analysis of the data has yet to be assessed. The purpose of this study was to provide a detailed report on the incidence of racehorse deaths at the FGRC from 2009 to 2022 and at other racetracks in the state of Louisiana to assess the number and cause of more frequent injury and death in horses.Records of race counts, starters, horse ages, and death counts were collected by the LRC Commission database for the FRGC and the other 3 Louisiana racetracks from 2009 to 2022. However, only horse racing at the FGRC underwent a targeted necropsy by a veterinary pathologist. Autopsy reports (2009-2022) from LADDL at LSU Vetmed were evaluated on all dead or euthanized horses at the FGRC. The data was compared to data from other years and from year to year.Expected results include that fatal injuries were less common in 2-year-old horses, horse racing in Louisiana had a low frequency of fatal injuries and fatalities compared to other state reports. Additionally, we would like to show that fewer racehorses have died from 2016 to present compared to before 2016, due to changes in drug laws.

Research Grant: Boehringer Ingelheim and Equine Health Studies Program LSU School of Veterinary Medicine
Student Support: Boehringer Ingelheim Bourse Régionale de mobilité internationale étudiant Auvergne-Rhône-Alpes

The correlation between chronic cardiac insufficiency and brain degeneration in canines

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Hypotension can result in brain hypoxia, resulting in fainting or syncope if acute. Cardiomyopathy places the brain at risk for these events when entering severe stages of heart failure. About 2% of the human population has congestive heart failure while 75% percent of these are individuals older than 65 years of age. As compared the canine population, a very common degenerative process, myxomatous valve disease, occurs in about 80% of small dogs, 30% of which progress into mitral regurgitation. Mitral regurgitation places the patient at risk for developing cardiomegaly and potentially congestive heart failure, particularly in late stages; thus, increasing the risk for neurovascular malperfusion. It has been well established that hypoxic events, depending on the duration and severity, can result in cognitive dysfunction in humans, presenting as atrophy in certain areas of the brain. Previous studies noted dementia-like cognitive impairments post-coronary bypass. This study will be focusing on whether there is a relationship between cardiac insufficiency and neurodegeneration while the localizing lesions such as gliosis within the brain. Using immunohistochemistry, degenerative lesions will be localized from the brain samples acquired from 20 deceased canine patients. This pool is comprised of patients with histopathological presentation of cardiac disease and cardiomegaly and a form of idiopathic neuronal degeneration.

Research Grant: Unknown
Student Support: NIH T35 Training Grant T335OD012199

Role of pro-inflammatory cytokines TNFα and CXCL10 on proliferation and migration of canine osteosarcoma cells

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Osteosarcoma (OS) is a malignant mesenchymal neoplasm that targets long bones of children and young adults and middle-aged large breed dogs. Despite decades of research, prognosis remains poor in both species primarily due to progression and development of pulmonary metastases. New therapeutic approaches that inhibit or halt metastatic disease are needed. The tumor microenvironment, which includes neoplastic cells, host cells, extracellular matrix, and biochemical signals such cytokines, has been implicated in the progression, invasion, and metastasis of tumors. Our lab previously found that canine Abrams OS cells secrete signals that promote secretion of the pro-inflammatory cytokines, TNFα and CXCL10, by macrophages. We hypothesized that TNFα and CXCL10 would, in turn, promote OS cell proliferation and migration. To test this hypothesis, we incubated canine Abrams OS cells with vehicle control or recombinant canine TNFα and CXCL10 at multiple concentrations for 24, 48, or 72hrs. We measured proliferation by colorimetric assay and migration by scratch assay. We found that TNFα and CXCL10 had no effect on canine OS proliferation. In contrast, while TNFα had no effect on OS cell migration, we observed a dose-dependent effect of CXCL10 on OS cell migration. These findings begin to tease apart the complex role that cytokines may play in the microenvironment of canine OS, which could lead to development of novel therapies that modulate tumor cell behaviors such as invasion or metastasis.

Research Grant: AKC Canine Health Foundation and Faculty Startup Funds
Student Support: NC State University Office of the Associate Dean for Research and Graduate Studies

A low-cost treatment for cranial cruciate ligament disease

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Cranial cruciate ligament disease (CCLD) is the leading cause of lameness and degenerative joint disease in the canine stifle. However, the cost of treatment may be prohibitive for many owners and shelters. The purpose of this study is to evaluate a cost-effective, simple, and minimally invasive surgery to treat CCLD in dogs. We hypothesize that a percutaneous lateral fabellar suture (pLFS) repair technique will be faster, more cost effective than the traditional open, while resulting in similar morbidity and earlier return to function of the limb. In this project, 10 dogs with unilateral CCLD were randomly assigned to the reference group (traditional LFS) or the test group (percutaneous LFS). Dogs were evaluated before and 3 times after surgery via owner's questionnaire, orthopedic examination, thermal imaging, radiographs, and pressure gait analysis. One-Sampled T-test were used for statistical analysis. Dogs treated with the pLFS had lower lameness score (pLFS $\bar{x}$ = 3.1, SD = 1.34; tLFS $\bar{x}$ = 3.9, SD = 0.74) and applied more pressure (pLFS $\bar{x}$ = 0.53, SD = 0.37; tLFS $\bar{x}$ = 0.35, SD = 0.23) on the operated limb at 2 weeks than those treated with a LFS (*P* value = 3.545e-06, *P* value = 0.0013). The maximal temperature over the operated stifle was 0.07 degrees lower after a pLFS than LFS (*P* value = 5.037e-10). These encouraging results justify further consideration of the pLFS as an alternative, low-cost treatment for CCLD.

Research Grant: PetSmart Charities, ASPCA
Student Support: PetSmart Charities

Higher serum anti-VapA IgG_1 activity is associated with reduced incidence of rhodococcal foal pneumonia

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Pneumonia caused by *Rhodococcus equi* is an important cause of disease and death in foals worldwide. Transfusion of foals with *R. equi*-hyperimmune plasma (HIP) is the best available preventative, but effectiveness of HIP under field conditions varies. Immunoglobulin G (IgG) against the virulence-associated protein A (VapA) of *R. equi* has been shown to protect foals from *R. equi* pneumonia. IgG sub-isotypes have varying functional activities and conflicting evidence exists for the roles of anti-VapA IgG_1 and $IgG_{4/7}$ in protecting foals from *R. equi* pneumonia. The association of anti-VapA IgG_1 and $IgG_{4/7}$ activities after transfusion of HIP with risk of pneumonia in foals has not been reported. Thus, we compared the activity levels of anti-VapA IgG_1 and $IgG_{4/7}$ after HIP transfusion between foals that did or did not subsequently develop pneumonia. All foals born at 2 farms in New York (n = 82) and transfused with HIP between January and March 2022 were included. Serum samples collected from foals pre- and post-transfusion were tested by ELISA for IgG_1 and $IgG_{4/7}$ activity against VapA. Foals were monitored for *R. equi* pneumonia by expert veterinarians. The proportion of foals with pneumonia was compared between foals with low activity (*i.e.*, ≤ median) or high (> median) of IgG_1 and $IgG_{4/7}$ using Fisher's exact test. Pneumonia was significantly (P = 0.003) less likely in foals with higher IgG_1 (5%; 2/41) than foals with lower IgG_1 (32%; 13/41); pneumonia was not significantly (P = 0.084) less likely in foals with higher $IgG_{4/7}$ (10%; 4/41) than foals with lower $IgG_{4/7}$ (27%; 11/41). Results indicate that activity against VapA of IgG_1 is more strongly associated with protection from *R. equi* pneumonia than $IgG_{4/7}$.

Research Grant: Link Equine Research Endowment, Department of Large Animal Clinical Sciences, Texas A&M School of Veterinary Medicine & Biomedical Sciences
Student Support: Boehringer Ingelheim VSP, Texas A&M School of Veterinary Medicine & Biomedical Sciences

Acrylamide increases incidence of macrophage infiltration in tumors of obese mice

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The exposure of obese individuals to environmental carcinogens may exacerbate an individual's risk of developing breast cancer or the severity of their cancer. In women, obesity increases inflammation while dietary exposure to environmental toxin acrylamide has been demonstrated to increase the risk of ERα-positive breast cancer. However, little is known about how acrylamide and obesity interact to promote breast cancer risk and their impact on ERα-positive tumor formation and macrophage infiltration. Three-week-old FVB female mice were randomized to receive a low fat (16% kcal from fat) or high fat diet (60% kcal from fat) with 0.7 mM acrylamide water or control water. Trp53-null mammary epithelial cells were transplanted into the mammary fat pad of recipient mice after clearing their endogenous epithelium. Tumors were collected after one year or when tumor burden exceeded 1.5 cm. We found that chronic exposure to acrylamide exacerbated the effects of obesity on mammary tumor formation in mice, leading to increased tumor incidence and reduced tumor latency. We observed increased macrophage infiltration in tumors of obese mice treated with acrylamide. We found variable expression of ERα within tumors. These results suggest acrylamide interacts with an obese state to promote mammary tumor formation by increasing inflammation within the gland. Understanding how carcinogenic dietary factors interact with obesity may reveal how breast cancer risk is further increased in obese patients, help in development of prevention strategies or early detection methods, and ultimately increase survival in obese breast cancer patients.

Research Grant: NIH NCI R01 CA227542
Student Support: National Institutes of Health (NIH), Boehringer Ingelheim (BI)

Determine the Effects of Artificial UVB Light on Plasma 25-Hydroxyvitamin D3 Over Time in Leopard Geckos

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Leopard geckos (*Eublepharis macularius)* is one of the most common reptiles kept in captivity, but there remains much we don't know about their basic husbandry. One of the most common diseases reported in captive leopard geckos is nutritional secondary hyperparathyroidism (a form of metabolic bone disease). This disease is preventable but is typically attributed to diets with low calcium/ high phosphorous or low vitamin D, or a lack of ultraviolet B (UVB) exposure. Since leopard geckos are insectivorous , most commercially available feeder insects are low in vitamin D and calcium when not gut-loaded appropriately. Because they are a crepuscular species, it was long believed that they didn't require UVB lighting in their husbandry; however, recent work by the senior author's laboratory has shown that UVB exposure does increase plasma 25-hydroxyvitamn D3 (25-OHD_3) concentrations in this species. Unfortunately, too much UVB exposure can lead to squamous cell carcinoma and cataracts. The purpose of this study was to determine the minimal amount of UVB exposure required to increase 25-OHD_3 concentrations in leopard geckos. Twenty-four leopard geckos were used for this study. The geckos were randomly assigned to three different UVB exposure groups: 15, 30, and 60 minute/daily UVB exposure. Each gecko served as its own control, and the geckos were exposed to the UVB for 28 days. The mean baseline 25-OHD_3 concentrations in the geckos was 29 nmol/L, which is low (< 50 nmol/L is low in this species). The 28 day post-UVB exposure blood samples have been collected and the results are pending.

Research Grant: Fluker's Farm, Boehringer Ingelheim Summer Scholars
Student Support: National Institutes of Health (NIH)

Pulmonary Health Impacts of Golden Tobacco Flavored Vuse Aerosols in Vulnerable Populations of Young Mice

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With ~2 million American youth using electronic nicotine delivery system (ENDS), there is growing public health concern surrounding the use of ENDS among young individuals. ENDS, devices, also known as "e-cigarettes," use nicotine salt-based formulas to deliver high doses of nicotine. Although Vuse Alto is a popular fourth generation ENDS, no study thus far has investigated the pulmonary health effects of inhaled Vuse aerosols. This study aims to provide laboratory-based evidence on the pulmonary effects of golden tobacco flavored Vuse aerosol exposures in juvenile mice. 4-week-old male BALB/c mice were exposed to either filtered air (n = 26) or golden tobacco flavored Vuse Alto aerosols (n = 26) via whole-body exposures for 1-hr/day (average total particulate matter concentration of 0.39 to 0.41 mg/puff), 5 days/week, for 3 months. Nicotine exposure from Vuse aerosols was confirmed with significantly elevated serum cotinine levels (> 33.2 ng/mL) in Vuse exposed mice compared to the air control group (< 2.1 ng/mL). Starting at day 54 until the end of the study, Vuse exposed mice had significantly decreased body weight (27.5 g) compared to the air control group (28.9 g), suggesting a potential effect of nicotine exposure on weight gain. Also, significantly elevated levels of 8-isoprostane, a biomarker of oxidative stress, was found in the broncho-alveolar lavage (BAL) of Vuse exposed mice (9.1 pg/mL) compared to air control (3.6 pg/mL). Although BAL cytology, lung tissue histopathology, and gene expression are currently ongoing, thus far our study shows that long-term exposures to Vuse aerosols can negatively impact the health of juvenile mice.

Research Grant: Project funded by a NIH NHLBI/FDA Center for Tobacco Products (CTP) grant to A. Noël (PI): K01HL149053
Student Support: NIH T35 Training Grant T35OD011151

Infectious disease seroprevalence in Minnesota moose

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Moose are a meaningful subsistence species for the Grant Portage Band of the Lake Superior Chippewa in Minnesota. Recently, moose populations have declined, affecting ecological balance and limiting available resources for those who consume them as a subsistence food. In order to maintain healthy moose population levels that are both ecologically and socially meaningful, we explored disease drivers impacting moose population decline. In this project, we aimed to identify the most prevalent infectious diseases affecting this population over the last decade and understand risk factors associated with disease presence. To achieve this aim, we collected serum samples from 152 animals, between 2010-2020, and tested for *Anaplasma phagocytophilum*, West Nile Virus, *Borrelia burgdorfer*i, *Leptospira Bratislava*, *L. canicola*, *L. grippotyphosa*, *L. hardjo*, *L. icterohemorrhagic*a, and *L. Pomona*. Individual animal characteristics were surveyed and their associations with disease were analyzed using multivariable logistic regression models. Preliminary results indicate *Borrelia burgdorferi*, West Nile Virus, and *Anaplasma phagocytophilum* had the highest seroprevalence rates (41%, 31%, 43% respectively), while overall disease prevalence and co-infections increased from 2018-2020. Models indicate seroprevalence for all diseases were impacted by the total number of exposures found in each moose, while 4 out of 5 leptospirosis strains were impacted by exposure to other leptospirosis strains. These results indicate co-infections and temporal changes impact disease seroprevalence, suggesting their importance for managing disease risk in moose populations. Future steps include incorporation of spatial analysis, pregnancy, and calf data.

Research Grant: None
Student Support: College of Veterinary Medicine Office of Graduate Programs

In vitro model of MOG and OVA peptide stimulated T-cell responses and effects of cannabinoids

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The plant *Cannabis sativa*, commonly known as marijuana, has a multitude of chemical compounds, known as cannabinoids. Two primary cannabinoids are cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC). These cannabinoids have been shown to suppress T-cell stimulated cytokine production, specifically in neurodegenerative autoimmune disease in mouse models. However, studying effects of cannabinoids *in vivo* can be limiting due to the systemic nature and costs of *in vivo* models. The goals of this research were to study peptide stimulated T-cell cytokine responses in a developed *in vitro* model, as well as to examine CBD and THC treatments causing T-cell cytokine suppression. Two peptides utilized in this research were Myelin Oligodendrocyte Glycoprotein (MOG_{35-55}), a self-peptide located in the myelin sheath of the central nervous system, and ovalbumin (OVA) peptide, a foreign peptide derived from chicken egg whites. We hypothesized that CBD and THC would suppress peptide stimulated cytokine production. Results showed that both MOG and OVA peptides drove T-cell cytokine production *in vitro*. CBD and THC also produced immunosuppression, as observed by ELISA, to measure cytokine levels. Data suggests that cannabinoids would be more efficacious as a prophylactic as opposed to a treatment for immune-mediated diseases. This study established an *in vitro* mouse model that will allow more complete analysis and further investigation of T-cell immunosuppression by CBD and THC, which could provide veterinarians and human doctors guidance on whether cannabinoids provide medical benefit or not.

Research Grant: Mississippi State University - Department of Comparative Medical Sciences
Student Support: NIH Grant P20GM103646 core

The Effects of Senolytic Drugs on the Gastrointestinal Integrity and Microbiome of Cynomolgus Monkeys

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Senolytic drugs are used to clear accumulated senescent cells (SCs) from the body. These cells arrest replication, but resist apoptosis. Upon acquiring a pro-inflammatory secretary phenotype, SCs cause aging-related comorbidities including cancer and cardio-metabolic diseases. The senolytic combination of Dasatinib and Quercetin (D+Q) has shown to reduce SC burden and lower gastrointestinal (GI) inflammation in aging mice. Caloric restriction (CR) is well known to decrease SC presence and chronic disease risk, and can work synergistically with other therapies. We investigated the barrier epithelium and microbiotic changes brought on by D + Q administration in middle-aged, obese, metabolically unhealthy male and female cynomolgus monkeys. Oral D + Q was given monthly for two days over a six-month period, and caloric intake was reduced by 10% at month five. Fecal, blood, and intestinal mucosal samples were collected at baseline, during, and at completion of senolytic therapy. Translocation biomarkers lipopolysaccharide binding protein (LBP) and soluble CD14 were used to assess the gut barrier integrity due to their roles processing leaked bacteria and their components. ELISAs performed on collected serum samples showed significant LBP decreases in D+Q treated monkeys. Further, LBP was correlated with soluble CD14 levels across the cohort. 16S sequencing performed on fecal and mucosal samples to analyze the gut microbiome at baseline showed no diversity or abundance-related differences between control and treatment groups. Microbiome changes will be characterized during and at completion of the study, and we expect that improved GI barrier function following SC clearance will be accompanied by a healthier GI microbiome.

Research Grant: NIH R01 HL149230
Student Support: NIH Training Grant T35OD010946

Assessment of a canine stifle goniometry simulation model for use in veterinary education

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Goniometry is an essential skill used in veterinary rehabilitation settings to help diagnose and monitor orthopedic conditions. This study explored the use of a canine stifle model for teaching goniometry. Our objectives were to create a normal canine stifle model and to compare students' confidence and accuracy in performing goniometry with exposure to either the model or traditional teaching methods. We hypothesized that students would demonstrate goniometry skills more confidently and accurately after using a simulation model than those given traditional materials. A flexible 3D printed model of a canine stifle was made. Twenty-three veterinary students were randomly given either instructional material from a textbook (n = 12) or access to the stifle model (n = 11). Students had 15 minutes with the reading material or the model individually. Immediately after a rehabilitation veterinarian assessed their accuracy in performing goniometry on a live dog. Students completed pre- and post- surveys where they indicated their confidence and anxieties. Statistical analyses performed include thematic analysis, descriptive statistics, and Chi Square analyses (significant at $P \leq 0.05$). There was no difference in goniometry performance between the model and reading groups. Students were more confident when identifying their anatomical landmarks after using prep materials as compared to before using the prep materials ($P = 0.05$). But, on average students could only identify 3/5 of the landmarks. Over half the students (12/23) could not correctly read the goniometer. While the model does not appear to increase student confidence or accuracy, in the future if supplemented with goniometer instructions, it may be a useful teaching aid.

Research Grant: Michigan Animal Health Foundation Educational Grant
Student Support: NIH T35 Grant

Measure of agreement among observers utilizing bottlenose dolphin pectoral flipper radiograph aging techniques

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A readily available, inexpensive, and non-invasive technique to more accurately age bottlenose dolphins is needed in order for stranding networks to continue to promote the conservation of this species. An aging technique assessing bottlenose dolphin pectoral flipper radiographs has been established and is readily available, inexpensive, and non-invasive but it has not been assessed for repeatability or agreement among users. The hypothesis of this study is that there will be good to excellent levels of agreement among four observers of varying experience levels scoring bottlenose dolphin pectoral flipper radiographs for aging purposes. Four observers of varying levels of expertise, including a board-certified veterinary radiologist, a marine mammal veterinarian, a veterinary student, and a stranding technician, evaluated 107 bottlenose dolphin pectoral flipper radiographs and scored 16 individual growth plates of the pectoral flippers utilizing the already established pectoral flipper radiograph aging technique. Agreement among the four observers was assessed by intra-class correlation (ICC) using PROC MIXED with SAS on all scored categories. The ICC analyses revealed that all four observers had excellent agreement levels on the total analysis of the 16 osseous locations scored. Results indicate that the published scoring system to age bottlenose dolphins by pectoral flipper radiographs can be used amongst workers of varying levels of experience with similar results; therefore, increasing its value as a tool for all stranding networks.

Research Grant: Mississippi Marine Mammal and Turtle Conservation, Recovery, and Monitoring Program; National Fish and Wildlife Foundation under Mississippi Department of Environmental Quality Agreement No. 18-00081
Student Support: Global Center for Aquatic Health and Food Security, Mississippi State University

Morphological and histological evidence for mechanical induction of osteoderm development in alligators

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The pathological development of bone in heterotopic ossification (HO) bears similarities to the natural development of osteoderms in the American alligator (*Alligator mississippiensis*). This post-embryonic process requires recruitment of mesenchymal stem cells followed by their differentiation into osteocytes. Factors determining stem cell fate are not well-understood in HO formation and completely unknown in osteoderm development. Studies have shown that mechanical stimuli may determine stem cell fate in heterotopic ossification in laboratory-generated mouse models of HO. Transmitting forces during normal body movements may provide the mechanical stimuli necessary to trigger stem cell recruitment to the dermis and differentiation into osteocytes post-embryonically.In this study, gross dissection was used to expose the ligamentous attachments of osteoderms to the skull, vertebral column, and ribcage, as well as tendinous attachments to muscular structures of the shoulder girdle and the epaxial musculature. These attachments were imaged and compared with histological data showing intrinsic dermal fibers under tension in specific and organized planes relative to the body axes. The findings of this study support that the development of osteoderms in alligators is triggered at least in part by mechanical forces acting on the dorsal scales during normal movements of the head, neck, truck, and tail during post-embryonic and juvenile growth phases; this developmental mechanism is comparable to the development of heterotopic ossification lesions via mechanotransduction.

Research Grant: Faculty-Student Research & Creative Activity Grant (Tarleton State University)
Student Support: Kenneth F. Burns Trust

Evaluation of a novel vaccine strategy for targeting neutralizing epitopes against the HIV-1 CD4 binding site

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Four decades after the declaration of the AIDS pandemic, no successful vaccine has been developed against HIV-1. Generation of broadly neutralizing antibodies (bNAbs) against the virus is critically important for developing a protective vaccine. VRC01 is a potent bNAb isolated from an HIV-1 infected individual against the CD4 binding site of the HIV-1 envelope glycoprotein gp120. VRC01-like antibodies, however, have not yet been successfully induced by vaccination. In this study, we evaluated a novel vaccine strategy referred to as Incremental, Phased Antigenic Stimulation for Rapid Antibody Maturation (IPAS-RAM) to target the CD4 binding site of gp120. Two antigens were used to immunize eight H-2Kb-tsA58 transgenic mice: HR1/2-eOD-GT6, a priming immunogen consisting of HR1/HR2 that can form a six-helix bundle and eOD-GT6, and BG505-DS gp140-μ85/229/241, a more native immunogen for boosting. Results from this study highlight the importance of vaccine formulation and provide future insight for studies utilizing the IPAS-RAM strategy on knock-in VRC01 mice.

Research Grant: NIH R21 AI-134838, Iowa State University
Student Support: NIH Award 5T35OD027967-03

Optic nerve head measurements of the adult equine eye using optical coherence tomography

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Purpose. To compare equine optic nerve head (ONH) measurements using spectral domain optical coherence tomography (SD-OCT) to evaluate ONH cupping and its correlation to pathology. Methods. Sixty eyes from 45 horses were evaluated and categorized as CONTROL (n = 19), CUPPED (n = 26) or PATHOLOGIC (n = 15). The eyes were categorized by ophthalmic examination with PATHOLOGIC eyes being those with visible ONH inflammation (e,.g., uveitis and optic neuritis) or proliferation (e.g., proliferative optic neuropathy and excessive myelination). The following measurements were performed: Bruch's membrane opening (BMO), optic cup width (OC), anterior laminar depth (ALD), prelaminar thickness (PLT), and cup to disk ratio (OC:BMO) at superior, central, and inferior ONH locations. Results. Compared to CONTROL and CUPPED, PATHOLOGIC OC and OC:BMO were significantly decreased. PATHOLOGIC OC (mean ± SD) was 1.51 ± 0.69, 1.84 ± 0.78 and 1.35 ± 0.82, while OC:BMO (mean ± SD) was 0.40 ± 0.19, 0.45 ± 0.20, and 0.36 ± 0.22. CUPPED eyes were found to have no consistent significant change when compared to control eyes. Conclusion. The term "optic nerve head cupping", as defined by direct and indirect ophthalmoscopy may be misleading in the horse. Eyes defined as having cupped ONHs based on direct and indirect ophthalmoscopy had a similar degree of cupping compared with clinically normal (CONTROL) eyes using OCT. Optic nerve head changes associated with inflammation can be readily identified and characterized using OCT. Further evaluation and research may allow us to better characterize and define equine posterior segment disease and optic nerve head cupping, respectively.

Research Grant: None
Student Support: Boehringer-Ingelheim Veterinary Scholars Program and Auburn University Department of Clinical Sciences

Toxic mechanisms for STM3845

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Non-typhoidal salmonellae (NTS), including serotype Typhimurium, are the leading causes of bacterial food-borne gastroenteritis in humans and livestock worldwide. Fundamental knowledge about bacterial growth is needed to develop new strategies to prevent NTS survival in, colonization of, and transmission from the intestine. A protein produced by NTS called STM3845 or Retron cold-anaerobic Toxin (RcaT, STM14_4640) is necessary for NTS survival by unknown mechanisms. The aim of this project is to understand how STM3845 functions as a toxin. STM3845, under the control of an IPTG inducible promoter and bearing a 3xFlag epitope tag, was grown in aerobic, anaerobic, and low temperature conditions. Co-immunoprecipitation was used to capture STM3845-3xflag and associated proteins, DNA, and RNA. Captured material was analyzed using mass spectrometry and DNA sequencing to identify targets that bind STM3845. Completion of these experiments should allow us to identify the targets of STM3845-mediated killing. Previous research performed suggests that STM3845 may act to disrupt cellular integrity. Increased knowledge of *Salmonella* survival mechanisms within the gastrointestinal tract will not only help with prevention of human disease but also the opportunity to create new strategies to reduce colonization in food animal species and thus reduce the contamination to the food supply and environment.

Research Grant: NIFA 2016-11004
Student Support: NIH T35OD010991-17, Texas A&M School of Veterinary Medicine & Biomedical Sciences

Identifying role of histone deacetylase class I and sirtuin in the development of the human parasite Entamoeba

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Amoebiasis in humans is produced by *Entamoeba histolytica* which affects 500 million people worldwide. *E. histolytica* has a life cycle that alternates between two stages: cyst (infective) and trophozoite (invasive). It is not possible to induce encystation in vitro in *E. histolytica*, however, the reptilian protozoa *Entamoeba invadens* has been used as a study model. The molecular mechanisms that regulate the conversion stage are not well known. Studies suggest that histone acetylation and deacetylation are key factors in the regulation of genes involved in the encystation. In Entamoeba, two classes of histone deacetylases (HDAC) have been identified: HDAC class I and HDAC class III also named sirtuins. Bioinformatic analysis showed that E. invadens has 2 genes coding for HDAC class I and 6 genes code for sirtuins. RT-PCR experiments tested the expression of HDAC class I and sirtuins and showed some genes were highly expressed during encystation, suggesting a role in this biological process. We aim to elucidate the participation of HDAC in the Entamoeba development. Full-length sequences of HDAC class I (EIN486830 and EIN096050) and three sirtuins (EiSir2A, EiSir2C, and EiSir2F) were cloned into E. invadens plasmid then, used to transfect parasites. Overexpression will be tested by Western blot and subcellular localization by immunofluorescence. The effect of overexpression of these genes will be evaluated by assaying encystation efficiency and phenotypes of altered cyst maturation and aberrant cyst morphology. The identification of HDACs role during encystation in *E. invadens* could provide information on therapeutic drugs that can block the process of encystation and reduce the infective transmission of *E. histolytica.*

Research Grant: None
Student Support: NIH T35 Training Grant OD010989

Veterinary student proficiency and ovariohysterectomy duration: a statistical analysis

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Castrations and ovariohysterectomies (OHEs) are among the most common services provided by veterinarians. At Mississippi State University College of Veterinary Medicine, veterinary students are first introduced to these surgical methods during their second year of training and continue to perform these procedures throughout their third and fourth years, specifically during a shelter medicine spay and neuter elective. Student confidence and factors encompassing student surgical procedure have been the focus of previous studies. However, the variability of the student learning is poorly understood. The aim of this study was to determine the number of OHEs veterinary students need to complete to obtain proficient surgical duration. This study utilized Microsoft Excel to record information pertaining to students and their OHE patients. Linear regression using a proc mixed procedure was used in analysis via SAS software. The average OHE duration for veterinary students started off at approximately forty-four minutes. The rate at which student surgical duration decreased was logarithmic and was statistically significant until their 51st OHE at which point the rate becomes negligible. All in all, this study can be implemented in veterinary colleges to target the ideal number of OHEs to maximize student success and experience, as well as conserve institutional resources.

Research Grant: Unknown
Student Support: Boehringer Ingelheim Veterinary Scholars Program, Miss. State CVM

Characterization of *Dirofilaria immitis* products secreted during infection of *Aedes aegypti* mosquitoes

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The parasitic nematode *Dirofilaria immitis* causes heartworm disease worldwide in domestic dogs, cats, and ferrets. Since mosquitoes are required as both an intermediate host and vector for *D. immitis*, they provide a unique opportunity to disrupt the heartworm transmission cycle. In a competent vector, microfilariae ingested during blood feeding migrate from the midgut into the Malpighian tubules, the mosquito renal organ. In the Malpighian tubules, parasites develop into infectious third-stage larvae (L3) over a two-week period. L3 migrate through the body cavity to the proboscis where they can be transmitted during the next blood meal. Given that the hemolymph, the fluid filling the body cavity, contains high concentrations of immune proteins, it is not known how parasites can survive without being eliminated. The aim of this study is to investigate the interaction between L3 and *Aedes aegypti* mosquitoes, and the study hypothesis is that larvae secrete products to modulate the hemolymph immune response. To investigate this, the Toll immune signaling pathway will be activated through gene silencing using RNAi by injecting dsRNA 14 days after infection when L3 are present in the body. The ability of L3 to emerge from mosquitoes will then be evaluated to determine whether they were killed or otherwise damaged. Additionally, mosquito hemolymph will be analyzed using bioinformatics pipelines for the presence of *D. immitis* proteins that could potentially modulate mosquito immunity. By learning more about these transcriptional changes and products that *D. immitis* secrete to evade the mosquito immune response, this data can be used to devise novel strategies to reduce heartworm incidence.

Research Grant: Morris Animal Foundation Grant (D22CA-015)
Student Support: NIH T35 OD010919, Boehringer-Ingelheim, and the University of Pennsylvania

Investigating chronic wasting disease testing capabilities in Virginia

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Chronic Wasting Disease (CWD) is a transmissible spongiform encephalopathy first confirmed in Virginia in 2009 and since resulted in 25 positive cases in white-tailed deer across Virginia. As a result, the Virginia Tech Animal Laboratory Services is in the process of standing up National Animal Health Laboratory Network (NAHLN) approved testing for CWD via ELISA and IHC. The goal of this study is to evaluate CWD disease surveillance in Virginia with reference to other state diagnostic laboratories and strategic plans. It is hypothesized that diagnostic testing provides a level of variance to the CWD risk profile despite the standardization of diagnostic procedures which will have qualitative impact on the Virginia CWD risk assessment. To study this, we contacted CWD laboratory managers and technicians at each NAHLN accredited laboratory approved for CWD testing to identify the laboratory differences. We created a Vensim model to show the variables impacting diagnostic turnaround time as well as developed a decision tree outlining the CWD testing process from retropharyngeal lymph nodes/obex sampling to CWD result dissemination. An abbreviated disease risk analysis was then performed following the World Organization for Animal Health Manual of Procedures for Wildlife Disease Risk Analysis. The variables found to have a significant impact on turnaround time include laboratory availability, priority listing, secondary confirmation, and the number of samples, technicians, and equipment. This project highlights the importance of diagnostic testing to disease surveillance, which factors are capable of extending laboratory turnaround time, and their corresponding impact on the CWD risk analysis.

Research Grant: Virginia-Maryland College of Veterinary Medicine
Student Support: Boehringer Ingelheim Veterinary Scholar

Parasites of wild turkeys from Middle Tennessee

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Wild turkeys play an important role in hunting as both a food source and in ecosystem conservation efforts; however, their populations have seen significant declines in North America, including Tennessee, over the last few hundred years. These declines may be attributed to their popularity in hunting both for nutritional purposes and as trophies; however, other factors have been cited for these declines such as parasites, disease, habitat loss and climate change. There is limited data currently available on the impact and prevalence of parasites in wild turkeys. Parasites known to infect wild turkeys include *Histomonas meleagridis*, *Heterakis gallinarum* and various gastrointestinal parasites such as *Eimeria* spp., *Coccidia* spp., and various helminths.. Due to the potential for histomonosis, a cause of mortality in turkeys, *Heterakis gallinarum* is especially important to monitor in wild populations. This study evaluates the parasites found in fecal samples from a wild turkey population in Middle Tennessee which has been experiencing declines. Using a fecal flotation method, parasite eggs for 400 turkey fecal samples were categorized.

Research Grant: None
Student Support: Boehringer Ingelheim

The role of bovine miRNAs in modulating the immune-system recognition pathway

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Bovine colostrum and human milk are composed of a variety of biologically active compounds including microRNAs (miRNA). miRNAs are non-coding RNA molecules that regulate mRNA and protein expression. The aim of this study is to identify specific miRNAs present in bovine colostrum which modulate the immune system's response to infection. To test the immunological regulation by bovine derived miRNAs, RAW-Blue Cells, a mouse macrophage reporter cell line was challenged with *E. coli* lipopolysaccharide (LPS) and incubated with a pool of miRNAs isolated from bovine colostrum over a 26-hour period. After incubation, QUANTI-Blue assay was used to detect the activation of the transcription factor NF-κB by the different treatments. Cells treated with miRNAs induced to some extent the signaling pathways leading to the activation of NF-κB relative to control cells ($P < 0.01$). Interestingly, bovine miRNAs suppressed LPS-induced NF-κB activity in a dose-dependent manner ($P = 0.01$). Currently, the bovine colostrum-miRNA profiling for this study is being performed using real-time qPCR assays to identify the top miRNAs that are driving such effects in vitro. Overall, our preliminary findings indicated that miRNAs derived from bovine colostrum have the potential to modulate the immune system recognition pathway. Moreover, miRNAs in bovine colostrum along with well-known molecules such as immunoglobulins may impact the response to neonatal infections.

Research Grant: School of Veterinary Medicine – Texas Tech University (SVM TTU)
Student Support: SVM TTU Veterinary Research Scholars Program

Incidence of liver abscess in feedlot mortalities during the feeding period and association with co-morbidity

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The timing of liver abscess development is poorly characterized in feedlot cattle. The study objectives were to evaluate when liver abscesses occur during the feeding period and potential associations with co-morbidities. An observational study was performed using systematic necropsies of all mortalities at 6 feed yards to evaluate multiple organ systems and primary cause of death. Abscess frequency and association with treatment history, average arrival body weight by lot, and lot arrival dates from yard databases were determined. In 232 necropsies, 14 cases had liver abscesses. Prevalence of liver abscesses in mortalities at < 50, 50-100, and > 100 days on feed were 3.4%, 2.0%, and 10.1% of all cases, respectively. No cases with rumenitis (58) or gastrointestinal (GI) lesions (1) were also noted to have liver abscesses. Of the 12 liver abscess cases treated prior to death, 8 were diagnosed at treatment as bronchopneumonia, 1 as GI disease, and 3 as other. Evaluation of primary cause of death found liver abscesses in 12/145 (8.2%) respiratory, 1/17 (5.8%) cardiovascular, 1/20 (5%) musculoskeletal, and none in digestive, neurological, reproductive, or other diagnoses. Initial arrival-weights were in the 500- or 600-lb ranges for 10/14 of liver abscess cases. This study's sample is biased since only feedlot mortalities were included instead of all cattle during the 6-week period. Respiratory disease is a common cause of death, which could show increased numbers of liver abscess in respiratory cases even though there is no significant association. Findings of this study indicate liver abscesses primarily occur after 100 days on feed and are not strongly associated with co-morbidities including GI lesions.

Research Grant: Funding from Innovative Livestock Services, Legacy Animal Nutrition, Beef Cattle Institute, and Foundation for Food and Agricultural Research
Student Support: Boehringer Ingelheim Veterinary Scholars Program and KSU Veterinary Research Scholars Program

Does hive strength predispose honey bees to European foulbrood disease?

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European Foulbrood (EFB) is a bacterial disease of young honey bee larvae, caused by *Melissococcus plutonius*. Once in the midgut, *M. plutonius* outcompetes the larva for nutritional resources, eventually leading to larval starvation and death. Increased incidence of EFB is observed during early spring, particularly in weak colonies. It is the period of rapid growth when the number of developing larvae (brood) relative to the adult bee population is the highest, and environmental food resources including pollen and nectar are sparse. However, it is unclear what the relative contribution of these factors is to the increased incidence of EFB. Accordingly, the objective of this study was to investigate if increased brood to worker bee ratio, such as found in quickly developing spring colonies, would increase larval susceptibility to EFB. Using a previously developed *in vivo* (within hive) model, we will establish small paired colonies with high or low brood to adult bee ratio. Next, a group of genetically related larvae inoculated with incremental doses of *M. plutonius* will be introduced to each colony. Larval survival to pupation (capping) and bacterial load in surviving pupae will be monitored in the infected individuals. We hypothesize that weak colonies with fewer worker bees and more brood will be more likely to develop EFB due to inadequate care. The results of this foundational study will contribute to better understanding how colony strength impacts EFB infection in honey bee colonies. By suggesting appropriate colony population management strategies, it may provide alternatives to antibiotic use for EFB management.

Research Grant: Project Apis m.; Costco Canada; BC Blueberry Council; Saskatchewan Beekeepers Development Commission; MITACS; Agriculture Development Fund; Agriculture Funding Consortium
Student Support: WCVM — Undergraduate Student Research Award; Boehringer Ingelheim Veterinary Research Award

Identifying orexin receptors on hypothalamic neurons activated by hypoxia

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The hypothalamus plays a role in the control of respiration. Specifically, the paraventricular nucleus (PVN) of the hypothalamus contributes to the cardiorespiratory response to hypoxia via projections to the nucleus tractus solitarius (nTS). Orexin, a neuropeptide expressed in the hypothalamus close to the PVN, also contributes to the cardiorespiratory response to hypoxia. The mechanism(s) by which orexin contributes to hypoxic responses is unknown. Our lab is determining if orexin receptor type 1 (Ox1R) is expressed by corticotropin-releasing hormone (CRH) neurons in the PVN that are activated by hypoxia. We hypothesize that CRH neurons that are activated by hypoxia express Ox1R. Rats were exposed to either normoxia or hypoxia for two hours to induce activation. Their brains were fixed with paraformaldehyde, harvested, and sectioned. Sections were mounted on slides for immunohistochemistry to detect c-Fos (marker of cellular activation), CRH, and Ox1R. Primary antibodies against these targets, followed by secondary antibodies labelled with fluorophores, were incubated with the slices from the PVN. Immunofluorescence visualized the Ox1R on activated, CRH neurons. Qualitatively, rats exposed to hypoxia had more Fos-immunoreactive (i.e. activated) CRH neurons compared to normoxic controls, and Ox1R was present on activated, CRH neurons. These results suggest CRH neurons express Ox1R, and that Ox1R may facilitate the activation of CRH neurons in response to hypoxia. In the future, we will look at nTS-projecting CRH neurons with and without orexin receptor blockade to assess whether orexin facilitates their activation under hypoxia.

Research Grant: R01HL098602 (Cummings, Co-PI)
Student Support: Boerhinger Ingelheim

Chinese water dragons and goldfish as hosts for maintaining *Burkholderia pseudomallei*

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Burkholderia pseudomallei is the cause of melioidosis, a prominent bacterial disease in Australia and Southeast Asia. In addition to causing severe human disease, this bacterium also has an extremely broad host range for many domestic and wild animal species such as goats, pigs, and sheep. Infection of *B. pseudomallei* occurs through contact with contaminated soil or water. There have been a few documented cases of iguanas that have been infected with *B. pseudomallei*, as well as an incident of transmission of *B. pseudomallei* to a human from a contaminated fish tank that contained imported tropical fish. Although infection of ectotherms is possible, it has been an understudied area for this bacterium. The purpose of these pilot studies was to examine the persistence of *B. pseudomallei* in fish tank water and its effects on goldfish and Chinese water dragons. We hypothesized that *B. pseudomallei* would colonize the fish tank water and fish, but the fish would not show overt disease and that the lizards would become infected regardless of the route of inoculation. We infected two groups of water dragons with *B. pseudomallei*, one orally and one subcutaneously, and performed necropsies to analyze the tissues for signs of infection. The fish tank was contaminated by the inoculation of *B. pseudomallei* into the water and was sampled routinely for culture. *B. pseudomallei* was isolated from the fish tank for several days but cleared rapidly. The water dragons were found to be highly susceptible to infection and developed characteristic abscesses, especially prominent in the liver. Individuals owning these species should be aware of the risk of possible infection of these reptiles or fish, especially if imported from endemic areas.

Research Grant: Animal Models Core
Student Support: Animals Models Core

Development of a pH reactive fecal sampling molecule for in vivo sampling of murine small intestine

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Pronounced regional differences between the microbiota of the upper and lower gastrointestinal tract (GIT) that influence metabolism and disease reinforce the importance of analyzing the complete gut microbiota. However, no commercial system currently exists to survey upper GIT contents without requiring highly invasive or post-mortem sampling. This project contributes to a larger goal of developing a non-invasive method to collect upper GIT microorganisms in animal models or humans. The ability to noninvasively evaluate the small intestinal microbiota would provide an alternative to terminal cohorts of mice in longitudinal studies and advance human gastrointestinal medicine. Stool pH is known to be 5.5 - 6.5 for the majority of the small intestine before increasing to 7.4 - 7.5 near the terminal ileum. The objective of the present study is to determine if *Lactococcus lactis* bacteria will bind to synthesized Poly(amino acid) at a pH similar to that of the small intestine and stay bound at a colon relevant pH. In principle, this will allow us to develop a magnetic particle coated with pH-sensitive biomolecular polymers to capture small intestinal microbiota, retain the contents throughout the large intestine, and recover them from the feces via magnetic separation. To test for positive association, we will utilize a strain of *L. lactis* that is transgenic for an inducible green fluorescent protein (GFP) and a Poly(amino acid) labeled with the TAMRA fluorophore. Co-localization of the bacteria with the polymer will be determined with high powered fluorescence microscopy. Development of a device to capture small intestinal microbiota would likely be marketable and amenable to translation to human medicine.

Research Grant: NIH U42 OD010918, Mutant Mouse Research and Resource Center
Student Support: An endowment from IDEXX BioAnalytics

Ethical considerations and outcome measurement in Accessible Veterinary Care

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Access to Veterinary Care (AVC) and the question of how to increase access for those who may otherwise not be able to utilize veterinary services is currently at the forefront of veterinary medicine. Veterinary care, like human healthcare, can be considered a limited or scarce resource for which the demand cannot be satisfied. As such, with the goal of improving access in mind, decisions must be made as to who will and will not be eligible for services, and regarding the desired impact. The ethical frameworks used to allocate these resources are well studied in human bioethics, but the same cannot be said for veterinary medicine. With this study, we will examine the prevalent ethical frameworks that guide decision making in human medicine and correlate these to veterinary medicine. Frameworks can be categorized by whom they aim to support, and their desired result. Broadly speaking, these are broken down into utilitarian principles which maximize overall health benefit or years of life, egalitarianism which offers equal chance of receiving support or equal resources across a population, welfare-based principles which prioritize the most vulnerable or susceptible groups, and desert-based principles which distribute resources relative to individuals' contributions to society or what is "deserved." Focus groups and interviews will be conducted among veterinarians, professionals in low-cost or accessible veterinary services, and clients to assess and compare how these groups employ ethical frameworks to make decisions and achieve desirable outcomes of treatment.

Research Grant: ASPCA
Student Support: Wolfe Summer Research Fund

Synthetic mRNA-induced expression of H2 Relaxin by bovine epithelial cells

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Relaxin (RLN) is a reproductive hormone that enhances connective tissue remodeling during pregnancy and parturition and has been used therapeutically to reduce the incidence of dystocia in cattle. However, attempts using purified porcine or recombinant human RLN to reduce the incidence of dystocia in heifers present variable results. Human 2 (H2) RLN has a high affinity to the bovine RLN receptor (RXFP1) and given advancements in mRNA therapeutics, H2 RLN mRNA therapy may prove to be a more efficacious treatment for dystocia. Using a H2 RLN-NanoLuciferase (NanoLuc) mRNA construct with a secretion signal, we transfected bovine kidney (BK) and primary bovine epithelial cells (BVEC) with 0.5, 1 or 2 μg synthetic mRNA. At 3, 6, 12, 24 and 48 h post-transfection, cell lysates and supernatants were collected for detection of H2 RLN indirectly via Nano-Glo Assay (Promega) or directly via ELISA (R&D Systems). Luminescence demonstrated that bovine epithelial cells expressed H2 RLN in cell lysates for all observed time points with a decline only observed at 48 h in the BVEC cells. In contrast, cell supernatants exhibited increasing levels of luminescence, indicating secretion of H2 RLN. Additionally, increasing concentrations of H2 RLN were observed from 6-48 h in supernatants from BK cells transfected with the lowest concentration of mRNA (0.5 μg). Furthermore, the *in vivo* transfection of a 6-month-old dairy heifer with NanoLuc mRNA demonstrates the bovine reproductive mucosa is receptive to transfection resulting in high levels of expression at the ectocervix, the target tissue for H2 RLN. These data provide evidence supporting future in vivo transfections with H2 RLN mRNA as a novel approach in reducing dystocia in heifers.

Research Grant: USDA ARS Biophotonics Research Grant project #6066-31000-015-00D
Student Support: Mississippi State University Office of Research and Graduate Studies

Characterization of the microbiome of anal sacs in pet dogs with apocrine gland anal sac adenocarcinoma

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Canine apocrine gland anal sac adenocarcinoma (AGASACA) are malignant tumors of the anal sacs (AS). AGASACA is commonly diagnosed at the University of Missouri Veterinary Health Center, and environmental factors including the microbiome may contribute to the pathogenesis. The microbiome of anal sacs in healthy pet dogs or those with AGASACA has not been described. The objective of this study was to characterize the microbiome of the AS of pet dogs with and without AGASACA. We hypothesized that the AS microbiome amongst healthy dogs would be similar regardless of diet or lifestyle and that AS microbial composition and diversity would differ between healthy controls and dogs with AGASACA. Samples from 24 healthy pet dogs and 13 dog with AGASACA were prospectively enrolled. Use of antibiotics and presence of gastrointestinal signs were exclusion criteria. Fecal samples and AS fluid samples from the left and right anal sacs were collected. DNA extraction and 16s rRNA amplicon-based microbiome analysis was performed. Using the Jaccard and Bray-Curtis indices, there was a significant difference (P = 0.0001) in β-diversity between the feces of healthy dogs and AGASACA dogs. There was a significant difference in AS β-diversity between dogs with and without AGASACA (P = 0.0018) using the Jaccard Index, which relates to presence and absence of taxa. However, there was no significant difference (P = 0.4137) in β-diversity accounting for abundance using Bray-Curtis similarities. Fecal samples from dogs with AGASACA were significantly less rich (P = 0.002), but no difference was found between healthy and AGASACA AS samples. Sample collection from dogs with AGASACA is ongoing.

Research Grant: None
Student Support: Stipend for Morgan Burke is supported by a grant from Boerhinger Ingelheim

Sperm collection in the domestic cat: A comparison of two techniques using dexmedetomidine

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Collecting sperm from cats can be difficult but is vital to determine potential fertility of a tom. Urethral catheterization (UC) and electroejaculation (EEJ) are established methods for collecting sperm in felids. Electroejaculation is considered the most reliable means of obtaining a sperm sample. Urethral catheterization allows practitioners to collect semen via a quick, minimally invasive procedure using medetomidine as a sedative. However, medetomidine, an α2-adrenoceptor agonist (α2A), is unavailable in some countries. Dexmedetomidine (dexdom), a potent α2A with the same properties has replaced it. This study compared two methods using dexdom alone (UC) or dexdom and ketamine in combination (EEJ) at a comparable dose to medetomidine. Twelve healthy, mature, intact male cats were collected thrice at 1 week intervals. Following an initial EEJ, all cats were randomly assigned to undergo EEJ or UC first, followed by the other procedure. Cats received ketamine (5mg/kg) and dexdom (30mcg/kg) for initial cleanout and trial EEJ, and dexdom (60mcg/kg) for UC. Results showed no notable differences in the percentage progressively motile, or morphologically normal sperm between ejaculate types. Although UC yielded lower volume and higher concentration ejaculates, there was an overall lower total sperm number (TSN) compared to EEJ. Urethral catheterization gave an average volume of 9.08 μL and a concentration of 2,621.3 million sperm per mL, and TSN of 20.03 million, whereas EEJ averaged a volume of 122.3 μL at 447.27 mill/mL and a TSN of 49.44. Urethral catheterization with dexdom at 60 mcg/kg consistently provided a sperm sample for analysis in 92% (11/12) of cats that was comparable to EEJ in overall sperm quality.

Research Grant: None
Student Support: Merial Summer Scholars Program

Chlamydia trachomatis and the role of its inclusion membrane protein CT226 in inflammation and infection

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Chlamydia trachomatis is the most frequently reported sexually transmitted bacterial infection, and infection can result in pelvic inflammatory disease, infertility, chronic pelvic pain, and ectopic pregnancy. *C. trachomatis* has a unique biphasic lifecycle in which it alternates between an infectious elementary body (EB) stage and a metabolically active reticulate body (RB) stage. Within the host cell, it forms a parasitophorous vacuole termed an inclusion. Recent work in our lab suggests that inclusion membrane protein, CT226, modulates the host immune response through interactions with the inflammasome. Following previously established methods for murine cervicovaginal chlamydial infection, two groups of twelve C3H/HeJ mice were intravaginally infected with *C. trachomatis* L2 (wild type) or deletion mutant *C. trachomatis* L2-ΔCT226. Following infection, intravaginal swabs were collected on days 3, 7, 14, 21, 28, 35, and 42. Once collected, the samples were diluted and cultured in McCoy cells, stained with immunofluorescent, *Chlamydia*-specific antibodies, and viewed using immunofluorescence microscopy to count and compare the number of infectious forming units (IFUs). On day 63, reproductive tracts will be harvested for histopathology to compare inflammation response between the two infections. In previous trials within our lab, L2-ΔCT226 infection resulted in a higher number of IFUs than L2 wild type, and we predict the result will be the same during this trial. These results will confirm the role CT226 plays in chlamydial infection.

Research Grant: NIH R15 1R15AI149439-03
Student Support: Oklahoma State University CVM Summer Research Training Program

The Immune and Molecular Landscape of Canine Osteosarcoma

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Osteosarcoma is an aggressive form of bone cancer that affects both humans and dogs. Immune cells can be present in these tumors and have an effect on overall patient outcomes. Our hypothesis is that osteosarcomas are localized in neighborhoods in which anti-tumor immune responses are initiated and sustained. Signatures for white blood cell subsets will be created using blood from healthy dogs and single-cell sequencing. Disaggregated canine osteosarcoma cells will be labeled with oligonucleotides and also processed for single-cell sequencing. Our results will allow us to describe the composition of the osteosarcoma microenvironment. This project will allow us to identify the relative proportion and diversity of immune and inflammatory cells in osteosarcomas. This knowledge will help to guide the development of appropriate therapies for treatment.

Research Grant: Veterinary Cancer Society/Dr. Gordon Theilen Resident Research Award (JEM), AKC Canine Health Foundation (CHF grant 03015, JFM), and Animal Cancer Care and Research Program of the UMN.
Student Support: NIH, Office of the Director, Award Number T35OD011118

Validation of a Copeptin Assay in Normal Dogs

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Copeptin is a 39 aa peptide produced in the pituitary through cleavage of pre-pro arginine vasopressin (ADH) and co-secreted in an equimolar amount with ADH into the blood. Copeptin is more stable than ADH in circulation, where it serves as a marker for ADH. Copeptin measurement has the potential to be useful for advancing understanding of disorders of water balance. The purpose of the study was to validate a commercial ELISA assay to measure copeptin in canine serum. A commercial ELISA assay (Copeptin (Human) EIA Extraction Free kit, Phoenix Pharmaceutical Inc) was validated using serum from healthy dogs. Standard curves were plotted using the 4-parameter logistic (4PL) model. Standards and canine samples were analyzed in duplicate. Validation parameters determined were intra- and inter-assay variation, recovery, parallelism, and dilutional linearity. Copeptin was detected in serum from all dogs (n = 9). The mean serum copeptin concentration was 1.67 ng/mL (range 0.85-4.09 ng/mL). Intra-assay coefficient of variation (CV) was 8.42% and inter-assay CV was 9.6%. The limits and range of detection were 0.1 -10 ng/ml. The assay displayed dilutional linearity and parallelism. Recovery from spiked samples averaged 147%. In conclusion, the copeptin assay displayed acceptable parameters for precision, linearity, and recovery. The results provide an expected range for the baseline serum copeptin concentration in dogs, although a larger population of healthy dogs would be needed to determine a true reference range. Copeptin measurement by ELISA may be a useful research tool to assess water balance in dogs.

Research Grant: Mark Derrick Canine Research Grant
Student Support: National Institutes of Health T35 Training Grant

Vesicular stomatitis virus-Indiana and *Culicoides sonorensis* biting midges: insights into vector competency

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Vesicular stomatitis virus (VSV) is an economically important, reportable, zoonotic disease of cattle, horses, and swine, transmitted via insect vectors and by direct contact. Unlike the New Jersey (NJ) serotype, Indiana (IN) rarely causes outbreaks. Yet in the summer of 2019, infections were confirmed in cattle and horses from 472 premises across eight states. We hypothesized that the *Culicoides sonorensis* biting midge, a known vector for VSV-NJ, would be a competent vector for VSV-IN. Thus, a time-course infection study was conducted in midges using a 2019 Indiana field isolate to determine whether virus would disseminate from the midge midguts and into the heads and salivary glands by day 10 post-feeding. Midges were fed a 1:1 mixture of sheep's blood and stock virus. Post feeding, fully engorged females were sorted, heads and bodies were separated and sampled at day 3, 8, or 10. Vero cell monolayers were used to confirm infectious virus by cytopathic effects (CPE). RNA extraction and RT-qPCR was performed from the cell culture samples. CPE was visible for 100% of day 0 and day 3 samples, 0% of day 8 samples, and 40% of day 10 samples. RT-qPCR detected virus in 100% of day 0 and day 3 samples, 40% of day 8 samples, and 90% of day 10 samples. The results demonstrated that by day 3 the virus has disseminated from the midgut to the head. The CPE indicated that the virus remains infectious through day 10 and suggests potential vector competence. This work has applications for insect control protocols during future outbreaks. Future replicate studies with additional time points are needed, as well as a better understanding of virus titers in VSV-IN infected livestock lesions on which midges feed in nature.

Research Grant: NP103, #3020-32000-019-00D
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Investigation of DNA repair and replication genes in *Plasmodium falciparum*

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Plasmodium falciparum is the causative agent of the most severe forms of malaria, a mosquito-borne parasitic disease that afflicts millions each year. As with many infectious diseases, the battle against drug resistance necessitates continuous research to discover novel therapies. Evolution of drug resistance and immune evasion in *P. falciparum* is often driven by genomic rearrangements, such as copy-number variation or antigen diversification, typically mediated by DNA repair and replication pathways. The *P. falciparum* genome contains genes with sequence homology to known members of these pathways, but few of these genes have been functionally characterized. We selected 15 genes with presumed functions in DNA repair or replication for detailed study using transgenic parasite lines generated in our lab that target these genes for conditional translational regulation. To determine which genes, if any, in our target set are required for mitigation of DNA damage, we pooled these parasite lines and exposed them to the topoisomerase inhibitor ciprofloxacin under conditions inducing either protein depletion or wild type expression. Samples were taken at regular intervals over 16 days for high-throughput sequencing of molecular barcodes to track the survival of each line over eight generations of parasite growth. Parasite lines targeting genes required for DNA repair or replication are expected to show decreased survival when subject to ciprofloxacin-induced DNA damage compared to our control compounds; DMSO and protein synthesis inhibitor chloramphenicol. Findings from this and future studies can be explored in-vivo using an immunocompromised (SCID) mouse model to bridge the gap between culture and living systems.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program, NIH T35 OD033655

Impact of Classical Counterconditioning (Quiet Kennel Exercise) in Sheltered Dogs

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A major welfare concern in animal shelters is excessive barking from kenneled dogs. This not only contributes to noise pollution and can cause hearing damage in humans, but also has a negative impact on all animals within earshot. This study aims to demonstrate that by implementing a simple counterconditioning exercise, we can help change the emotional state of dogs from negative to positive, and consequently reduce the fear and frustration that leads to excessive barking. The study was conducted in the five wards of adoptable dogs at Wake County Animal Center in Raleigh, North Carolina. The Quiet Kennel Exercise consisted of instructing passersthrough of the wards to toss a treat to each dog regardless of the behavior they exhibited. For the first two weeks of the study, baseline data was collected. This consisted of using a hand-held decibel meter to measure the volume of barking in each ward three times a day. Video cameras were mounted in each ward to document the number of people that pass through each ward each day. Data was collected for four weeks of intervention. During this time, people were encouraged to toss treats to each dog, using signage. Data was collected using the same methods, but this time the number of people who did and did not toss treats were recorded to measure compliance. At this time, data is still being collected and statistical analysis is pending. However, an overall positive change in attitude from most dogs towards visitors has been observed and an increase in compliance from the public gives hope that the exercise would have statistically and clinically significant positive effects at the conclusion of the study, and the intervention continued longterm.

Research Grant: Fear Free Research Grant
Student Support: NC State University Fluoroscience Endowment

Under pressure: the neural and hormonal mechanisms underlying courtship and predator avoidance tradeoffs

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Reproductive success depends upon appropriate decision-making in the face of competing demands. For example, mating provides clear fitness benefits, yet courtship can involve conspicuous displays that make animals more vulnerable to predation. How do animals balance conflicting needs? Ecological evidence provides some insight into how animals balance needs such as courtship and predation risk, but the genomic and hormonal mechanisms underlying this tradeoff are not well understood. Threespined stickleback (*Gasterosteus aculeatus*) males were presented with a tradeoff between courtship and predator avoidance. Territorial males, identified based on breeding coloration and aggressive behavior, were placed in individual tanks with nesting material. Males with completed nests were then randomly assigned to one of four treatments: predation risk (chemical and visual cues of a common predator), courtship opportunity (exposure to a gravid female), both predation risk and courtship opportunity, and control. Courtship and vigilance behaviors were collected for 2 minutes before, during, and after treatment using Behavioral Observation Research Interactive Software (BORIS). After one hour, males were euthanized, brains were excised and divided into diencephalon, telencephalon, and cerebellum/brainstem for RNA sequencing, and bodies were collected for hormone analysis. We hypothesize that males balancing the risk of predation against the opportunity to mate will compromise courtship, prioritizing survival over reproduction. Further, comparing neural gene expression and hormone levels across treatments will reveal the mechanisms underlying this tradeoff, ultimately providing insight into how animals manage conflicting demands.

Research Grant: NSF Postdoctoral Fellowship in Biology (PRFB) 2109619
Student Support: Office of the Director, NIH, T35 OD11145

Prevalence of leptospirosis in an urban rat population in Orange County

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Leptospirosis is one of the major zoonotic diseases worldwide, with over 1 million novel cases and around 60,000 deaths annually. It is not only a problem in developing countries but also in developed and industrialized countries due to flooding or natural disasters. Incidence of leptospirosis in children has been increasing and outbreaks in dogs have been reported recently. Wild rats, especially those of the genus *Rattus rattus* and *Rattus norvegicus*, are known to be sources of infection in urban areas. The pathogen is concentrated in large amounts in the kidneys of reservoir hosts and spread through the urine. The bacteria can survive in water or soil for months afterwards, causing infection when entering the body through the skin or mucous membranes. The purpose of this study is to investigate if the rats in Orange County are carriers of Leptospirosis. Fifty deceased rats from multiple locations in Orange County were necropsied for kidney tissue samples. Currently, DNA extraction and assessment of the quality of genomic DNA obtained is being performed. These samples will be tested for the presence of *Leptospira spp.* DNA using a real time PCR assay targeting the LipL32 gene, which can differentiate between pathogenic and apathogenic species. Primers and probe for LipL32 gene were manually designed based on DNA sequences of 410 Leptospira species available at the Integrated Microbial Genomes & Microbiomes portal. Positive samples will be further speciated based on Sanger sequencing of a fragment of the glmU gene. The results of this study will expand our current understanding on the role of urban rats on the epidemiology of Leptospirosis in Orange County.

Research Grant: Boehringer Ingelheim Veterinary Scholars Program
Student Support: Western University of Health Sciences- Veterinary Summer Research Program

Contraception use and its effects on the reproductive health of captive lesser apes (*Hylobates spp.*)

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Many lesser apes (*Hylobates spp*.) species are critically endangered or vulnerable according to the International Union for Conservation of Nature (IUCN). The need to protect these species is recognized by many institutions and currently there are three subspecies being managed by zoos which includes white-handed gibbons, white cheeked gibbons, and siamangs. In captivity contraception is one tool used to maintain genetic diversity in these species. Common types of contraception used in lesser apes are melengestrol acetate (MGA) and deslorelin implants. The current literature is sparse regarding the overall effects of contraception use in *Hylobates spp*. This study will examine how contraception impacts the reproductive health of lesser apes. We hypothesize that contraception use increases the risk of reproductive diseases. Samples were taken from the Reproductive Health Surveillance Program (RHSP); 25 female and 3 male reproductive tracts were examined. Specimens were examined grossly focusing on size, presence of abnormal growths, and overall appearance. Tissue from the uterus and each ovary in females, and the penis and each testicle in males were processed and stained routinely. Samples were examined microscopically for evidence of disease including pyometra, endometrial hyperplasia, leiomyomas, and other neoplasms. Fisher's exact test and Spearman's correlation will be used to determine the frequency of disease in contraceptive animals versus non-contracepted individuals and identify any correlations These findings will allow more effective management of fertility and population health for lesser apes in captivity.

Research Grant: We appreciate the support from the Association of Zoos and Aquaria Reproductive Management Center and the zoos that provide samples for the archive.
Student Support: NIH Grant 5T35OD016477-20 to Michigan State University

Analysis of Nasal Bacteria, Heavy Metals, and Pollutants of Galapagos sea lions (*Zalophus wollebaeki*)

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The Galapagos sea lion (*Zalophus wollebaeki*) is an endangered marine mammal endemic to the Galapagos archipelago. As a large fish-eating carnivore, this predator plays an essential role as a sentinel species of the archipelago marine ecosystem. Sea lions are also subject to the effects of bioaccumulation of pollutants and heavy metals from the environment. These anthropogenic factors coupled with the introduction of novel pathogens related to introduced species (e.g. dogs and cats) could lead to the rise of disease, negatively impacting these populations. In this study, we are investigating the concentrations of heavy metals and pollutants in the hair and blood of the Galapagos sea lion and determine if these exposures could be affecting the immune status of sea lions inhabiting the El Malecon rookery at San Cristobal island, which represents the largest population in the entire archipelago. To our knowledge, there has not been a prior assessment of heavy metals in this population and the most recent study of pollutants in the sea lion population is over a decade old. We hypothesize that pollutant concentrations have changed since then. We suspect heavy metal concentrations to be relatively high because of the volcanic activity on the islands and the fact that sea lions consume a large amount of fish. In parallel we are conducting next generation sequencing analysis of DNA extracted from nasal swabs of the sea lions to determine the bacteria present. We suspect that some pathogens recorded in dogs and cats on the islands will also be observed in the sea lions.

Research Grant: NIH T35OD011070 Interdisciplinary Biomedical Research Training Program
Student Support: NIH T35OD011070 Interdisciplinary Biomedical Research Training Program

Development of an ELISA kit to detect antibodies against Feline Infectious Peritonitis Virus (FIPV)

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Feline Infectious Peritonitis Virus (FIPV) causes Feline infectious peritonitis (FIP), one of the most fatal feline infectious diseases. There is no effective treatment or vaccines, and the fatality rate is essentially 100%. Determining the presence of anti-FIPV antibodies in cerebrospinal fluid and serum is helpful. However, antibodies simply demonstrate that the animal has previously been exposed to a coronavirus. Thus, it is necessary to develop a diagnostic tool to determine whether animals maintain protective immunity. The spike (S) protein of FIPV consists of two subdomains: domain 0 (D0) and B (DB), which provide structural support and contain a receptor binding motif for viral attachment and entry to host cells respectively. Thus, the D0 and DB are ideal targets for serological diagnosis and protective immunity. In this study, we constructed HEK293t cells permanently expressing D0 and DB fused with mouse Fc region of IgG1 (D0-mIgG and DB-mIgG, respectively). We established an indirect ELISA using a microfluidic system which curtails the materials and time for ELISA. Our preliminary data showed significant variations in DB-mIgG ELISA results among cat sera collected from animal shelters. These suggest that cats are ubiquitously exposed to FIPV and develop an immune response with different levels of protective immunity. We will continue to analyze 300 cat serums collected from Mississippi State animal shelters to investigate the prevalence of FIPV infections and protective immunity in shelter animals. We will also determine plaque neutralization assay to correlate DB-ELISA results with protective immunity. This will help clinicians in practice, and shelter medicine diagnose cats with FIPV and determine vaccine use.

Research Grant: Mississippi State University College of Veterinary Medicine
Student Support: None

Does the presence of familiar calves affect pain response associated with castration in the feedlot?

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Commingling involves mixing calves from different sources upon arrival at beef feedlots, and is a common practice due to pricing and calf availability. However, commingling is a known stressor and increases the risk of illnesses such as Bovine Respiratory Disease (BRD). This study is part of a larger project examining potential social buffering effects on commingling stress. Specifically, we hypothesized that social buffering confers benefits regarding pain and recovery associated with castration surgery. Each pen (n = 17) consisted of 3 familiar (F) calves from the same source farm and 3 unfamiliar (U) calves from different source farms. Calves were surgically castrated on day 14. Home pen behavior was video-recorded and with Noldus The Observer XT software, pain behaviors were quantified using continuous sampling for 30-min periods at 0h, 1h, 4h, 24h, and 48h relative to castration. Castration wound healing was scored by direct observation on days 21 and 28, and was statistically analyzed using the Wilcoxon signed-rank test for each observation day. There were no observed differences for wound healing between F and U calves on day 21 (median = 1; range 1-2; $P = 0.58$) or day 28 (median = 1; range 1-3; $P = 0.24$). These results were unexpected, since F calves showed higher average daily gain than U calves on day 21. Behavior data (pending) will inform interpretation of these findings, with the goal of improving commingling practices for the period following castration where infections and delays in growth are likely to occur.

Research Grant: This research and student stipend are supported by USDA NIFA Animal Health and Production and Animal Products: Animal Well-Being Program [grant no. 2019-67015-29572/project accession no. 1019068]
Student Support: Same as above

Effects of Infusion of Glial Derived Neurotrophic Factor DNA Nanoparticles on Opioid Relapse Behaviors

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Opioid use disorder (OUD) is a chronic, relapsing condition associated with significant impairment, however there are few non-opioid-based medication options to help maintain recovery. One alternative treatment approach focuses instead on neural dopamine deficiencies induced by chronic opioid use. The aim of this project is to test the hypothesis that glial derived neurotrophic factor (GNDF), a neurotrophin that supports the health of dopamine neurons, may reduce drug craving and seeking during periods of abstinence. Fifty adult male Sprague Dawley rats were surgically implanted with external jugular catheters and trained to lever press for oxycodone during daily (6h) operant sessions. Following 12 days of IVSA, rats were assigned to two treatment groups based on their overall intake and motivated responding for oxycodone. Then next day subjects received either intranasal infusion of pGDNF NPs or saline vehicle. All animals then experienced forced abstinence for 28 days. Animals were then returned to the operant chambers to reassess drug seeking behaviors (i.e. lever presses when no drug was available). A significant reduction in drug seeking behaviors during reinstatement was observed in the pGDNF NPs treatment group. This finding supports the hypothesis that intranasal delivery of pGDNF NPs may reduce craving and relapse risk in OUD. Ongoing studies are examining neural modifications within the dopamine system associated with the behavioral findings. The overall goal of this study was to investigate the effectiveness of non-opioid-based GDNF gene therapy in OUD and the results provide preliminary support for this treatment approach.

Research Grant: National Institute on Drug Abuse, National Institutes of Health Award Number UG3DA050942; National Center for Advancing Translational Sciences, National Institutes of Health, Award Number UL1TR002544
Student Support: Tufts Summer Research Training Program

Investigation of inhibitors against the mitochondrial ubiquinone pathway in *Toxoplasma gondii*

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Toxoplasma gondii is an intracellular parasite that infects a variety of hosts, including humans. *T. gondii* replicates as tachyzoites in acute infection and can convert into bradyzoites in chronic infection. Because felids are the only definitive host, they are crucial to the environmental persistence of *T. gondii*. There are no FDA approved treatments for clinical toxoplasmosis in veterinary patients; current recommendations are to utilize treatments used in human medicine (clindamycin, pyrimethamine, and sulfonamide). There is no effective treatment against chronic infection. Prior work in the Moreno lab has shown that inhibition of an essential mitochondrial enzyme (TgCoq1) disrupts the ubiquinone (UQ) pathway and results in dysfunction of the mitochondrial electron transport chain; this dysfunction resulted in inhibition of parasite growth. The compound responsible for the inhibition (a bisphosphonate, BPH-1218) protected mice against a lethal acute infection. Inhibition from BPH-1218 can be rescued by adding UQ in growth media, providing further evidence that BPH-1218 inhibits UQ production and that the UQ pathway may be a good therapeutic target. We hypothesized that UQ synthesis is a novel drug target for toxoplasmosis. To test this hypothesis, we screened small molecule libraries (bisphosphonate derivatives and the MMV pandemic response box) against wild-type *T. gondii*. Inhibition of parasite growth was measured and UQ was supplemented to validate that UQ was the therapeutic target. We have found some potential hits and are validating these hits at various concentrations to verify the rescue by UQ. We will also test these hits against the bradyzoites of the chronic infection in vitro and in vivo in mice.

Research Grant: AI147661
Student Support: NIH T35 OD 010433 Georgia Veterinary Scholars Summer Research Program

Engineering chimeric antigen receptor (CAR) lymphocytes to target feline infectious peritonitis virus

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The fatal disease feline infectious peritonitis (FIP) currently has no FDA approved treatments. The goal of this study is to design a novel immunotherapy targeting cells infected with FIP virus (FIPV) and thus expressing surface spike protein that can be detected by chimeric antigen receptor (CAR)-engineered immune cells. CAR immunotherapy has been successful in treating some human cancers but has not yet been developed for acute viral infections like FIPV, nor used at all in cats to date. CARs are comprised of two main components: a single chain antibody fragment (ScFv) and signaling domain(s) from immune costimulatory receptor(s). Here we will design an ScFv specific for FIPV spike protein to create an anti-spike CAR to direct effector immune cells to seek and destroy FIPV spike-expressing cells. Current human CAR therapies require use of autologous T cells since allogeneic T cells may attack the new host tissue and result in severe graft-versus-host disease. Thus a second goal of the study is to determine the potential to use natural killer (NK) cells which induce much less graft-versus-host disease, and thus may be used allogeneically. We have successfully demonstrated that feline T and NK cell populations can be visualized by flow cytometry and thus enriched by cell sorting. We have also designed an ScFv from the anti-spike clone 18A7.4 that is stably expressed in mammalian cells. Completion of this study will provide proof-of-principle data using an FIPV model to support the development of FIPV CAR-cell therapy for this devastating disease in cats, and will also determine the feasibility of developing a CAR-based immunotherapy for the potential treatment of acute coronaviral infections in cats and humans.

Research Grant: Cornell Feline Health Center
Student Support: Liz Hanson Graduate Scholarship

Endoscopic treatment of tracheal lacerations with fibrin glue — a feline cadaveric study

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Post-intubation tracheal laceration (PITL) in cats is a rare but serious condition that may occur with improper intubation technique as well as excessive movement of the cat's recumbent position without proper disconnection of the endotracheal tube from the anesthetic machine. Although tracheal rupture may be managed with supportive care, cats with severe dyspnea may require invasive emergency surgery. The primary objective of this study was to determine the feasibility of endoscopic application of fibrin glue for treatment of post-intubation tracheal laceration in cats. Secondary objectives included developing guidelines for application of fibrin glue. Using frozen and fresh feline cadavers, an experimentally induced tracheal rupture was created via overinflation of an endotracheal tube cuff. After endoscopic identification of the tracheal tear, a double lumen catheter was used to instill the fibrin glue into the tracheal defect. Following the procedure, the airway of each cat was examined, and leak tested. Length of laceration was documented, as well as quantity of glue used, time of procedure, and whether a seal was attained. A complete seal was obtained in 67% of fresh cadavers when complete filling of the defect with fibrin glue was performed. Bridging the defect with fibrin glue was not an effective method of application of fibrin glue in fresh or frozen cadavers. The median volume of glue used to fill defects in fresh cadavers was 0.5 ml (range 0.4 to 2 ml). Median time for endoscopic application of fibrin glue was 8 minutes and 36 seconds (range 4 minutes and 52 seconds to 19 minutes and 6 seconds). Endoscopic application of fibrin glue may be a feasible method of treatment for PITL in cats.

Research Grant: Faculty Start-up Funds
Student Support: Linda F. Hayward Florida Veterinary Scholars Program, UF College of Veterinary Medicine

Vaccine development against Chlamydia trachomatis (Ct) in swine – Stage 1: Vaccine adjuvant and administration

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Chlamydia trachomatis (Ct) is the most common bacterial sexually transmitted infection (STI) which may result in infertility, ectopic pregnancy, and chronic pelvic pain. Despite these strong disease sequelae, a vaccine against *Ct* is not available. The development of such a *Ct* vaccine is the overall goal of this project. To improve translatability, pigs have been chosen as biomedical animal model: pigs are immunologically similar to humans; they are the natural host of *Chlamydia suis*; and they are susceptible to *Ct*. The current Stage 1 of this project aims to identify the most immunogenic adjuvant and route of administration. Therefore, an immunogenic *Ct* protein was combined with one of two adjuvants (Adjuvant 1+2) that have previously been used in chlamydia vaccines. These two vaccine candidates were administered in a combination of intranasal (IN) and intramuscular (IM) prime/boost administration. To this end, 36 pigs were distributed into six groups – Control (Adj1), Control (Adj2), IM/IN (Adj1), IN/IM (Adj1), IM/IN (Adj2), and IN/IM (Adj2). Vaccine immunogenicity was determined by quantifying the production of the crucial cytokine, interferon-gamma, upon in vitro restimulation with the *Ct* vaccine antigen using ELISpot and flow cytometry. Our results allow three conclusions: i) all vaccine candidates were immunogenic; ii) IM induced a stronger systemic response than IN; and iii) Adj2 induced a stronger response than Adj1. Based on these results, we can use the most immunogenic adjuvant and route of administration to determine the most immunogenic *Ct* antigen in State 2.

Research Grant: NIH NIAID 1R01AI162709
Student Support: Boehringer-Ingelheim Veterinary Scholars Program; Herbert Benjamin Endowment

Reproductive complications and survival after parturition in hospitalized goats

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Despite the growing popularity of goats as production animals and pets, there is very little information available regarding the incidence of periparturient reproductive complications in goats. The objective of this study was to describe the incidence of reproductive conditions in pregnant does admitted to referral hospitals. A multi-center cross-sectional study including 9 veterinary teaching hospitals was conducted, with data collected by questionnaire completed by the admitting clinician. Descriptive statistics were used to report complications, and survival between groups was compared using Chi-square. A total of 184 does were included in the study. One-hundred forty-seven (80%) does had dystocia, 108 (59%) underwent C-section, and 28 (15%) were diagnosed with pregnancy toxemia. Periparturient complications included retained fetal membranes (n = 35, 19%), vaginal/perineal trauma (n = 28, 15%), uterine tears (n = 24, 13%), metritis (n = 21, 11%), uterine/vaginal hemorrhage (n = 7, 4%), and uterine prolapse (n = 1, 0.5%). Does with uterine tears were less likely to survive to discharge than does without uterine tears (29% survival vs 86% survival, $P < 0.001$). Does with uterine/vaginal hemorrhage (43% survival vs 86% survival, $P = 0.002$) or metritis (62% survival vs 87% survival, $P = 0.003$) were less likely to survive to discharge than those without these conditions. This study demonstrated a high incidence of reproductive complications in periparturient does admitted to referral hospitals. Periparturient reproductive complications were associated with non-survival to hospital discharge.

Research Grant: None
Student Support: Linda F. Hayward Veterinary Scholars Program, UF College of Veterinary Medicine

Pilot study: heart rate and muscle activity as an indicator of sleep in dairy cattle

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Sleep is an innate part of all mammal physiology. For dairy cows, evaluating sleep can give us insight into the comfort of the facilities and may be linked to disease risk and productivity. Researchers have accurately estimated sleep in dairy cows, but the technology used to date is cumbersome and not practical in a commercial setting. The purpose of this project is to investigate the practicality of estimating sleep in dairy cattle from muscle activity (using surface electromyography; sEMG) and heart rate variability (HRV). The specific objectives are to: 1) determine if there is agreement between two sEMG sensors placed on the neck and back muscles of dairy cows, and 2) compare HRV and muscle activity in cows during different postures indicative of rapid eye movement (REM) sleep and wake states. Three indoor-housed dairy cattle from one dairy farm on Prince Edward Island will be used in this study. All measurements will be recorded for 8 hours overnight. To record muscle activity, Delsys Trigno Avanti wireless sensors will be attached to the skin of the trapezius and gluteobiceps muscles. To record HRV, a Polar equine belt will be secured around the chest of each cow. A video camera will be used to determine postures associated with sleep and wake. The hypotheses are that the sEMG data from the neck and back muscles will be similar, and that the muscle activity and HRV values will differ when cows are in postures related to different vigilance states. It is anticipated that muscle activity will decrease during REM sleep, but that HRV will increase during REM sleep. Validating these physiological measures as indicators for sleep will facilitate future dairy cattle sleep research.

Research Grant: NSERC Discovery Development Grant
Student Support: Atlantic Veterinary College Summer Research Award; Boehringer Ingelheim Veterinary Scholarship

Role of the androgen and progesterone receptors in regulating HSV-1 gene expression

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Following acute infection, Herpes Simplex Virus 1 (HSV-1) establishes a lifelong latent infection in neurons. Physical or environmental stress can lead to reactivation from latency through steroid hormones which bind to and activate the Glucocorticoid Receptor (GR). Previous studies demonstrated that GR cooperates with Kruppel-like transcription factors 4 and 15 (KLF4, KLF15) to express the Infected Cell Protein 0 and 4 (ICP4, ICP0), viral transcriptional regulators required for efficient productive infection. Additionally, the Androgen Receptor (AR) and Progesterone Receptor (PR) have been shown to activate the related Bovine Herpesvirus 1 (BoHV-1) ICP0 and ICP4 expression cooperatively with KLF4 and KLF15. Therefore, we hypothesize that AR and PR increase expression of HSV-1 ICP0 and ICP4, cooperatively with KLF4 and KLF15 in mouse neuroblastoma (Neuro-2A) cells. We performed dual luciferase assays using Neuro-2A cells transfected with either an ICP0 or ICP4 promoter construct. Cells were co-transfected with the AR or PR, alone or in combination with KLF4 or KLF15, and treated with dihydrotestosterone (DHT) or progesterone (P4) respectively. AR transactivated the ICP0 and ICP4 promoters two-fold, however treatment with DHT either decreased or had no effect on promoter activity. Interestingly, AR reduced KLF4 and KLF15-mediated transactivation of the ICP0 and ICP4 promoters, with or without DHT treatment. Co-transfection of PR and ICP0 plus P4 treatment increased promoter activity two-fold, but reduced KLF15 mediated transactivation. Collectively, these studies demonstrate how hormone receptors regulate HSV-1 gene expression to promote reactivation from latency and productive infection.

Research Grant: National Institute of Neurological Disorders and Stroke of the National Institutes of Health under Award Number R01NS111167
Student Support: Oklahoma State University College of Veterinary Medicine

Deregulated proteostasis inhibits V(D)J recombination and promotes microhomology-mediated translocations

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V(D)J recombination is a DNA double strand "cut and paste" process that assembles the variable region of antigen receptors in developing B and T lymphocytes, exclusively using nonhomologous end-joining (NHEJ) to efficiently complete recombination. The absence of the NHEJ DNA end sensing complex Ku in G1/G0 cell cycle phase enables an alternative end-joining (A-EJ) pathway to inefficiently complete recombination and translocate to other Double stranded breaks (DSBs) using a mechanism that does not depend on microhomologies (MHs). MHs are short shared sequence homologs at the junction between two different genome-matched alignments and are utilized A-EJ. We hypothesize the poor MH utilization in G1/G0-phase could be due to DNA repair protein expression differences across the cell cycle. Using a drug-indued G1 arrest B-cell progenitor cell line that undergoes V(D)J recombination, we treated NHEJ deficient cells and wildtype cells with either DMSO, a proteosome inhibitor, or a ubiquitin-like tagging inhibitor. Cell DNA then was collected and *HTGTS-JoinT-seq* assessed the location and DSB sequences at rejoining events and translocations across the genome. We find that although inhibited ubiquitin-like tagging or protein degradation suppresses V(D)J recombination, recovered genome-wide translocation junctions are increased and uniformly harbor microhomologies in both NHEJ and A-EJ proficient backgrounds. These findings suggest key repair pathway choice factors are degraded in G1/G0-phase, and their stabilization promotes aberrant translocations. Therefore, identification of these normally degraded proteins could be important drug targets for noncycling tumor subpopulations that contribute to tumor recurrence and metastasis.

Research Grant: V Foundation for Cancer Research
Student Support: T35 OD0109899

Iron overload in captive Amargosa voles (*Microtus californicus scirpensis*)

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At UC Davis, a captive breeding colony of endangered Amargosa voles was established in 2014 for population insurance. In the past year, two voles have been diagnosed with hemochromatosis, a disease of excessive hepatic iron accumulation resulting in liver failure. The aim of this study was to describe hepatic iron overload in captive Amargosa voles and identify potential risk factors including time-in-captivity, sex, lineage, and diet that could influence colony management decisions. Hepatic iron content was quantified by mass spectroscopy in voles with hemochromatosis and compared to age and sex-matched unaffected voles and age and sex-matched laboratory mice. Forty-four individuals evenly distributed within five different age groups were randomly selected from a collection of 72 formalin-fixed voles. Paraffin-embedded liver from these voles was stained with Prussian blue and evaluated for iron content by automated image analysis. According to mass spectroscopy measurements, affected voles, unaffected voles, and laboratory mice averaged 18366.7, 2443.3, and 1010 ppm of hepatic iron by dry weight, respectively. Hepatic iron content indicated no significant difference between the five age groups ($P = 0.54$), suggesting that iron accumulation is not associated with time-in-captivity. However, analysis of variance of iron content measured by Prussian blue staining between sexes revealed a significant difference ($P < 0.004$) with female voles having a greater average area fraction of iron staining. These findings confirm the presence of hemochromatosis in Amargosa voles and suggest a genetic etiology rather than a husbandry-based etiology.

Research Grant: None
Student Support: Students Training in Advanced Research (STAR) Program through SVM endowment funds

The development and validation of a behavioral based vision test for dogs

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Age-related decline in vision, particularly in low lighting conditions, is well described in humans, but poorly understood in dogs. Dogs are common cohabitants with humans, and aging of their visual system may mirror that of people. We aim to develop a behavioral-based vision test for dogs that would assess vision based on interaction with high and low luminance videos. We hypothesize our vision test will be reproducible and significantly associated with objective electroretinogram (ERG) outcomes. A preliminary study using random presentation of 20, 6-second videos, presented twice during a clinical laboratory visit was used to determine video preference in 3 healthy dogs. Dogs were situated 50cm from a high-resolution computer monitor. Videos included animal subjects with extensive horizontal movement (> 50% of screen). The average time the dog watched each video was measured and compared to the mean watching time for all videos (defined as dog watching threshold). Preliminary results showed that videos with high contrast, dog subjects, and with extensive horizontal movement are most engaging to dogs. The 10 most consistently engaging videos will be edited for high and low luminance and presented in a 2-screen testing environment whereby one screen will present the video, and the adjacent screen will present a still image from the same video. Side (left vs. right screen), and order of videos will be randomized in the psychophysics software Psychopy. The engagement in the moving video will be graded in Peyecoder software and the responses to high and low luminance videos compared in dogs of different ages. Ultimately, high and low luminance behavioral responses will be compared with ERG high and low intensity responses.

Research Grant: UW-Madison SVM Companion Animal Funds, NIH K08EY028628
Student Support: National Institutes of Health T35 OD011078-12

Prevalence of canine babesiosis in Florida shelter dogs

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In the United States, the distribution of canine babesiosis due to infection with the apicomplexan parasites *Babesia vogeli* and *Babesia gibsoni* is rapidly expanding, but screening for *Babesia* infection is not a component of routine canine health assessments, and dogs suffering from babesiosis may go undiagnosed and untreated. We conducted a cross-sectional study of 71 shelter dogs in Florida to (a) measure *Babesia* exposure using serological rapid diagnostic tests and peptide antigen-based ELISA, (b) determine the prevalence of active infections with *B. vogeli*, and *B. gibsoni* using qPCR and peripheral blood film analysis, and (c) evaluate demographic risk factors (region, shelter site, age, sex, breed, anemia, intake conditions, bite history) associated with increased risk of exposure to *Babesia* parasites. We obtained surplus blood collected during routine intake health evaluations performed by four participating animal shelters in north-central and south Florida. We compared seroprevalence of *Babesia* antibodies against demographic risk factors using Chi-squared analysis. Overall, 29.6% (21/71) of dogs tested positive for antibodies against *Babesia* by a research use only serological rapid test. A single (1.4%, 1/71) dog tested was considered suspected-positive for *Babesia* on an initial pan-*Babesia* qPCR screen, and a suspected-positive for *B. gibsoni* on a secondary species-specific qPCR assay. We found no significant difference in seropositivity between risk factor groups ($p > 0.05$). Although prevalence of current *Babesia* infection was low, nearly one third of dogs were seropositive for *Babesia*, suggesting high risk of exposure among Florida shelter dogs.

Research Grant: United States Centers for Disease Control Grant 1U01CK000510 & 1U01CK000662 UF College of Veterinary Medicine Preeminence Initiative
Student Support: Linda F. Hayward Florida Veterinary Scholars Program, UF College of Veterinary Medicine

Characterization of Squamate Microbiomes

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All living things are colonized by trillions of bacteria with the human intestine colonized by 300 to 500 species alone. Collectively, the genomes of these populations are referred to as microbiomes and they are found in most systems ranging including the skin, oral cavity, digestive tract, and reproductive and respiratory systems. Microbiome research has exploded in the last decade and dramatically expanded the role of microbes in health and disease beyond classical role such as aiding in digestion, immune system development and pathogen defense. The majority of microbiome research is done in rodents and humans, but very little is known about the microbiome of reptiles such as squamates. Our overarching goal is use NextGen sequencing tools to characterize the fecal microbiome of snakes of multiple species and housed in multiple settings and use this information as baseline data for future studies aimed and assessing the role of microbiome changes (i.e., dysbiosis) in disease. A better understanding of squamate dysbiosis will ultimately lead to development of novel preventative, therapeutic, and diagnostic strategies.

Research Grant: Franklin discretionary funds
Student Support: Franklin discretionary funds

Assessment of healing times in avian wildlife bone fractures when adding photobiomodulation to treatment plan

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Limb fractures are among the most common types of injuries that avian wildlife present to a veterinary hospital. With large influxes of injured wildlife, limited funds for treatment, and a finite amount of available space and supplies, hospitals are often overwhelmed with a large patient caseload. Through an analytical review of our wildlife data entry program, Wildlife Rehabilitation Medical Database (WRMD.org), complete healing times for avian fractures is approximately 28 days. The longer the avian patient is hospitalized and the limb held immobile, the greater the likelihood for negative effects to occur such as contracture, non-unionized healing, or erroneous imprinting. Photobiomodulation (PBM), also referred to as laser therapy, has shown to be a successful adjunct option to traditional fracture treatment and has been proven to reduce pain, decrease inflammation, and stimulate tissue healing. PBM does this by enhancing healing properties in cells, increasing their viability, and decreasing the time for an injury to heal. We propose that applying PBM to a fracture site tri-weekly will decrease the time for a successful fusion of a fractured bone to occur and therefore decrease the time avian wildlife spend in hospitals due to fractures. Through the assessment of weekly radiographs, use of a goniometer to assess changes in range of motion, and tri-weekly visual assessments of the injury site, we hypothesize that birds supplemented with PBM therapy to a surgically corrected limb will show consistent recovery in 21 days, a standard deviation of 7 days, regardless of type and location of fracture, fracture treatment method, and variation in avian species. This research project is currently in progress.

Research Grant: Funded by LSU VetMed Faculty Start-up Funds (Hale-Mitchell)
Student Support: National Institute of Health

Validation of significant X-Linked genes in sexual dimorphism of the BPH/5 preeclamptic mouse model

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Pre-conception maternal obesity is a risk factor for development of preeclampsia, a hypertensive disorder of pregnancy with adverse offspring outcomes. This condition is mirrored in the blood pressure high subline 5 (BPH/5) mouse where both sexes are hypertensive, however only females are obese. Previous observational studies in the BPH/5 have shown improved offspring outcomes with attenuation of maternal obesity. We hypothesized that a correlation exists between BPH/5 adiposity and cardiometabolic risk through epigenetic programming. Genome wide association studies performed on BPH/5 females revealed genetic mutations on the X chromosome, potentially contributing to sexual dimorphism. Whole genome bisulfite sequencing (WGBS) of reproductive white adipose tissue (rWAT) from adult BPH/5 littermates was utilized to understand X chromosome dysregulation and genetic contributions to the phenotypic differences between sexes (n = 6). To test our hypothesis, methylation levels were identified and quantified with 10% in the promotor region and 7% exonic. Downstream genes in the rWAT of BPH/5 males and females were validated utilizing quantitative polymerase chain reaction. Xist expression was 5-fold higher in BPH/5 females compared to males (n = 3-4; $P < 0.05$). Androgen receptor relative expression was 2-fold higher in BPH/5 males compared to females (n = 3-4; $P < 0.05$). These results validate the methylation directionality obtained via WGBS and allow for further comparison to control mice to determine changes in gene expression. Genetic methylation differences have potential as future biomarkers of disease risk, specifically genetic predisposition to obesity in offspring born to preeclamptic mothers.

Research Grant: NIH P20GM13F002
Student Support: Kenneth F. Burns Trust

Myxomatous mitral valve disease in two cohorts of genomically related large breed dogs

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Myxomatous mitral valve disease (MMVD) is the most common acquired heart disease in dogs. Canine MMVD research has focused predominantly on small breed dogs, with MMVD in large breed dogs poorly characterized to date. The study objective was to describe characteristics of MMVD in large breed dogs belonging to one of two genomic clades (r and t). Medical records were retrospectively reviewed, and stored echocardiograms were measured. Seventy-seven dogs met inclusion criteria (clade r: N = 38, clade t: N = 39). Most common breeds included Brittany Spaniel (N = 10), German Short-Haired Pointer (N = 9) and Viszla (N = 7) in clade r; and Border Collie (N = 17) and Australian Shepherd (N = 11) in clade t. Median age was 10.2 and 10.6 years in clades r and t, respectively. Sex distribution in clade r was balanced, with 19 dogs of each sex, while clade t had 17 females (43.6%) and 22 males (56.4%). In clade r dogs, MMVD stage was B1 in 22/38 (57.8%), B2 in 8/38 (21.1%) and C in 8/38 (21.2%), with 21/38 (55.3%) dogs having mitral valve prolapse (MVP). In clade t dogs, MMVD stage was B1 in 26/39 (66.7%), B2 in 10/39 (25.6%), and C in 3/39 (7.7%), with 18/39 (46.2%) having MVP. At the time of diagnosis, atrial fibrillation was identified in 5/38 (13.2%) clade r dogs and 2/39 (5.1%) clade t dogs, while ventricular arrhythmias were identified in 9/38 (23.7%) clade r dogs and 3/39 (7.7%) clade t dogs. Systolic dysfunction was appreciated in 4/38 (10.5%) clade r dogs and 5/39 (12.8%) clade t dogs, all of which were stage B2 or C. In conclusion, MMVD characteristics in two cohorts of large breed dogs have been described. Disease onset was late in both groups, with a higher frequency of advanced MMVD and arrhythmias in clade r versus t.

Research Grant: None
Student Support: NIH T35OD010991-17

Teaching module efficacy on student ability to evaluate for radiographic small intestinal obstruction in dogs

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Mechanical small intestinal obstruction is a common occurrence in dogs and frequently requires surgical intervention. New veterinarians may lack confidence in identifying radiographic small intestinal obstruction due to a lack of experience. The purpose of this study is to investigate the efficacy of a teaching module on student ability to identify small intestinal obstructions in dogs via abdominal radiographs. The project consists of a pre-assessment, teaching module, and post-assessment, each administered a week apart. Third- and fourth-year veterinary students were recruited to participate. All radiographic images were sourced from the Midwestern University Companion Animal Clinic and identified as consistent or inconsistent with small intestinal obstruction by a board-certified veterinary radiologist. The teaching module contains 28 multiple-view cases with a brief signalment. It was created using open-sourced nonlinear software called Twine. After reviewing basic information on the radiographic diagnosis of small intestinal obstruction, participants identify presented cases as obstructed or non-obstructed and receive feedback. The pre- and post-assessments include a survey on student attitudes regarding confidence and impact of the module, along with a quiz comprised of 20 radiographic cases to be identified as obstructed or non-obstructed and their degree of confidence in the diagnosis. Results will be analyzed using simple descriptive statistics and comparing changes between pre- and post-assessment results with a Wilcoxon matched-pairs signed-rank test. Data collection is ongoing.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program and Federal Work Study

Use of serum osmolality to identify heart disease stage in dogs and the relationship to chloride correction

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Serum osmolality reflects relative free water status and therefore could indicate the influence of non-osmotic antidiuretic hormone in dogs with myxomatous mitral valve disease (MMVD) and congestive heart failure (CHF). In this prospective and observational study, we sought to determine if serum osmolality is related to heart disease stage and the amount of mathematical correction of serum chloride (which has been proposed to indicate relative free water status) in dogs with MMVD. Serum samples from 50 dogs with MMVD (23 Stage B (preclinical), and 27 Stage C/D (CHF) were analyzed for renal panel variables, serum chloride concentration was mathematically calculated, serum osmolality was calculated, and serum osmolality was measured by freezing point depression. Variables were compared between Stage B and Stage C/D dogs. Correlations were explored between osmolality methods and renal panel variables. Bland-Altman analysis was used to assess agreement between calculated and measured osmolality methods. Serum osmolality by either method was not different between Stage B and Stage C/D dogs. Directly measured osmolality was moderately correlated (P = .001, r = 0.45) with calculated osmolality, but with a proportional bias (-10.6 mOsm/kg) and wide limits of agreement (± 48 mOsm/kg). Calculated osmolality was negatively correlated (P < .0001, r = -0.73) with the amount of chloride correction. In conclusion, although serum osmolality was not different between dogs with and without CHF, it was inversely related to the amount of chloride correction, which supports its use in the assessment of relative free water. Agreement between calculated and measured serum osmolality was too wide to allow for interchange.

Research Grant: Florida Veterinary Medical Association
Student Support: Linda F. Hayward Florida Veterinary Scholars Program

Novel surgical spoon for urolith removal during canine cystotomy

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Urolithiasis is common in dogs. Cystotomy is a standard surgical procedure to remove uroliths from the urinary bladder, but retrieval of uroliths is limited to improvised use of available surgical instruments or off-label devices. Furthermore, there is an unacceptable incidence of incomplete urolith extraction, which can necessitate additional surgery and exacerbate morbidity. To improve urolith extraction, three novel 3D-printed surgical spoons underwent a clinical trial in Northwest Arkansas veterinary clinics. Postoperative questionnaires provided to those veterinarians recorded favorable experiences using one or more of the surgical spoons in two dogs weighing 23 to 34 kg. The most favored surgical spoon from the clinical trial underwent strategic design modification to evaluate in dogs weighing less than 20 kg. The resultant cystotomy spoon is a 12.5 cm shaft with a spoon on each end, one spoon narrower (1.4 cm at widest dimension) than the other (2.0 cm at widest dimension). The spoon will be manufactured into 316L surgical grade stainless steel and enter a clinical trial at the University of Missouri Veterinary Health Center. Faculty surgeons and surgical residents will use the spoon during canine cystotomies and will complete a postoperative questionnaire to record expert opinion on the efficacy and usefulness of the spoon. We hypothesize that these surgeons will view the spoon as effective and user-friendly for urolith extraction during cystotomy. We anticipate that these surgeons may also have constructive input for design improvement. Once perfected, this surgical spoon will provide a specific device for canine urolith removal and positively impact the standard of healthcare for dogs requiring a cystotomy.

Research Grant: None
Student Support: A gift from Dr. Natalie Rabiner, alumnus of the University of Missouri CVM

Validating commercial ELISA kits to measure cytokines in giraffe (*Giraffa camelopardalis*) with osteoarthritis

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This study aimed to validate giraffe (*Giraffa camelopardalis*) cross-reactivity with commercially available assay kits to measure cytokines in giraffes with osteoarthritis (OA). Cytokine concentrations of TNFα, IL-1β, IL-6, IL-8, and IL-10 were/will be compared in healthy giraffes without OA to giraffes with OA. These cytokine concentrations will also be measured in giraffes with OA before and after mesenchymal stem cell (MSC) therapy, a novel therapeutic alternative for OA management. The purpose of this study is to provide objective evidence of efficacy of of MSCs used as a therapeutic modality in giraffes with OA via a measurable reduction of inflammation. Commercially available ELISA kits validated for use in sheep exhibited cross-reactivity with some giraffe serum proteins (TNFα, IL-1β) however, there was no cross reactivity in the IL-6 kit. Banked serum samples previously collected from giraffes (n = 9) from multiple institutions were tested. The clinical history of each animal was considered and placed into one of the two sample groups, healthy (n = 3) or osteoarthritic (n = 6). Fresh whole blood samples from multiple giraffes (n = TBD) will also be collected and used to validate the detection of cytokines in the supernatant of Concanavalin A- and LPS-stimulated peripheral blood mononuclear cells. True cross-reactivity and successful quantification of cytokines in this species with this protocol is to be determined and not well documented to date. However, we anticipate that with this protocol and data comparison of the different sample groups, this study will result in objective data that will provide essential authenticated support to current subjective data of clinical improvement following MSC treatment.

Research Grant: Principal Investigator MSU Startup Funds
Student Support: NIH Grant 5T35OD016477-20 to Michigan State University

Recognition of right sided cardiomegaly on thoracic radiographs by artificial intelligence software

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Deep learning with Convolutional Neural Networks (CNNs) are beginning to be integrated into many types of diagnostic imaging. In veterinary medicine, the use of artificial intelligence systems (AI) has recently been used to assess radiographs for cardiomegaly, including left atrial enlargement, left and right ventricular enlargement, and pleural effusion. AI could be used in emergency settings to get a quick reading of radiographs of critically ill-patients especially when no radiologist is available for support. While AI will not be able to provide a diagnosis of the disease itself, it can be trained to identify certain patterns on images, and use this learned pattern recognition to provide a ranked list of differential diagnoses. AI could be utilized to aid veterinarians with a faster, cheaper way to assess radiographs while waiting for the official radiology report from a veterinary radiologist. As veterinary AI is still in its beginnings, future training of the AI systems is needed to ensure the success of this technology. The goal of this study is to train and test an AI program in recognizing right sided cardiomegaly. Cases are selected based on general markers for right sided cardiomegaly, such as pulmonic stenosis, patent ductus arteriosus, heart worm disease, and tricuspid dysplasia. Only cases with right sided cardiomegaly confirmed by echocardiogram are eligible for recognition training. Test cases will be a mix of right sided cardiomegaly, left sided cardiomegaly, generalized cardiomegaly, and no cardiomegaly.

Research Grant: Vetology and Carlson College of Veterinary Medicine Biomedical Sciences Internal Grants 2022
Student Support: Boehringer Ingelheim Veterinary Scholar Program and Oregon State University

Louisiana surveillance of bovine parasite resistance and comparison of fecal diagnostic tests

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Two farms (Farm 1 SE LA, Farm 2 N LA) in Louisiana were used to establish efficacy of ivermectin and albendazole and provide a clinical comparison of the Modified McMaster (McM) and Mini FLOTAC (FLO) fecal egg count methods. Sixty-four calves had fecal samples collected prior to administration of ivermectin, albendazole, or a combination of the two, or they were used as controls. Fecal samples were collected again on day 20 (06/14/22) for Farm 1 and day 12 for Farm 2 (06/21/22). The McM (limit of detection 50 EPG), FLO (limit of detection 5 EPG), and quantitative fecal sedimentation (Fluke Finder) methods were used to determine eggs per gram of feces (EPG) of trichostrongyle-type and trematode eggs of each fecal sample pre and post anthelmintic administration. The mean pre-administration was 60.00 EPG (0-350 EPG; n = 30; McM), and 74.33 EPG (0-405 EPG; n = 30; FLO) for Farm 1, and 117.11 EPG (0-550 EPG; n = 34; McM) and 126.97 EPG (0-580 EPG; n = 34; FLO) for Farm 2. Animals with EPG ≥65 on the FLO pre-anthelmintic treatment were included in calculations for the fecal egg count reductions by anthelmintic group. For comparison, the reductions using the McM egg counts were also calculated. The groups treated with ivermectin had a fecal egg count reduction (FECR) of 9.68% (Farm 1) and 39.44% (Farm 2) for the FLO, and 0.89% (Farm 1) and 44.00% (Farm 2) for the McM. The groups treated with albendazole had a fecal egg count reduction of 74.56% (Farm 1) and 99.39% (Farm 2) for the FLO, and 83.33% (Farm 1) and 100.00% (Farm 2) for the McM. The group treated with the combination (Farm 2) had a fecal egg count reduction of 98.48% for the FLO, and 100.00% for the McM. *Fasciola hepatica* was detected at low levels (0-2 EPG) on Farm 1.

Research Grant: None
Student Support: Boehringer Ingelheim - Louisiana State University Summer Scholars Program

Clinical features, imaging findings, treatment, and outcome in dogs with discospondylitis

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Discospondylitis is an infection of the intervertebral discs, adjacent cartilaginous endplates, and/or vertebral bodies. A paucity of information exists regarding canine discospondylitis which negatively impacts clinical management. The aims of our study were to describe signalment, clinical findings, treatment, and outcome in dogs with discospondylitis. Study methods included retrospective case review (01.01.10 - 12.31.21) at 4 referral institutions. A total of 172 dogs were identified. The median age of dogs was 6 years (range, 11 weeks - 15 years) and male dogs were overrepresented. Of the cases where duration of signs were known (n = 136), 92 (68.1%) were chronic in duration. Twenty-three patients (17%) presented with subacute disease and 20 (14.8%) presented acutely. The most common clinical signs were lethargy, pain, and decreased appetite. Positive bacterial cultures were noted in 46 dogs (26.7%) and fungal cultures in 4 dogs (0.23%). The most common affected site was L7-S1. Twenty-one patients had evidence of discospondylitis on advanced imaging but no evidence of disease on initial radiographs. Treatment was medical in 159 dogs and surgical in 18 dogs. Of the patients with follow-up data available (n = 58) 51 patients showed signs of clinical improvement while 3 patients had progressive disease, 3 patients initially improved but relapsed, and 1 patient never improved despite radiographic resolution of disease. Patients clinically improved at a median of 20 days from diagnosis (range 1-545 days). Twenty-six patients exhibited radiographic resolution of discospondylitis at a median of 110.5 days post treatment (range 20-604 days). Median duration of antibiotic use was 20 weeks.

Research Grant: NIH 5T35OD016477-20
Student Support: NIH 5T35OD016477-20

The effect of dietary zinc on *C. difficile* colonization and pathogenesis in neonatal piglets and dairy calves

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Clostridioides difficile is a significant enteric pathogen capable of causing severe and sometimes fatal diarrhea in both humans and livestock, including neonatal swine and cattle. Transition metals such as zinc play an essential role in gut microbiota ecology and alter susceptibility of the host to enteric infections. In mice, excess dietary zinc has been shown to increase susceptibility to and severity of *C. difficile* infection by modifying the structure and diversity of the gut microbiome. In this study, controlled feeding trials were performed to evaluate the effect of high levels of dietary zinc on carriage of *C. difficile* in preparturient cows and sows and in their offspring. Cows and sows were randomized to receive a standard or high level of dietary zinc 6 weeks and 1 week prior to parturition, respectively. Fecal samples were collected from the dam before the trial and after parturition, as well as from neonates within 3 days of birth. To detect *C. difficile* colonization, anaerobic culture will be performed on all fecal samples and DNA will be isolated for comparative genomics across isolated strains. Toxin-specific cytotoxicity assays for *C. difficile* will be performed; growth dynamics, motility, and biofilm formation capacity of isolates will also be assessed. Risk factors for carrying *C. difficile* other than diet (e.g., maternal age, parity, early calving/farrowing) will be determined. Characteristics and composition of the gut microbiota will be assessed and compared among treatment and control animals. The findings of this study will help further elucidate factors that shape gut health of piglets and dairy calves and the characteristics of pathogens that affect them.

Research Grant: Pennsylvania Department of Agriculture
Student Support: NIH T35 OD010919, Boehringer-Ingelheim, and the University of Pennsylvania

Parasites in wild-caught *Notophthalmus viridescens* experimentally infected with *Bsal*

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Amphibians are part of a current sixth mass extinction event, with extinction rates over 200 times the natural extinction rate. One emerging infectious disease contributing to these declines is caused by the fungal pathogen *Batrachochytrium salamandrivorans* (*Bsal*). *Bsal* has caused mass die-offs of fire salamanders in Europe, and would pose a major threat to salamander biodiversity in North America if introduced. A recent study investigated the effect that pathogen dose and environmental temperature have on disease progression in *Bsal* chytridiomycosis using wild-caught *Notophthalmus viridescens* (Eastern newts; n = 41) as the model species. *Bsal*-associated lesions were examined histologically and *Bsal* qPCR load was measured. Incidentally, multiple types of parasites were noted histologically. Though most parasites are considered commensal to amphibians, some have detrimental effects on their hosts and contribute to immunosuppression. Therefore, the goal of the current study was to determine if parasite load may have an effect on severity of *Bsal* chytridiomycosis infection. Parasites were classified and quantified for each individual, and statistical analyses were performed to determine if a relationship exists between environmental temperature, *Bsal* exposure dose and parasite load or if parasitic infection affects survival time in *Bsal*-exposed individuals. Preliminary results showed that more than half the individuals were infected with parasites, most commonly mesomycetozoans and nematodes. These results will have the potential to provide greater understanding of how parasitic infection may contribute to morbidity and mortality in *Bsal*-infected Eastern newts, and could be used in *Bsal* disease management efforts.

Research Grant: NSFDivision of Environmental Biology Ecology and Evolution of Infectious Diseases Program Grant#1814520; U.S.Department of Agriculture National Institute of Food and Agriculture Hatch Project#1012932
Student Support: Boehringer Ingelheim

Assessment of Retinal Function in Dogs with Glaucoma Following Treatment with the Nutraceutical Blend Ocu-GLO

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Glaucoma is a group of degenerative eye diseases occurring in both humans and animals leading to vision loss due to an increase in intraocular pressure (IOP) affecting the ganglion cells in the inner retina. Currently, no cure exists for glaucoma despite medical and surgical treatments to lower IOP. Retinal ganglion cell loss continues despite these methods to decrease IOP and results in progressive vision loss then blindness. Ocu-GLO is a nutraceutical blend consisting of 12 antioxidants such as grape seed extract (GSE), lutein, an assortment of vitamins, and omega-3 fatty acids. In this study, we utilized ten beagles with the *ADAMTS10*-Open-Angle Glaucoma (*ADAMTS10*-OAG) gene to test the neuroprotective capabilities of Ocu-GLO . Five dogs were treated with Ocu-GLO and five dogs were treated with sham treatments using a masked study design. Electroretinograms were recorded at baseline, four weeks, and six weeks of Ocu-GLO treatment to detect improvements in the retina's function. Diurnal IOPs were an additional outcome measurement assessed with the use of weekly non-invasive rebound tonometry. For both treatment and control groups, amplitudes and implicit times of retinal responses were compared between baseline and post-treatment time points to determine if the inner retina showed improvement with supplementation of Ocu-GLO . We hypothesize that the antioxidants used in Ocu-GLO will help to improve retina function and preserve vision in dogs affected with glaucoma.

Research Grant: None
Student Support: NIH Grant 5T35OD016477-20

Use of a superoxide dismutase mimetic to prevent spaceflight-induced bone and joint degradation in mice

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The damaging effects of microgravity on bone during spaceflight are well known; however effects on joint soft tissues remain poorly understood. A previous spaceflight mission identified cartilage and meniscal degradation in mice after ~35 days on the International Space Station (ISS). Damage occurred coincident with reduced antioxidant (Aox) defenses (e.g. superoxide dismutase (SOD)) and increased oxidative stress. As oxidative stress is associated with arthritis, we measured the efficacy of MnTnBuOE-2-PyP5+ (BuOE, 1 mg/kg, 0.2 mL), an Aox SOD mimetic, at preventing damage. Ten mice in the Rodent Research 18 mission to the ISS (Dec-Feb, 2022) were used. Ten-week-old C57BL/6 male (n = 10) mice spent 35 days on ISS. Tissues were collected upon return, (5 BuOE, 5 saline controls), along with corresponding ground controls (n = 10). Knee joints from right hindlimbs were isolated for histologic assessment using MMP13 and ADAMTS5, and staining for sulfated glycosaminoglycans (GAGs). TRAP staining assessed osteoclasts. Our microCT data indicates that the Aox is protective against bulk cartilage and bone degradation during spaceflight. TRAP staining indicated Aox lowered osteoclast number overall, though by day 35 osteoclast number from FLIGHT mice were lower than control, indicating bone loss with microgravity occurred early in the mission. Aox may also promote formation of sulfated GAGs in the cartilage and menisci during spaceflight, but as thinning occurred despite no loss of GAGs in flight, degradation of collagen may contribute to soft tissue damage during spaceflight. Together, our data indicate treatment with MnTnBuOE-2-PyP5 may protect against degradation of joint soft tissue and bone.

Research Grant: NASA 80NSSC21K0544, NASA 80NSSC21K0294. NASA NNX15AB50G
Student Support: NIH T35 Training Grant T35OD010946

Iron sequestration affects bacterial dynamics in *Drosophila melanogaster*

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Iron is an essential nutrient for all organisms, including bacterial pathogens. Hosts have evolved mechanisms, known as nutritional immunity or immunometabolism, to sequester iron to prevent pathogens from accessing this crucial resource. Numerous studies have documented the molecular and physiological mechanisms underpinning iron sequestration, but they have largely focused on *in vitro* dynamics. Consequently, we know little about the functional outcomes of iron sequestration in terms of host recovery or pathogen growth and transmission. Here, we use the *Drosophila melanogaster - Salmonella enterica* system as case study to gain a better understanding of how iron sequestration affects pathogen growth and virulence. We used an iron chelator (BPS) to enhance the host's immune system to sequester iron away from pathogens and increase host survival. We infected both male and female *Drosophila melanogaster* (w^{1118}) individuals, either orally or systemically with *Salmonella enterica* (strains SL1344 and ST4/74). Individuals were then treated or not with BPS. For the flies treated with BPS, we predict increased survival due to a reduction in bacterial growth rate. Because of differences in immune patterns, different responses after infection and BPS treatment should be highlighted in males and females. This study focuses on better understanding the *in vivo* consequences of iron sequestration and nutritional immunity upon bacterial infection. Considering the rising issue of antimicrobial resistance, limiting iron intake could be used as strategy to foster host recovery and limit the evolution of antimicrobial resistance.

Research Grant: None
Student Support: Boehringer Ingelheim, Bourse régionale à la Mobilité Internationale des Etudiants (région ARA)

Chitosan hydrogel and polylactic acid particles loaded with fosfomycin for local treatment of osteomyelitis

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Staphylococcus aureus (*S. aureus*) is the most common pathogen in osteomyelitis (OM), an acute or chronic bone infection. *S. aureus* can become resistant to different types of antibiotics delivered systemically and/or orally. Locally administrated antibiotics placed directly at the site of infection and delivered via a sustained release would allow for a higher dose to be used while simultaneously reducing the risks of resistance and toxicity seen with long-term use. Our objective was to evaluate the efficacy of a chitosan hydrogel and/or polylactic acid (PLA) particles loaded with fosfomycin (FOS) to reduce the bacterial load of *S. aureus* in the femur and soft tissue in a rat model of chronic infection. We hypothesized that FOS delivered dually via antimicrobial chitosan hydrogel and PLA particles would reduce the bacterial load compared to FOS delivered via either chitosan or PLA particles alone. Radiographic images of the femur were taken as real-time indicators of infection status and were used to analyze change in relative bone density over time. There were no differences between treatment groups, but relative bone density decreased from day 8 to day 14 but began to stabilize by day 21 in all groups containing FOS. Blood samples were evaluated for the presence of haptoglobin. Though differences between treatment groups were not seen, haptoglobin levels were higher from day 1 to day 14 compared to pre-surgery values, but returned to baseline by day 21. At day 35, bone and surrounding soft tissue samples will be harvested to quantify bacterial load. Tailored biomaterials, such as the ones used in our study, may allow for increased therapeutic efficacy in future OM cases with challenging pathogens such as *S. aureus*.

Research Grant: National Institutes of Health (NIH) Center of Biomedical Research Excellence Grant (P20GM103646)
Student Support: NIH T35 Training Grant (T35OD010432)

***In Vitro* Assessment of Growing Conditions for Fungal Pathogens of Sea Turtles**

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Endangered sea turtles are threatened by a variety of natural and anthropogenic factors, including fungal pathogens in live turtles and nests. Most fungi of sea turtles are considered opportunistic, and it is important to identify the microorganisms present so that therapy can be promptly instituted. Certain fungi remain difficult to diagnose and may require several weeks to isolate using traditional media and growing conditions. The objective was to determine optimal growing conditions for 10 common pathogenic fungi of sea turtles and their eggs. We compared isolate growth rates and characteristics using six types of selective media, two incubation temperatures, and two methods of measurement (manual measurement and digital imaging software). Statistical analysis consisted of repeated measures analysis and Tukey's post-hoc test. Our study revealed a significant effect of media type and incubation temperature on growth rates of all 10 tested fungi. Three types of media (Sabouraud dextrose, potato dextrose, and Rose Bengal) yielded consistent and rapid growth of most fungi, whereas growth was less reliable on inhibitory mold, SABHI, and synthetic mycobiotic agars. Most fungi grew significantly faster at 30°C than 23°C regardless of media type. Thus, the use of certain media and incubation temperatures may enhance the recovery rate of fungal pathogens from sea turtles, and in turn, inform appropriate therapy and reduce the duration of illness and rehabilitation. Our results may also aid the diagnosis of fungal infection in sea turtle nests and in other reptile species, as several of the examined fungi are broad pathogens of ectothermic hosts.

Research Grant: Research Grant: MSU Global Center for Aquatic Health & Food Security; MS Marine Mammal & Turtle Conservation, Recovery, & Monitoring Program; Nat Fish & Wildlife Foundation; MDEQ Agreement No.18-00081
Student Support: Student Support: MSU Veterinary Research Scholars Program

Determining the origins of the piglet gut microbiome using strain-resolved longitudinal metagenomics

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The piglet gut microbiome has been linked to numerous aspects of swine health and productivity, including growth and development, fat content of tissues important in meat quality, diarrhea in neonates, and even swine welfare. Despite this association, little is known about the factors that contribute to the initial colonization of the neonatal gut. The goal of this study was to chart the assembly of the piglet gut microbiome in the three weeks following birth, while simultaneously sampling maternal and environmental microbes and using strain-tracking to identify potential sources of early life colonization. Ten farrowing units, each consisting of one sow and at least 6 piglets were housed in individual farrowing stalls in the same room. Sow feces and piglet rectal swabs were collected on days 2, 4, 7, 14, and 21 post-gestation, and sow vaginal swabs were collected pre- and post-gestation. Environmental samples, including sow feed, piglet feed from a creep-feeder, and floor swabs, were used to monitor the farrowing environment for microbes. To profile the microbiome in these samples, we extracted DNA using the Qiagen PowerSoil Pro kit, used this DNA to prepare sequence-ready libraries, and carried out shotgun metagenomic sequencing. Analysis of these data is currently underway and may provide valuable insight into the seeding and assembly of the neonate gut microbiome. This data may help identify 'keystone' bacteria that are beneficial for promoting healthy, fast-growing piglets, thus potentially setting the stage to develop probiotics that can improve animal health and production in swine farming.

Research Grant: Pennsylvania Department of Agriculture and the University of Pennsylvania
Student Support: NIH T35 OD010919 and Boehringer-Ingelheim

High throughput molecular diagnostics for cattle using Applied Bio Code's Barcoded Magnetic Beads technology

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Ticks are found on 80 percent of the world's livestock and are linked to a variety of health and economic problems. There are more than 60 agents that cause diseases in cattle including *Anaplasma, Borrelia, and Babesia* have significant impacts economically. *Borrelia burgdorferi*, transmitted by Ixodes ticks, is the primary causal agent of Lyme Borreliosis in humans, domestic animals, and livestock in the United States. With the current methods of detection, it is difficult to rule out the infection or give conclusive evidence of the disease. The purpose of this study was to measure the sensitivity of *B. Burgdorferi* in 20 spiked calf blood samples using Applied Bio Code's Barcoded Magnetic Beads (BMB) technology on BioCode 2500 Multiplex Detection System. After PCR analysis of 20 positive control spiked calf blood samples, *B. Burgdorferi* was detected. Furthermore, PCR of 10 negative control samples revealed that *B. Burgdorferi* was undetectable. Gel electrophoresis verified the presence of *B. Burgdorferi* in all positive control samples and not other pathogens. The future aim of developing high throughput, short turnaround time, and low-cost nucleic acid-based multiplex assay that will detect three pathogens (*Anaplasma, Borrelia, and Babesia*) and encourage annual routine testing in cattle herds to provide effective treatment, control tick-borne diseases in the herd, and prevent community spread.

Research Grant: Boeheim Ingelheim
Student Support: CVM Veterinary Summer Research Program

Identification of disease-causing alleles in equines for microphthalmia and alopecia areata

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Microphthalmia and alopecia areata (AA) exhibit Mendelian inheritance patterns in humans and other species suggesting that these diseases are monogenic in horses. Recent studies have shown that whole genome sequencing (WGS) of an individual with a suspected Mendelian disease can be sufficient to identify the causative mutation when combined with information about population frequencies of putative mutations. The aim of this study is to prioritize causative mutations for microphthalmia and AA using WGS of an affected offspring and healthy parent. We used a modified version of our pipeline for WGS mutation prioritization. Candidate genes were identified using a literature search and two candidate gene prioritization programs (Phenolyzer and Endeavour). Mutations present in the affected offspring and absent in the unaffected parent (*de novo* or dominant inheritance) or homozygous in the affected offspring and heterozygous in the unaffected parent (recessive inheritance) were extracted. In total, 93,608 mutations were identified in the microphthalmia pair and 88,232 mutations were identified in the AA pair. These variants will be further filtered by those associated with the candidate genes and those that possess the Mendelian inheritance pattern to give the final putative causative mutations. For example, so far 11 variants were found in *STRA6* for microphthalmia and 9 variants were found in *ITGB4* for AA, however, after filtering by inheritance pattern, 7 variants for microphthalmia and 0 variants for AA were kept for further analysis. Our goal is to identify the causative mutations for both diseases to allow for genetic testing to reduce the risk of future foals being born with these diseases.

Research Grant: USDA NIFA-AFRI Project 2017-67015-26296: The AQHA and a UMN Multistate grant. Salary support (Durward-Akhurst): ACVIMF fellowship, T32 Grant (5T320D010993-12), and MAF Research Fellowship (D20EQ-403)
Student Support: Boehringer Ingelheim

Determining the impact of GIP receptor signaling on alpha cell GLP-1 production

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Insulin secretion in response to oral glucose intake is described as the incretin effect and is driven by two gut-derived hormones: glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). GLP-1 is derived from the protein proglucagon, which can be cleaved into either glucagon or GLP-1 via the PC2 or PC1/3 enzyme, respectively. Traditionally, it was believed that PC1/3 expression was exclusive to the gut, but multiple studies have demonstrated alpha cells are capable of being stimulated to express PC1/3 and consequently produce GLP-1. Because the half-life of active GLP-1 in circulation is extremely short, it is believed alpha cells play a role in promoting glucose-stimulated insulin secretion (GSIS) via paracrine signaling to beta cells using GLP-1. A recent study demonstrated that GIP contributes to GSIS through the alpha cell. We hypothesize alpha cell GIP receptor signaling promotes GSIS by activating the production of GLP-1. The goal of our project is to mimic the conditions of this study to determine if GLP-1 is released in response to alpha cell GIP receptor signaling, which would give more insight into potential mechanism behind alpha and beta cell communication in GSIS. To achieve this, we will treat alpha TC1-6 cells with GIP under conditions of high and low glucose and measure the alpha cell response via ELISAs for glucagon, active GLP-1 and insulin levels. Additionally, PC1/3 & PC2 mRNA and protein levels will be quantified by qPCR and immunoblotting. Because glucogenic amino acids, such as alanine, are key stimulants of alpha cell hormone secretion, treatments containing GIP and alanine will be analyzed as described above as well.

Research Grant: Hartwell Foundation, NIH/NIDDK R56DK124853
Student Support: UC Davis SVM Endowment Funds, NIH Grant T32GM136559

Glial inflammation in aging canines

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Tracking human-canine interactions through the centuries shows how canines have been experiencing parallel lifestyles beside their guardians. These companions are enabling a closer look at neurobiological aging processes. When observing aging of humans and canines, both display gliosis which is increased reactivity and inflammatory signaling of both astrocytes and microglia within the central nervous system. An increase of microglia and astrocyte activation has also been noted to increase susceptibility to neurodegenerative diseases; such as Alzheimer's disease (AD) and its comparable canid-disease called canine cognitive dysfunction (CCD) syndrome. The correlating pathology of aging, as well as the similar lifestyle shared between these two species suggests that the canine acts as an innovative translational comparison between the biological aging process and both progressive neurodegenerative diseases: AD and CCD. By using immunohistochemistry (IHC) to analyze various age groups of young and old canines, we hypothesize that both astrocytes and microglia numbers and inflammation will increase in older canines. We find that in the frontal cortex of older canines there is increased astrogliosis measured by S100b, as well as an increase in microglia reactivity detected by Iba1, in comparison to younger canines. These findings will further our understanding of aging and neuroinflammation in both canines and humans. Further directions for this study will include looking at misfolded proteins such as phosphorylated tau and amyloid beta in canines with CCDS.

Research Grant: Research Grant: Boehringer Ingelheim Veterinary Summer Scholar Program, Colorado State University
Student Support: Student Support: Boehringer Ingelheim

Detecting virulence and antimicrobial resistance genes from *Salmonella spp.* from veterinary teaching hospital

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Salmonella spp. are commonly found in the environment and can potentially cause outbreaks in large animal veterinary teaching hospitals (LA-VTH) affecting several animal species. Literature review suggests that there could be an association between the presence of virulence genes and antimicrobial resistance (AMR) genes as pathogenic bacteria could be multi-drug resistant. This study was designed to assess the presence of 19 virulence determinants and 87 AMR gene profiles of *Salmonella spp*. isolates recovered over 4 years (2018-2022) from environmental samples of a LA-VTH and those found in equine patients. We hypothesize that there will be some difference between the virulence and AMR gene profile of environmental and equine patient *Salmonella* isolates, as certain genes would be needed for infectivity and survivial in animals. The genomic DNA was purified from 29 environmental and 6 equine isolates, and they were screened for the presence of virulence genes using conventional PCR and agarose gel electrophoresis to determine their product size. The AMR genes were screened in a selected set of isolates with microbial DNA qPCR array kit (Qiagen) and compared to their antimicrobial susceptibility profiles. Three virulent genes were present in all environmental samples: *invA*, *tufA*, and *aroA*, and seven virulent genes were present in some isolates: *iroB* (96.6%), *sopB* (96.6%), *pipB* (51.7%), *fimA* (20.7%), *siiE* (24.1%), *stn* (20.7%), *siiD* (20.7%), and *intI1* (6.9%). From the 6 equine patients, 6 virulence genes were present in all samples: *tufA*, *sopB*, *aroA*, *stn*, *invA*, and *iroB*. The *Salmonella* characterization shows that there are slight differences between the genetic profiling of isolates from the environment and equine patients.

Research Grant: Tifton Veterinary Diagnostic & Investigational Laboratory Operational Funds for development of new assays
Student Support: UGA Foundation, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Evaluation of Herpes Simplex Virus ICP22 Function in Early Transcription

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About two-thirds of the human population is infected with human simplex virus 1 (HSV-1), an alphaherpesvirus. The vesicles formed by this virus are often mild but can induce severe disease in immunocompromised individuals. HSV-1 is able to establish latency, and once reactivated can produce symptoms intermittently. During productive lytic infection, HSV-1 transcription is mediated by host RNA polymerase II (Pol II), which is recruited to initiate a temporal cascade of viral gene expression. The immediate-early (IE) genes are transcribed first, and their protein products are required to continue viral replication. In the absence of IE gene products ICP4, ICP0, and ICP22, viral transcriptional activity is increased at just 1.5 hours post-infection (hpi). The requirement for ICP22 is curious because it is not known to be a virion component suggesting it must be rapidly synthesized to affect early transcriptional repression. To test this hypothesis, cells were infected with either an ICP22 deletion or ICP22 repair virus in the presence or absence of the protein synthesis inhibitor cycloheximide (CHX). Infected cells were harvested at 1.5hpi and a precision nuclear run-on with deep sequencing was performed. The hypothesis predicts that the ICP22 repair virus should repress transcriptional activity in non-CHX treated cells. If ICP22 is not present in the virion and is required to be synthesized, we expect to see high levels of transcriptional activity on the repair virus upon CHX-treatment and on the deletion virus in all treatment groups. However, if ICP22 is present in the virion then we should observe equivalent transcriptional repression.

Research Grant: NIH R01 AI 141968, NIH R21 AI 148926
Student Support: Cornell Veterinary Leadership Program – NIH grant T35 AI007227

Synthesis and *in vitro* evaluation of irinotecan loaded chitosan nanoparticles against colorectal cancer

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Colorectal cancer is the third most frequent cancer in males, and the second most common cancer in women. The topoisomerase inhibitors that cause apoptosis and are used to treat a variety of malignancies, including colon cancer. Irinotecan (IRI) is a topoisomerase-I inhibitor that is used in this study. It is a fact that anticancer medications alone have not always been successful due to severe adverse effects and therapeutic failures with relapses. The use of nanoparticle drug delivery systems has heralded a new era in cancer research. According to many studies, the formulation of anticancer medicines into nanoparticles is known to boost the efficiency. We synthesized chitosan-tripolyphosphate nanoparticles loaded with IRI to test on colorectal cancer cells. Biodegradability, better drug bioavailability, muco-adhesive characteristics, and greater safety are advantages of employing chitosan nanoparticles. Ionic gelation method is used to synthesize nanoparticles. The synthesized nanoparticles are characterized for loading efficiency and particle size. Furthermore, we evaluated the effect of nanoparticles on colorectal cancer cells and found that the chitosan encapsulated IRI nanoparticles exhibited significantly lower cell viability and encouraging results on cell cycle analysis by flowcytometry. Future research will be conducted based on the findings of current investigations.

Research Grant: This research was supported by grants from NIH T35OD010432, DHHS/HRSA D34HP00001-35-00, and NIH/NIMHD RCMI grant # U54MD007585
Student Support: Boehringer Ingelheim Veterinary Scholars Program and TU-CVM - Center of Excellence

Heavy Metal Associated Resistance in Avian Pathogenic *Escherichia coli* (APEC)

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Avian Pathogenic *Escherichia coli* (APEC) is one of the leading causes of loss to the world's poultry production resulting in significant morbidity, mortality and carcass condemnation putting at risk one of the world's cheapest sources of protein. One area of interest is the use of heavy metal-based compounds as a supplement/antimicrobial and its potential role in the selection of virulent and resistant APEC. Here, we assessed APEC strains from Georgia for heavy metal resistance using broth microdilution for phenotype analysis and polymerase chain reaction (PCR) to detect heavy metal associated genes. A survey of 50 isolates were examined to determine resistance to silver, zinc, lead, chromium, cobalt, and arsenic. All isolates were assessed for minimum inhibitory concentration (MIC) of metals using the broth microdilution assay at concentration ranges of 0.78 µg/mL to 1600 µg/ml. Isolates were also screened for metal resistance associated genes using PCR analysis. The results found that all APEC isolates displayed growth in all 6 metals at high levels with MICs and PCR analysis reflected detection of all 6 metal genes. Highly resistant strains, chosen based on their growth in arsenic, were used to challenge 12-day old eggs via embryo lethality. Results found that the embryos challenged with isolates 128, 349, 379 and 403 grown in the arsenic metal resulted in a higher lethality than their control counterpart. In addition to providing valuable insight as to what metals could potentially be used therapeutically to control the spread of APEC, it is beneficial to characterize APEC's resistance to heavy metals also as these resistances may contribute to the selection of virulent strains.

Research Grant: US Poultry and Egg Association – Project #726
Student Support: UGA Foundation, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

A cathepsin-based near-infrared (NIR) imaging for intraoperative detection of canine appendicular osteosarcoma

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Osteosarcoma (OSA) is the most common primary bone tumor seen in dogs. Amputation is generally considered the standard of care in these patients but carries a poor long-term prognosis, with 90% of dogs dying from metastatic disease within a year of surgery, leading to adjunctive treatment with chemotherapy. Limb-salvage procedures provide an alternative to amputation and allow a return to more normal mechanical function. Intraoperative imaging can improve the definition of surgical margins and increase the likelihood of successful surgical excision, lowering the morbidity of the surgery and providing a translational model for human limb-sparing procedures. The hypothesis of this study is that near-infrared (NIR) imaging using a cathepsin-based fluorophore will allow visualization of the tumor margins of appendicular osteosarcoma. This study aims to assess the correlation between tumor margins determined with NIR imaging, MRI, and histopathology. Based on limb radiographs, dogs presenting for suspect appendicular osteosarcoma were recruited with informed owner consent. They underwent an MRI of the affected limb to assess the tumor margins and received VGT-309, a cathepsin-based NIR fluorophore, before standard of care limb amputation. The bone was imaged immediately after amputation, longitudinally sectioned, and reimaged. Comparison of the distribution, maximum intensities, and total intensities of the fluorophore allows for evaluation of cortical and medullary uptake of VGT-309.

Research Grant: Companion Animal Research Fund at the University of Pennsylvania
Student Support: NIH T35 OD010919, Boehringer-Ingelheim, and the University of Pennsylvania

Investigating the ablative and immunomodulatory effects of H-FIRE in canine metastatic osteosarcoma

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Osteosarcoma (OS) is the most common primary bone tumor in dogs. Despite limb amputation and chemotherapy, prognosis remains poor due to aggressive metastatic development. Failure of current methods to improve clinical outcomes necessitates the investigation of novel therapies to combat metastasis. One such therapy, high-frequency irreversible electroporation (H-FIRE), uses electric pulses to deliver non-thermal damage to targeted cancer cells resulting in cell death. H-FIRE may also induce an inflammatory anti-tumor response to mitigate metastasis. We hypothesized that *in vitro* H-FIRE treatment would successfully induce cell death in the D17 canine OS cell line. We treated D17 cells with increasing H-FIRE field strengths (2000 to 4000 V/cm), and D17 cells were analyzed for viability by flow cytometry at 24, 48, and 72 hours post treatment (PT). Samples treated at 3000 V/cm showed time-dependent death with averages of 0.29% live, 1.70% dying, and 77.38% dead cells at 24 hours PT and 96.34% dead cells by 72 hours PT. In contrast, samples treated at 2000 V/cm recovered from treatment, showing averages of 66.41% dead cells at 24 hours PT and 62.61% live and 32.53% dead cells by 72 hours PT. Field strengths > 3000 V/cm generated undesirable heat; therefore, we selected 3000 V/cm as the ideal H-FIRE field strength which induces irreversible cell death without thermal effects. In subsequent experiments, D17 cells will be treated with H-FIRE at 3000 V/cm and co-cultured with canine macrophages to investigate the immunomodulatory effects of H-FIRE in the context of canine OS *in vitro*. This work represents an important initial step in developing H-FIRE as an immunostimulatory treatment for OS.

Research Grant: Internal Research Competition (Virginia Tech intramural grant)
Student Support: NIH T35OD011887

Vasopressin stimulation testing for Hypothalamic-Pituitary-Adrenal Axis function in hospitalized foals

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Sepsis, defined as life-threatening organ dysfunction due to a dysregulated host response to infection, is a leading cause of foal mortality. Exceedingly low cortisol levels due to dysfunction of the Hypothalamic-Pituitary-Adrenal axis (HPAA) are correlated with further increased mortality in foals and critically ill people. Administration of Arginine Vasopressin (AVP) to healthy foals stimulates the HPAA more predictably than other available diagnostic tests. This project evaluates the HPAA response to administration of AVP in hospitalized foals compared to healthy foals. Sample groups consisted of 6 healthy foals, 13 sick non-septic (SNS) foals and 5 septic foals (defined as positive blood culture or modified sepsis score $\geq$ 12). Sepsis scores were higher in both SNS and septic foals compared to healthy foals ($P < 0.05$). Neutrophil count was lower ($P < 0.05$) in septic compared to SNS foals. Baseline blood samples were collected prior to administration of a randomly assigned dose of AVP (2.5 or 5 IU) then blood samples were collected at 15, 30, 60 and 90 minutes. Baseline AVP concentrations were higher ($P < 0.05$) in septic foals when compared to both healthy and SNS foals. Baseline AVP concentrations between the 2.5 and 5 IU treatment groups were not different ($P > 0.05$). Adrenocorticotropic Hormone, Corticotropin Releasing Hormone and cortisol will be measured to assess the HPAA response to AVP. If AVP administration continues to provide more predictable results than other dynamic tests, AVP stimulation testing might be preferred for assessing HPAA function. This would allow septic foals with dangerously low cortisol levels due to dysfunction of the HPAA to be more readily identified and treated with hormone replacement therapy.

Research Grant: NC State University College of Veterinary Medicine Intramural Seed Grant
Student Support: NIH T35OD011070 Interdisciplinary Biomedical Research Training Program

Pharmacokinetics of Intravenously, Orally, and Topically Administered Fluralaner in Laying Hens

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Ectoparasite infestations negatively affect both backyard and commercial chicken flocks in the US. Fluralaner is an isoxazoline shown to be efficacious in treating mite infestations in poultry. Fluralaner is approved to treat fleas and ticks in dogs and cats in the US and in Europe to treat mite infestations of chickens; however, the use of fluralaner in poultry is not yet approved in the US. This study aims to determine the bioavailability of fluralaner administered orally and topically, establish the elimination half-life of fluralaner in plasma of chickens, and quantify fluralaner residues in the eggs and blood of treated birds at subsequent time points. A total of 19 individually-housed healthy shaver hens received a single dose of either intravenous technical grade fluralaner at 0.5mg/kg, oral technical grade fluralaner at 0.5mg/kg, or transdermal fluralaner (Bravecto (fluralaner topical solution) for Dogs) at approximately 55mg/kg. Plasma from each bird was collected at 0, 0.5, 1, 3, 6, 8, 12, 16, 24, 36, 48 hours as well as 4, 7, 14, 21, 28, 35 days post-fluralaner administration. Eggs collected for 35 days after fluralaner administration will be analyzed to quantify drug residues excreted in the egg whites and yolks. Fluralaner concentrations in eggs and plasma are being measured using Ultra Performance Liquid Chromatography with Mass Spectrometry (UPLC/MS/MS). Understanding the pharmacokinetics of fluralaner is important for prescribing veterinarians to create effective treatment plans and issue safe withdrawal times.

Research Grant: USDA grant "Food Animal Residue Avoidance Databank (FARAD)"
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Effects of *in ovo* exogenous estrogen exposure on 5mo *Alligator mississippiensis* ovarian folliculogenesis

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Estrogenic environmental contaminants are becoming more common in endemic species' habitats. Environmental estrogens can compromise reproductive health across vertebrates and, more specifically, can override temperature dependent sex determination (TSD) producing female crocodilians incubated at male-producing temperatures. However, less is known how exogenous estrogen exposure may affect crocodilian ovary formation. To this end, this study investigated the morphological effects of developmental 17 -estradiol exposure on the folliculogenesis in *Alligator Mississippians* ovaries. Particularly ovarian cortex size and the quantity and size of enlarged stage 3 follicles in diplotene arrest were examined. American alligator eggs were collected from Lake Woodruff, Florida soon after oviposition and incubated at a female-producing 30°C. At developmental stage 24 (the end- of TSD and ovary formation), eggs were topically treated with 50ng/egg in ethanol (n = 5) or vehicle alone (n = 5). Post-hatching alligators were raised until 5 months old when ovaries were collected at necropsy and divided into three zones (cranial, medial, and caudal). Each zone was formalin fixed, transverse paraffin sectioned, and H&E stained. Per ovary zone, two random images were selected for analysis: the percent area of the ovary compartment (cortex and medulla), average stage 3 follicle average diameter, and total stage 3 follicle count. Since estrogens can drive initial ovary formation, but also act as negative endocrine feedback to folliculogenesis, we hypothesize estrogen-treated animals ovaries will have with fewer follicles compared to controls.

Research Grant: None
Student Support: IDEXX-BioAnalytics

Functional anatomy of the mitral valve in canine degenerative mitral valve disease

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Degenerative mitral valve disease (DMVD) is the most common acquired heart disease in small breed dogs as well as the number one cause of cardiac-related death in this population. With recent advancements in procedural options for DMVD, it has become increasingly important to understand the functional anatomy of mitral valve to determine candidacy for these procedures. The hypotheses of this study are: 1. that excessive leaflet motion originates at the A2 segment of the anterior leaflet of the mitral valve, with additional segment involvement in later stages of disease and 2. there will be an additional component of functional mitral regurgitation (MR) secondary to cardiac remodeling with increasing disease severity. Excessive leaflet motion of the A2 and P2 segments of the mitral valve was assessed on the short-axis and multiple long-axis inflow-outflow views from previous prospectively acquired echocardiograms from clinical patients at varying stages of DMVD. Functional MR was evaluated by measurement of vena contracta width compared to anatomic diameter on the commissural view. The results of this study will contribute to the understanding of the functional anatomy of the mitral valve in DMVD. This understanding will aid in determining candidacy and timing of interventions for DMVD.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholar

Preictal firing rates of interneurons and excitatory neurons in the hippocampi of epileptic rats

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Temporal lobe epilepsy is a common form of epilepsy in humans. Little is known about what happens in the brain in the seconds leading up to a spontaneous seizure. Past research has shown that seizures typically begin in the hippocampus of the temporal lobe. We hypothesize that interneurons will decrease in activity thereby reducing inhibition, and excitatory neurons will increase in activity before a seizure occurs in the hippocampi of rats with pilocarpine-induced temporal lobe epilepsy. To determine when and where a seizure starts, the local field potential recording from surgically implanted electrodes in many brain regions was analyzed. Tetrodes, whose depths were individually adjustable, recorded the action potentials of individual neurons. Accelerometer data in conjunction with the local field potential recordings were used to establish the immediate pre-seizure baseline brain state, for example, movement, slow wave sleep, or rapid eye movement sleep. After recordings were finished, brains were sectioned and Nissl stained to identify precise anatomical locations of tetrodes and local field potential electrodes. Data acquisition and analysis are in progress. Cells were classified as interneurons or excitatory neurons based on action potential shape (symmetrical or asymmetrical) and firing rate (> or < 4 Hz). Neurons will also be classified by hippocampal subregion (dentate gyrus, CA3, CA1, subiculum) and location relative to seizure onset site. Action potential firing rate during the last 1 or 10 seconds before seizure onset will be compared to the baseline firing rate (t test). Results will test our hypothesis that interneurons decrease their activity and excitatory neurons increase their activity pre-seizure.

Research Grant: NIH R01 NS107290
Student Support: NIH T35 ODO010989

Validation of the Polar heart rate monitor for collection of heart rate variability measures in horses

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Heart rate monitors can be helpful tools when studying as well as training equines. Heart rate variability (HRV) measures specifically can be used as objective data in equine studies investigating stress and pain. The equine Polar heart rate monitor and portable electrocardiogram (ECG) are the two most common methods of obtaining HRV data in horses. The portable ECG monitor is considered the gold standard method of obtaining data for HRV measures; however, these monitors are expensive making them cost prohibitive for use by many researchers. In addition, these monitors may not be accessible when research is conducted in the field. However, the use of the more accessible Polar monitor is still controversial and validation per study is recommended. The aim of this study is to validate the accuracy of the Polar monitor compared to the portable ECG for analysis of HRV measures during three activities. HRV data was obtained from horses using the equine Polar monitor and portable ECG simultaneously while they were free in the stall, tied, and walking, which allowed for comparison of measures. Time domain, frequency domain and non-linear HRV measures were analyzed using Kubios software and compared statistically. The descriptive statistics demonstrated consistency between methods within each activity. Preliminary analysis of mnHR, PNS, and SNS, during the activities, using the Bland-Altman method showed a 95% agreement between the data from the two monitors. Further analysis will be conducted for the remaining HRV measures across the three activities. The findings demonstrate that the equine Polar heart rate monitor is comparable in accuracy to that of the gold standard portable ECG in obtaining HRV data in horses.

Research Grant: None
Student Support: Atlantic Veterinary College Veterinary Student Research Award

A description of the canine fecal microbiome after a yearlong feeding trial consuming a plant-based diet

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Adverse implications of industrial food animal production have fueled an increased interest in plant-based nutrition for both people and dogs. In this study, we followed a cohort of 15 dogs that switched from a meat-based to a plant-based diet for a year. Here, we aim to characterize the fecal microbiome, which was achieved by analyzing fecal samples from dogs collected at the beginning and end of the trial. Analysis of the fecal microbiome is a non-invasive method of gauging the composition of the gut microbiome and can be utilized as a marker of host health and gastrointestinal function. Canine fecal samples were surveyed by 16S rRNA gene PCR, followed by amplicon sequencing. Alpha-diversity analysis reflected a reduction in the total number of observed species after a year of consuming a plant-based diet. Community composition trends showed the plant-based diet switch increased the relative abundance of the *Bacteroidetes* phylum with an increase for the *Bacteroides* and a decrease for the *Prevotella* genera. *Bacteroidetes* are gut microbes responsible for certain metabolisms, such as protein and carbohydrate degradation as well as fermentation of plant materials resulting in the production of short chain fatty acids. The products of these metabolic processes, central to the host-microbiome symbiosis, play a role in determining a healthy gut environment (*e.g.*, immune function, energetic harvest). Our study is the first to assess the impact of a plant-based diet on the canine fecal microbiome over one year. This data can serve as a stepping stone to pave the way for improved understanding of canine health outcomes associated with long-term implementation of more environmentally sustainable and ethically produced dog foods.

Research Grant: Research Grant: Boehringer Ingelheim
Student Support: Student Support: WesternU CVM Veterinary Summer Research Program

Colocalization of GABAergic and glutamatergic cells with ALDH1A2 in the zebra finch vocal circuit

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Zebra finch males learn a unique song over a period of vocal learning, but the neural mechanisms and molecular prerequisites for learning song are not fully understood. Retinoic acid signaling plays a key role in the development of the vocal circuit and the ability to learn and maintain the zebra finches' song. In the zebra finch brain, the vocal pathway consists of discrete, interconnected pallial and basal nuclei that are exclusive for vocal learning and production. The songbird vocal circuit is well-characterized anatomically and consists of a posterior vocal-motor pathway (VMP) and an anterior forebrain pathway (AFP). The VMP includes the nidopallial nucleus, HVC, which links to the robust nucleus of the arcopallium (RA), then to the brainstem vocal and respiratory centers. The AFP links the HVC to striatal Area X (X), then the dorsolateral thalamic nucleus (DLM), and finally to the lateral magnocellular nucleus of the anterior nidopallium (LMAN). LMAN then connects to X and RA, allowing for fine control of complex vocal-motor output. These pathways contain the brain-specific aldehyde dehydrogenase (ALDH1A2), the terminal enzyme that is necessary for retinoic acid synthesis. There exist competing hypotheses as to the specific targets of retinoic acid in the vocal circuit. To test these hypotheses, we examined colocalization of ALDH1A2 with markers of GABAergic and glutamatergic cells in the HVC. Fluorescent in situ hybridization and immunohistochemistry in thin brain sections were utilized to observe ALDH1A2 with GAD2 and VGLUT mRNA, markers of these cell types, respectively. This analysis gives new insights into the areas of the vocal circuit that utilize retinoic acid signaling for this complex learned behavior.

Research Grant: Midwestern University College of Graduate Studies Intramural Grant #28
Student Support: Boehringer Ingelheim Veterinary Scholars Program and Federal Work Study

Comparing the protective effects of equine EVs and BM-MSCs cultured in FBS and equine serum on an OA model

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Osteoarthritis (OA) can occur following joint trauma where global posttraumatic inflammation leads to progressive degradation of cartilage extracellular matrix (ECM). Mesenchymal stem cells (MSCs) have been proposed as a potential therapy for joint disease due to their potent immunomodulatory properties. Extracellular vesicles (EVs) produced from bone marrow derived (BM)-MSCs have been shown to hold therapeutic potential due to their role in mediating intercellular communications and modulating inflammatory responses. Concerns regarding the immunogenicity of BM-MSCs traditionally cultured in fetal bovine serum (FBS) media has prompted research into xenogen-free culture alternatives. Our lab previously showed that BM-MSCs cultured in equine serum exhibit superior immunomodulatory properties compared to BM-MSCs cultured in FBS. The objective of this study is to compare the protective effects of BM-MSCs and EVs from FBS and equine serum media on cartilage explants cultured in an OA model. We hypothesize that BM-MSCs and EVs from equine serum media will exhibit superior protective capabilities compared to FBS media. BM-MSCs will be isolated from six horses and cultured to passage 3 in equine serum or FBS media. BM-MSCs and EVs from each culture condition will be co-cultured with cartilage explants with or without interleukin-1β/tumor necrosis factor-α (TNF-α) to drive *in vitro* inflammation as a model of OA. ECM degradation will be assessed by histological analysis of explants and quantification of glycosaminoglycan in the supernatants and cartilage explants using a DMMB assay. Gene expression of IL-1β, TNF-α, IL-6, collagen type II, aggrecan, MMP13, and ADAMTS4 in cartilage explants will be determined. Results are pending.

Research Grant: None
Student Support: NIH T35 OD010919, Boehringer-Ingelheim, and the University of Pennsylvania

MSC secretome targeting mitochondrial function to treat degenerative disc disease

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Previous research shows that placing mesenchymal stem cells (MSCs) into experimentally induced intervertebral disc (IVD) lesions prevents degeneration; however, the mechanisms underlying the beneficial effects of implanted MSCs remain unclear. Mitochondrial (MT) dysfunction in IVD cells plays a causal role in the degenerative pathogenesis. Our broad objective is to study MT transfer from MSCs to damaged IVD cells, which is known to restore MT function and prevent apoptosis in other cell types. The goal of this study was to investigate MT transfer between MSCs and IVD cells in vitro. We hypothesized that MT transfer occurs with direct cell-cell contact and through extracellular vesicles (EVs) containing MT (mtEVs). EVs > 200 nm were isolated from MSC conditioned media (CM) using filtration. General microvesicle markers Hsp90 and Flotillin and cellular control Iκβα characterized microvesicles via western blot. Voltage dependent anion channel (VDAC), a MT protein, was present in the microvesicle fraction of MSC CM indicating the presence of mtEVs. An ex vivo model was established to observe MT transfer from human MSCs (transduced with a RFP targeted to their MT) to td-To degenerative IVD cells. Evidence of MT transfer via direct cell-cell contact was captured using confocal imaging. Characterization of the secretome in the context of improving MT function in degenerative IVD cells is a promising future direction for acellular MSC therapies of IVD disease and other orthopedic diseases.

Research Grant: None
Student Support: None

Quantitative MRI Mapping of Acute Ischemic Injury in a Piglet Model of Legg-Calve-Perthes Disease

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Legg-Calve-Perthes Disease (LCPD) is a juvenile hip disorder caused by temporary interruption of blood flow to the developing femoral head that can result in joint deformity. To improve clinical management of the disease, new imaging techniques that can better evaluate early ischemic injury are needed. Using an LCPD piglet model, the goals of this study were to: 1) determine the repeatability of quantitative magnetic resonance imaging (MRI) measurements; 2) analyze differences in left and right unoperated femoral heads; and 3) characterize the earliest biological changes that occur after onset of ischemic injury. In this study, six 6-week old piglets were imaged in vivo at 3T MRI using 3D T2, aT2ρ, and aT1ρ mapping immediately before undergoing surgery to induce unilateral global femoral head ischemia. The piglets were imaged again at 2 hrs (n = 2), 24 hrs (n = 3), or 48 hrs (n = 1) post-op. The MRI sequence maps of the femoral heads were segmented into ten regions of interest (ROIs). Median relaxation times for each ROI were compared between right and left and pre- and post-op hips for each MRI sequence. Results revealed that: 1) repeatability was greater in cartilage than in bone marrow regions; 2) differences in right and left unoperated hips were small and less variable than differences between pre- and post-op control hips; and 3) the secondary ossification center and articular-epiphyseal cartilage complex ROIs showed increasing response to injury as the post-op interval progressed. These preliminary findings help validate use of the contralateral hip as a control in future studies and prompt further investigation into the cellular changes that correspond to observed differences in variability and response to injury.

Research Grant: NIH grants R56AR078315 and P41EB027061
Student Support: Boehringer Ingelheim and the University of Minnesota, College of Veterinary Medicine

Eosinophilic Esophagitis Induced T-cell Infiltration into the Esophagus

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Eosinophilic Esophagitis (EoE) is a T-cell driven, inflammatory food allergy of the esophagus with strongly increasing incidence rates. EoE causes narrowing of the esophageal lumen leading to dysphagia, nutritional deficiencies in children, and food impaction in adults. Apart from allergen avoidance, there are no FDA-approved treatments available. Based on the physiological and immunological similarities between pigs and humans, the overall goal of the project is to establish the pig as a biomedical model for EoE. The aim of this study was to determine how oral steroid treatment will affect EoE. To this end, 23 pigs were divided into three groups: i) non-treated control pigs; ii) EoE-induced pigs were sensitized and orally challenged with hen egg white protein (HEWP); and iii) EoE-induced pigs that were additionally treated with oral steroids. After three weeks of oral challenge, esophageal tissue samples were taken and stained for immunohistochemistry - the nuclear stain DAPI, the epithelial cell marker claudin-4, and the T-cell marker CD3. Stained samples were imaged using fluorescent microscopy on the one side to differentiate esophageal epithelia, lamina propria, muscularis mucosa, and submucosa; on the other side, to quantify T-cells within these sub-sections. While differences in the T-cell infiltration could be seen between the esophageal sub-sections, our preliminary data (n = 3) did not identify differences in T-cell numbers between the treatment groups. A full analysis of all pigs might reveal such differences; another option is that while esophageal T-cell numbers might stay similar, their activity might change in EoE patients.

Research Grant: Research Grant: NIH 1R21AI149098-01
Student Support: Student Support: Herbert Benjamin Endowment

Porcine model of costochondral graft for temporomandibular joint replacement

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An adult large animal model of costochondral graft for temporomandibular joint reconstruction is necessary to support preclinical trials. The hypothesis tested in this study was that costochondral graft promotes better mandibular condyle microstructure, morphology, and articular cartilage characteristics than no treatment. The left mandibular condyle and caudal ramus were replaced with either the 7th rib and costochondral junction (n = 5) or no treatment (n = 2) in adult Yucatan boars. Mandibular morphology, microstructure, and condyle articular cartilage were assessed 6 months after implantation using computed tomography, micro-computed tomography, and articular cartilage-specific gene expression, respectively. In both groups, the ostectomized tissue was replaced with new bone growth of similar shape to the native condyle, with no treatment condyles more closely resembling the native structure. Overall bone volume of treatment group (124 ± 5.264%:115% at 12wk; 122% at 24wk) tended to be higher than no treatment (106.8 ± 3.258%:111% at 12wk, 100% at 24wk). With no treatment, *Col3* and *SOX5* were upregulated and *Col1* and *2*, *SOX6* and *9*, *TGF*β3, *Runx1, aggrecan*, and *BMP2* were downregulated. Preliminary results suggest that cartilage differentiation is decreased in no treatment condyles, and new cartilage consists of more scar-like fibrocartilage due to expression of *Col3*. The graft led to overgrowth of the mandibular condyle, a common complication in humans. The rate of bone growth also signals a similar timeline of recovery as the human. Results of this adult porcine model for costochondral graft mandibular condyle reconstruction suggests that the model is appropriate for human preclinical trials.

Research Grant: Laboratory for Equine and Comparative Orthopedic Research
Student Support: Student Support: Kenneth F. Burns Trust

Effect of insulin on the equine vascular endothelial glycocalyx

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Laminitis is a common and potentially devastating disease of horses. Laminitis primarily results from either sepsis or insulin dysregulation (hyperinsulinemia). There is extensive evidence that microcirculatory dysregulation is an important part of laminitis pathophysiology. Also, endothelial glycocalyx (EG) damage is increasingly being recognized as a pivotal contributor to microcirculatory dysregulation. We have already demonstrated that EG damage is occurring in some horses with sepsis. However, whether EG damage is occurring in horses with insulin dysregulation, remains unknown. The objective of the present study is to assess the role of insulin on the integrity of the EG in horses. We hypothesize that elevated insulin levels promote EG degradation. Intravenous glucose tolerance, oral sugar, and insulin tolerance tests were performed in 8 healthy adult horses. Blood samples were obtained at 0, 60 and 120 min for laboratory determination of: glucose, insulin, hyaluronan, and neuramindase-3 concentrations and neuramindase-3 activity. We anticipate that blood concentrations of measured EG components will increase following glucose and insulin treatments. Such results would imply that EG damage may be occurring in endocrinopathic (insulin-mediated) laminitis.

Research Grant: Animal Health Foundation of St. Louis
Student Support: Animal Health Foundation of St. Louis

***In vitro* effects of palmitoylethanolamide in a canine mast cell tumor cell line**

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High grade canine mast cell tumors (MCTs) carry a poor prognosis due to their high metastatic rate and overall aggressive phenotype. Palmitoylethanolamide (PEA) is an endocannabinoid ligand that has shown the ability to inactivate mast cells in allergic conditions. The objective of this preclinical study was to assess PEA's potential as a therapeutic option for canine MCTs by assessing cannabinoid receptor expression and evaluating PEA's cytotoxicity in the C2 canine mast cell tumor cell line. Western blot revealed the presence of TRPV1, but could not confirm the presence of CB1 or CB2 proteins. PEA's IC50 determined by MTT assay and fit by a variable slope Hill's equation was 102.6μM. When the IC50 was compared to vehicle control it resulted in a statistically significant ($P < 0.0004$) decrease in cell viability. PEA has demonstrated cytotoxic characteristics; however, further evaluation into its underlying mechanism of cytotoxicity and interaction with standard of care chemotherapy is needed.

Research Grant: Kansas State University Mark Derrick Canine Research Fund
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Determining Plasmid-Mediated Mupirocin Resistance in *Staphylococcus felis* and *Staphylococcus haemolyticus*

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Use of the topical antibiotic mupirocin over time has the potential to select for resistant staphylococcal bacterial populations. Low to moderate bacterial mupirocin resistance is commonly caused by mutation of the native *ileS* gene resulting in resistance to mupirocin. High-level resistance to mupirocin is usually conferred by the *ileS*-2 gene encoded on plasmids that could possibly transfer via conjugation between *Staphylococcus* species such as *Staphylococcus felis* and *Staphylococcus haemolyticus*, bacteria common to the skin microbiota of cats and humans respectively. The aims of this study were to determine the incidence of mupirocin resistance in isolates of *S. felis* and *S. haemolyticus* from veterinary patients and confirm whether resistance was due to the presence of *ileS*-2. *Staphylococcus* isolates from veterinary patients were identified using MALDI-TOF mass spectrometry, and those identified as *S. felis* and *S. haemolyticus* were streaked onto agar plates containing 8μg/mL mupirocin. PCR was performed on isolates that grew on mupirocin plates to determine if resistance was plasmid mediated or the result of endogenous mutation of the *ileS* gene. None of the 61 isolates of *S. felis* were resistant, and 4 of the 22 *S. haemolyticus* isolates were resistant, with all resistant isolates confirmed to carry the plasmid mediated *ileS*-2 gene. Although *S. haemolyticus* is common to the human skin microbiota, the resistant samples isolated form animals in this study suggest the potential for future transference of mupirocin resistance, however the results indicate that resistance has not currently affected the normal microbiota in cats.

Research Grant: FDA Vet-LIRN program U18FD006171
Student Support: NIH T35OD010991-17, Texas A&M School of Veterinary Medicine & Biomedical Sciences

Effects of nerve blocks using ropivacaine with or without morphine in dogs undergoing stifle surgery

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Opioid use in rats and humans has been shown to decrease the effectiveness of local anesthetics. The objective of this study was to compare the effectiveness of saphenous and sciatic nerve blocks using ropivacaine with or without systemic morphine in dogs undergoing stifle surgery. Clinically healthy dogs who presented to Midwestern University for stifle surgery were enrolled in the study and randomly assigned to one of two treatment groups: with morphine (WM) or without morphine (NM). Group WM received maropitant, acepromazine, and morphine as premedication, while group NM received maropitant, acepromazine, and saline. Anesthesia was induced with propofol and midazolam and maintained with isoflurane. All dogs received nerve blocks preoperatively and liposomal bupivacaine was infiltrated into the surgical site prior to closure. Intra- and post-operatively, group WM received morphine intramuscularly and group NM received a lidocaine infusion as rescue analgesia, if painful. Postoperative pain was assessed by using the short form of the Glasgow Composite Measure Pain Scale (CMPS-SF) and Electronic Von Frey apparatus (EVF). Pain assessment was performed by personnel who were blinded to treatment. Thus far, three dogs have been enrolled in the study (WM = 1, NM = 2). The dog in group WM required intra-operative rescue analgesia. In the NM group, one dog required intra-operative rescue analgesia, while the other dog did not. CMPF-SF scores collected up to two weeks post-surgery were 1-4 out of 24 in group WM and 2-4 out of 24 in group NM. EVF values measured up to two weeks post-surgery were 674 ± 241 grams in group WM and 529 ± 281 grams in group NM. Thus far, no patients have exhibited obvious pain post-operatively.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program and Federal Work Study

Investigating limb integument, osteology, and myology of the Aldabra giant tortoise (*Aldabrachelys gigantea*)

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The endangered Aldabra giant tortoise, *Aldabrachelys gigantea*, is recognized as the second-largest tortoise in the world and has endemic roots in the Aldabra Atoll of the Seychelles Islands. With an average weight of 550 pounds, Aldabra tortoises require certain adaptations of their integument and musculoskeletal system to facilitate adequate external protection and efficient locomotion. The published literature on tortoise hindlimb myology is not only extremely limited and outdated, but also quite variable from their terrapin counterparts. In our study, detailed anatomical dissections were completed to investigate the functional adaptations of the integument, bone, and musculature of the hindlimb apparatus of *A. gigantea* in comparison to other smaller tortoise species (*Centrochelys sulcata* and *Kinixys erosa*). As a novel hindlimb tortoise dissection study, the main objective of this research was to explore the muscular and tendinous relationships that exist in the thigh, crus, and pes of *A. gigantea*, as well as confirm the absence of a true "patellar" bone. Given the giant size of the Aldabra specimen, it was hypothesized that they possess larger and additional muscle bellies, along with more prominent fascial sheaths, to contribute to a powerful propelling step and to support their massive weight. Additionally, the morphological differences with respect to osteoderms in the giant Aldabra are hypothesized to be supported by their inability to fully tuck their large pelvic limbs into their shell, thus serving as an external protective mechanism.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program

The Ventral Surgical Approach to the Axillary Region of *Canis lupus familiaris*

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The brachial plexus is a network of nerves responsible for motor and sensory responses in the thoracic and pectoral muscles, because of this it is very important to ensure it remains intact. This study explores a ventral surgical approach to the axillary region in *Canis lupus familiaris* to avoid damage to the brachial plexus and preserve the function of the thoracic limb from the scapula and shoulder region down to the palmar surface of the paw. This was accomplished by making a semi-lunar-shaped incision beginning at the glenohumeral joint and ending at the first mammary gland; making the peak of the incision at the sternum near the 4th and 5th rib. Overall, our findings were successful in preserving the nerves and vessels in cadaver specimens. This project is continuing in to Fall 2022.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program (BIVSP)

Acute phase proteins in cats with classical bacterial, blood-borne bacterial and retroviral infections

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This study aimed to compare the plasma levels of serum amyloid protein (SAA) and α1-acid glycoprotein (AGP) among healthy cats, febrile infected cats, and afebrile cats with asymptomatic infections. Banked plasma samples of 211 cats previously submitted for physical examination, tested for Feline Immunodeficiency Virus (FIV) and Feline Leukemia Virus (FeLV), and common blood-borne bacterial infections (*Hemotropic Mycoplasmas* and *Bartonella spp.*) were used. Cats are divided into Healthy (n = 67), Afebrile asymptomatic (normal range temperature, no clinical signs of disease, carried one or more infectious agents [n = 49]), and Febrile (body temperature > 102.5 F, carried one or more infectious agents [n = 95]). The Febrile group was further divided into subgroups based on their confirmed or suspected diagnosis: Classical bacterial infection (n = 50), Blood-borne bacteria (n = 14), FIV/FeLV (n = 11), and mixed infections (n = 23). SAA and AGP concentrations were measured in plasma samples using ELISA. Acute phase proteins were compared using the Kruskal-Wallis test followed by Dunn's multiple comparison test. Results indicated that fever in association with classical bacteria, blood-borne bacteria, FIV/FeLV, or co-infections significantly increased ($P < 0.0002$) SAA and AGP when compared to health and afebrile asymptomatic cats. No differences were observed between the Healthy and Afebrile asymptomatic groups. In a nutshell, while plasma concentrations of SAA and AGP were increased on febrile infected cats, they are not capable of differentiating types of infection. For the first time our data shows that SAA and AGP are not reliable markers for asymptomatic infections with classical bacteria, blood-borne bacteria, or retroviruses.

Research Grant: Center for Integrative Mammalian Research, Ross University School of Veterinary Medicine; FONDECYT REGULAR 1191462, ANID, Chile
Student Support: Morris Animal Foundation

Serum biochemistry reference values for zoo-housed neonatal giraffe calves (*Giraffa camelopardalis*)

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Species-specific reference intervals are necessary for the proper interpretation of biochemical values in zoo-housed species. Reference intervals may vary between neonates and adults. Thus, the aim of this study is to provide age-specific reference intervals for biochemical values of neonatal zoo-housed giraffe (*Giraffa camelopardalis*). Serum biochemistry results were obtained by performing a retrospective survey of zoological institutions throughout the United States. Inclusion criteria included: giraffe calves born between January 2016 and May 2021 which were deemed healthy by the attending veterinarian, had samples collected via jugular venipuncture and placed into serum separator or lithium heparin tubes, taken at the time of routine neonatal health exams, and performed under manual restraint when calves were less than 72 hours old. Reference intervals will be calculated using the American Society for Veterinary Clinical Pathology's (ASVCP) consensus guidelines for determination of de novo reference intervals. It is anticipated that the results of this study will be a valuable tool for clinicians and allow for more precise interpretation of laboratory results.

Research Grant: Robert Devries Binder Park Zoo Professional Scholarship and the Binder Park Zoo Research Funds
Student Support: Boehringer Ingelheim and Michigan State University Graduate School

Histologic analysis of nano-plastic particles in freshwater mussels

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Recent studies show that microscopic plastics can be found in detectable levels in both the environment and in tissues of organisms near populated areas; however, their tissue-level effects in animals remain relatively unknown. This study serves to inform our understanding of the pathologic effects of plastic particles on freshwater bivalves, keystone species in North Carolina waterways. The species of mussels used in this study include wild-caught Elliptio complanata, Lampsilis radiata, and Villosa constricta. These individuals were allowed to acclimate to treated tap water for at least 36 hours before exposure to the plastic suspension. The plastic suspension consisted of 2.5mg/mL polyethylene terephthalate (PET) particles with a size of approximately 208nm per particle, suspended in bovine serum albumin (BSA). The experiment focused on three treatments, two with nanoparticles and one BSA control: 1) Three E. complanata, two L. radiata, and two V. constricta were placed in 495mL water containing 5mL PET suspension; 2) three E. complanata, two L. radiata, and two V. constricta were placed in 499.5mL water containing 0.5mL PET suspension; and 3) one E. complanata, two L. radiata, and two V. constricta were introduced to 499.5mL water containing 0.5mL BSA as a control. After six hours of exposure, all individuals were euthanized in 5g/mL buffered MS-222 for 18 hours, then were removed from the plastic suspension and placed in Davidson's fixative. Each individual was trimmed in cross-section and processed onto slides stained with hematoxylin and eosin. The histologic examination evaluated loss of cilia on the gill epithelia, and loss of absorptive cells in the digestive diverticula for each individual.

Research Grant: NCSU Veterinary Scholars Program and Boehringer Ingelheim-NIH, NC Department of Transportation and the National Wildlife Foundation; PET particles were provided by the Research Triangle Institute.
Student Support: NIH T35 Training Grant

Computed tomographic angiographic study of common carotid artery anatomic relationships in the dog

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Although the common carotid arteries and brachiocephalic trunk are essential to the arterial system in dogs, little is known about their "normal" anatomic pathway. Current research has focused on the variations of branching patterns of these vessels, but no study has yet quantified these vessels' spatial relation to each other and other anatomic structures that cross the thoracic inlet such as the trachea. Computed tomographic (CT) images from the Atlantic Veterinary College database were selected utilizing inclusionary criteria; the animal is a dog that received radiopaque contrast and underwent both thoracic and neck/cervical CT imaging on the same day between July 2nd 2020 - June 17th 2022. Dogs were excluded on the basis of having any lesions or if the animals positioning was perceived to alter the anatomic positioning of internal structures. Currently, 20 CT studies from dogs of various breeds are being evaluated utilizing open source software (https://horosproject.org) to evaluate brachiocephalic trunk length, inter-carotid distance, thoracic inlet height and width, and the vertebrae at which vessels are directly lateral and directly ventral to the trachea. Additional components of the software such as 3D image rendering and 2D orthogonal multiplanar reconstruction (MPR) are being utilized to increase the accuracy of measurements. Our hypotheses are that there will be variation between the vessel spatial distance and length, and that these variations can be detected applying the methods previously described. The data from this study may be utilized for surgical procedures and as an identification tool for anatomical variations which may be associated with pathophysiological conditions.

Research Grant: None
Student Support: Ptarmigan Foundation, AVC Veterinary Summer Research Award

A comparative proteomic analysis of BRD based on thoracic ultrasonographic and clinical assessments

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Bovine respiratory disease (BRD) is the second leading cause of death in pre-weaned dairy calves. A complication for BRD management is the absence of a gold-standard antemortem diagnostic test. Clinical Respiratory Scoring Charts (CRSC) and thoracic ultrasonography (TUS) are BRD diagnostics, however, previous research has noted discrepancies between these methods. Proteomic analysis is an established tool for identifying diagnostic biomarkers. The aim of this study was to identify common disease-associated proteins differentially expressed in calves diagnosed with BRD via TUS, CRSC, or both. In this cohort study, 60 calves were enrolled across two dairies in Washington. Thoracic ultrasonographic and clinical assessments were performed weekly through the first 12 weeks of life. Blood draws were performed biweekly, and 29 calves were selected for serum proteomic analysis based on the identified groups of interest (healthy, CRSC-/TUS+, CRSC+/TUS- and CRSC+/TUS+). TUS scores were assigned using a scoring system developed by Ollivett and Buczinski and CRSC scores were defined with the University of Wisconsin Calf Health Scoring Chart. Two serum samples were submitted for proteomic analysis from each diseased calf and age-matched healthy comparisons: a sample from the onset of disease and a sample obtained two weeks later. Statistical analysis of the proteomic data is pending; however, preliminary results indicate that three proteins (Apolipoprotein A-IV, CD59 glycoprotein, and an uncharacterized protein) were differentially expressed in diseased calves compared to healthy calves. These proteins are predominantly associated with the complement system and immune function, speaking to the role of the host immune system in BRD.

Research Grant: Agriculture and Food Research Initiative Competitive Grant no. 2019-68008-29897 & 2021-68014-34144 from the USDA National Institute of Food and Agriculture
Student Support: Washington State University Summer Veterinary Student Research Fellowship

Bile acids and their role in microbial control of phenotypic programing in utero

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Recent studies have shown a link between gut microbiome composition and myriad different phenotypes and conditions. In an effort to study the mechanisms by which gut microbial populations affect certain phenotypes, we examined two groups of mice of the same strain, populated with either a low- or high-richness gut microbiome (GM), designated GM1 and GM4 respectively. Mice with GM4 are lighter, more active, and display less anxiety-related behavior than their age- and sex-matched GM1 counterparts. Despite the fact that these distinct gut microbiomes can be reliably transmitted to fostered pups, the phenotypes associated with the GM of the birth dam remain into adulthood, an indication of fetal programing. Secondary bile acids (BAs) are one class of microbial metabolite currently under investigation for their role in this process. Prior data showed that bile salt hydrolase and primary and secondary BAs are greater in the cecal contents of GM1 mice. The objective of this study was to determine if there are also differences in serum BAs and expression of BA receptor TGR5 and downstream molecules. Data has shown that, on average, serum BAs are five times higher in mice with GM1 than GM4 and in mice cross fostered to GM4 than GM1, however the difference was not significant due to high variability. Expression of BA receptor TGR5 was significantly greater in the colon of GM1 mice and we expect to see that pattern upheld in the small intestine with resultant increases in GLP-1 and PYY, as well as downstream receptors GLP-1R and NPY2R in the brain. Together, these data suggest a mechanistic role of BAs in the fetal development of anxiety-related behavior and weight gain.

Research Grant: Research support provided by the MU Metagenomics Center
Student Support: Stipend for Cassandra Fletcher is supported by an endowment established by IDEXX-BioAnalytics

Mutations of SARS-CoV-2 spike protein and their impact on viral fitness with host ACE-2 receptors

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As the SARS-CoV-2 pandemic has swept across the world, we have seen many jumps of the virus into new species. With these jumps into different species, there has been concern for the virus to mutate within these species and then re-infect humans with the ability to cause more severe disease or to no longer be prevented by developed vaccines. Our goal is to find likely mutations in animal hosts and determine whether each makes the virus better at infecting humans. With this study we passaged the SARS-CoV-2 virus through cell culture that contained ACE-2 receptors from humans, cats, dogs, mink, and deer, species that are known to be susceptible to SARS-CoV-2. We then sequenced the virus and isolated mutations that occurred repeatedly or had been found in natural sequences. From there we decided to focus on two mutations within the spike protein. We developed primers to create these mutations via PCR fragments, which we then assembled by Gibson assembly. We then amplified the new fragment with replication cycle reaction (RCR) and will be confirming that we have the expected mutation with Sanger sequencing. Once the mutation is confirmed we will perform competition assays to determine if the mutation made this virus better able to infect through species specific ACE-2 receptors, including human ACE-2. With this information, we can better understand mutations that may arise within animal populations and predict those mutations that have a positive effect on fitness of the virus. This could help us know what variants are likely to spread efficiently from animal to human populations.

Research Grant: Research Grant: This project was supported by a One Health Seed Grant awarded to J.W-L
Student Support: Student Support: NIH T35OD011887

Assessing the impact of a hiding space on the behaviour and stress response of newborn dairy calves

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Allowing for the expression of natural behaviours and the incorporation of the natural environment into standard housing practices is important for dairy cattle welfare. One natural behaviour of dairy calves that has yet to be explored is their neonatal hiding instinct. The impact of allowing for this hiding behaviour on calf stress is unclear. The objectives of this study are to: 1) describe the hiding behaviour of indoor-housed dairy calves kept with their dam and provided a space to hide in the first week of life, and 2) assess the effect of a hide on physiological indicators of stress in calves using heart rate variability (HRV). A total of 12 cow-calf pairs housed at Dalhousie University's Ruminant Animal Centre will be used in this study. Each pair was randomly assigned to a treatment with or without a calf hide (n = 6 per treatment). For the first objective, continuously recorded video data from calves in the hide treatment will be analyzed to assess the hide use of the calves over the first week of life. For the second objective, HRV data collected from Polar heart rate monitors on the calves in both treatments will be compared to evaluate the impact of hides on their stress response. It is hypothesized that calves will use a hide when provided one and will progressively spend less time in the hide over the week. For the second objective, it is anticipated that calves provided a hide will have higher HRV, indicative of lower stress, compared to calves without a hide. This study will address the relationship between hide use and its effect on calf stress and will help guide producers on how to incorporate the allowance of natural behaviours to reduce stress and improve calf welfare.

Research Grant: Sir James Dunn Animal Welfare Centre
Student Support: Sir James Dunn Animal Welfare Centre Veterinary Summer Research Award

Mouse models of Charcot-Marie-Tooth Type 2E for therapeutic development

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Charcot-Marie-Tooth (CMT) is a group of inherited peripheral neuropathies with an incidence of approximately 1 in 2,500. Currently, there are four types of CMT: 1, 2, 4, and X. This project studies CMT2. CMT2 is a slow, but progressive disorder associated with axonal dysfunction and the deterioration of axonal connections and communication with muscles. Clinical manifestations include muscle weakness, hammer toes, high arches, loss of balance and coordination, and loss of sensation that progresses to the legs, arms, and hands. Subtype CMT2E is an autosomal dominant axonopathy caused by any one of over 30 mutations in the gene *NEFL* that encodes for the intermediate filament protein, neurofilament light (NF-L). NF-L is one of five subunits (NF-H, NF-M, NF-L, peripherin, and α-internexin) that composes neurofilaments and contributes to the cytoskeleton of axons. In addition to providing structural integrity, neurofilaments aid in axonal assembly and maintenance. Different *NEFL* mutations result in variable times of onset and typically lead to paresis and atrophy of the muscles in the distal lower limbs and gait abnormalities. However, some patients present with more severe clinical symptoms, including dyspnea, laryngeal weakness, and other respiratory complications. We are directing focus toward the human mutation E396K (E397K), a point mutation that alters amino acid 396 from glutamic acid to lysine (acidic to basic) in the ––––rod domain of the NF-L protein. In this study, we use a newly derived E396K mouse model in which the corresponding mutation was generated in the mouse *NEFL* gene. The objective of this study is to characterize the newly derived E396K model and develop a therapeutic strategy for the treatment of CMT2E.

Research Grant: This work is supported by a grant awarded to Dr. C. Lorson from the CMT Research Foundation.
Student Support: Student stipend support provided by an endowment established by IDEXX-BioAnalytics.

Melanoma antigen proteins (MAGEs) in equine melanomas

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The aim of this study is to investigate the expression of melanoma antigens (MAGEs) in equine melanoma. Melanoma is the most aggressive form of human skin cancer, of which incidence, in contrast to many other tumor types, still steadily increases. While novel treatments showed encouraging results, resistance to the therapy remains an existing burden, calling for better markers and therapeutic targets. Melanoma antigens (MAGEs) are proteins normally expressed only in testes but are often aberrantly activated in different types of human cancer, especially in melanomas - where they were initially discovered. They have attracted a lot of attention as targets of cancer immunotherapy; however, effective therapy still awaits discovery. MAGE expression is associated with a more aggressive type of disease and therapy resistance. Interestingly, equine melanomas represent a common malignancy in gray-colored horses, but, in contrast to humans, the disease is much more benign in these animals. The molecular underpinning responsible for species diversity in cancer malignancy is poorly understood. To address this question, we aim to determine the MAGE expression in equine melanomas. We will collect tissue samples from horses undergoing planned surgery or post-mortem. From the tissues, we will isolate RNA and measure MAGE gene expression by real-time quantitative PCR. The results of our study will lay the ground for comparative research of the MAGE function, which may offer yet unexplored insights into the mechanisms responsible for cancer aggressiveness and novel therapy opportunities for humans and horses.

Research Grant: TTU SVM start-up (to K.F.T.); Cancer Prevention and Research Institute of Texas Scholar Award RR200059 (to K.F.T.); Foundation for Prader-Willi Syndrome Research Grant 22-0321 (to K.F.T.)
Student Support: Boehringer Ingelheim Veterinary Scholars Program

A retrospective study on the detrimental effects of *Fasciola hepatica* (liver flukes) on small ruminants

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The parasitic trematode *Fasciola hepatica* is known as the common liver fluke. Infection in small ruminants and cattle is a known source of chronic liver wasting, economic losses, and decreases in animal welfare. *F. hepatica* invades the liver, causing subsequent inflammatory responses and often death. This notion is assumed for small ruminants, although no recent investigations have confirmed this. This study aims to determine common clinical signs, pursued diagnostics, outcomes of treatments, and autopsy findings of small ruminants with confirmed *F. hepatica infestation* and to determine the diagnostic efficacy regarding specificity and sensitivity of other testing methods. We hypothesize there will be an increasing trend of *F. hepatica* mortality in herds during seasons of optimal temperatures for completion of the parasite life cycle, more noticeable initial symptoms, and a correlation between histories of prior infection and disease prevalence. Medical records of cases of small ruminants that presented to the Louisiana State University Veterinary Teaching Hospital between 2012-2022 were assessed for confirmed infection. Out of 2,049 records, 15 confirmed cases were evaluated. The majority of *F. hepatica* infections (75%) occurred during the optimal period of February-July, with a mortality rate of 80%. Lethargy (64%), anorexia/emaciation (57%), dehydration (50%), and anemia (43%) were common findings among exams. Euthanasia was the most common treatment (60%), along with flunixin meglumine (47%), thiamine (33%), and albendazole (33%). Frequent necropsy findings included bile duct hyperplasia (92%), concurrent parasite infection (75%), and grossly observed trematodes (67%).

Research Grant: Louisiana State University School of Veterinary Medicine
Student Support: Kenneth Burns Trust, Louisiana State University School of Veterinary Medicine

Strain screening procedures for field isolates of the chronic wasting disease agent

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Prion diseases such as chronic wasting disease (CWD) in cervids and scrapie in sheep are fatal neurodegenerative diseases caused by an accumulation of misfolded prion proteins (PrPSc) in the central nervous system. CWD was first reported in mule deer in Colorado and Wyoming in 1967 and now has been found in 29 states, 2 Canadian provinces, the Republic of Korea, Norway, Finland, and Sweden. CWD isolates can be categorized into different strains based on characteristics that make them unique. Some strains are characterized by glycosylation profiles, incubation periods, genotypes affected, and regions of the brains with lesions. Prion protein gene (PRNP) polymorphisms are a major factor in determining the susceptibility of sheep to scrapie. Further investigations into cervid PRNP polymorphisms and the susceptibility to the CWD agent needs to be studied for effective surveillance and control programs in the future. The purpose of this study is to develop a method to identify and differentiate strains; to do this, we examined 44 field samples from U.S. farmed white-tailed deer. The brainstem was homogenized and used for enzyme immunoassay and sequencing of the PRNP gene. Out of the 44 samples, 35 were identified as positive for CWD, of which 31 were genotyped as wild-type WTD; we also identified 7 deer with PRNP polymorphisms at codons 95 and 96. Candidates identified for further testing based on PRNP results may be subjected to other assays to test biophysical properties such as fibril stability or mouse bioassay to test transmission characteristics. Being able to identify and differentiate CWD strains may play a significant role in future management and regulation of CWD in farmed cervids and wildlife.

Research Grant: Research Grant: USDA ARS NADC CRIS PROJECT: 320-228
Student Support: Student Support: USDA ARS NBAF AGREEMENT NO: ARS 59-3022-1-003

Osteocalcin is a possible biomarker for detection of musculoskeletal injury in racehorses

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Tools to predict and prevent catastrophic injuries in Thoroughbred racehorses are needed. Early-stage changes in fetlock joints, thought to lead to catastrophic injury, can be viewed using standing computed tomography (CT). To identify circulating biomarkers for early injury detection, blood samples were collected from 2-year-old Thoroughbred racehorses at 0 and 6 months during their first year of training, concurrent with standing CT imaging of the fetlock joints. Samples from 3 horses with minimal fetlock pathology (H) and 3 horses with severe fetlock pathology (P) at 6 months were selected for analysis. Markers of bone resorption (CTX-I), bone formation (osteocalcin), and inflammation (IL-1α, IL-1RA3, and TGFβ1) were evaluated with commercially available ELISA kits or by QPCR. Mean (± SD) CTX-I levels were 0.48 ± 0.18 ng/mL and 0.46 ± 0.08 ng/mL at 0 and 6 months respectively in H horses and 0.36 ± 0.16 ng/mL and 0.36 ± 0.02 ng/mL at 0 and 6 months respectively in P horses. There was a small significant difference between P and H horses at 6 months (P = 0.024) but no other statistically significant differences in CTX-1 levels. Mean (± SD) osteocalcin levels were not significantly different at 0 months between H (37.9 ± 11.8 ng/mL) and P (33.2 ± 17.8 ng/mL) horses (P = 0.67), however at 6 months, they were significantly lower (P = < 0.0001) in P animals (17.5 ± 5.5 ng/mL) than H animals (57.5 ± 6.9 ng/mL). Osteocalcin levels were significantly increased in H animals at 6 months (P = 0.002) but the noticeable decrease in P animals at 6 months was not statistically significant (P = 0.17). QPCR results are pending. In conclusion, osteocalcin shows promise as a biomarker of early fetlock injury. Further studies are needed for verification.

Research Grant: University of Pennsylvania School of Veterinary Medicine, Pennsylvania Horse Breeders Association
Student Support: NIH T35 OD010919, Boehringer Ingelheim

Tissue residue depletion of cannabinoids in cattle fed industrial hemp

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Anat. and Physiol. (B Fritz, Magnin, Curtis, Martin, Montgomery, Coetzee), Clin. Sci. (M Kleinhenz, Weeder, Leslie), Diagnostic Med./Pathobiol. (K Kleinhenz, Johnson), Diagnostic Lab. (S Fritz), College of Veterinary Medicine, Manhattan, KS and John C. Pair Horticulture Center (Griffin), Haysville, KS, Kansas State University

Legalization of industrial hemp (IH) cultivation has increased interest of its use in cattle feed. However, the safety of tissues from exposed cattle must first be established. The objectives of this study were to describe the cannabinoid tissue residue profile in cattle fed IH and to estimate withdrawal times. Twenty male Holstein cattle (n = 16 bulls, n = 4 steers, average 297 kg) received oral IH boluses at a target dose of 5.5 mg/kg/d cannabidiol for 14 d. Plasma was collected throughout the feeding period. On day 15, 16, 17, 19, and 22, four cattle were humanely euthanized and liver (L), kidney (K), muscle (M), and adipose tissue were collected. Cannabinoid content was quantified with ultra-performance liquid chromatography triple-quadrupole mass spectrometry. Adipose and plasma samples are still being analyzed. Withdrawal periods were estimated using the FDA WithdrawalApp in R Studio (99th percentile upper tolerance limit, 95% CI, tolerance = 10 ng/g). Whole tissue withdrawal periods and target tissues were selected based on the compound with the slowest depletion. In L, K, and M, Δ-9-tetrahydrocannabinol was detected in 8, 17, and 0 samples and cannabidiol was detected in 14, 18, and 10 samples, respectively. The compounds with the highest peak concentrations were cannabidiolic acid (L, K) and 7-carboxy-cannabidiol (M). The withdrawal times for L, K, and M were 68, 21, and 39 d, respectively. Liver was selected as the target tissue. Our results will inform future discussions regarding the inclusion of IH in cattle feed.

Research Grant: USDA National Institute of Food and Agriculture (Agriculture and Food Research Initiative); Kansas State University
Student Support: Boehringer Ingelheim Veterinary Scholars Program, Kansas State CVM Office of Research

Survey of protozoan parasites in coyote (*Canis latrans*) tissues from Northwest Oklahoma

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Coyotes (*Canis latrans*) are adaptable canids inhabiting a variety of habitats. Coyotes and domestic dogs share many internal and external parasites. In Oklahoma, several greyhound kennels use their dogs to hunt coyotes; this practice increases the risk of exposure for the hunting dogs and zoonosis potential for hunters handling coyote carcasses. *Babesia conradae* is an intraerythrocytic apicomplexan parasite previously identified in coyote-hunting greyhounds in California and Oklahoma. Symptoms of *B. conradae* infection in dogs resemble those of other *Babesia* spp., including anemia, thrombocytopenia, organomegaly, and rarely severe complications such as acute renal disease. The mode of transmission of *B. conradae* remains unknown, and aggressive greyhound-coyote interactions are a suspected route of infection. This study aims to analyze the presence of *B. conradae*, *Hepatozoon* sp., *Sarcocystis* sp., and *Toxoplasma gondii* in greyhound-hunted coyote tissues collected by hunters in Northwest Oklahoma. Between November 2021 and January 2022, samples from 30 coyotes were collected from an area with previous reports of *B. conradae* infection in greyhound dogs and stored at -20 °C until further processing. After DNA extraction and purification from tongue, liver, and lung tissues, four separate PCR reactions were performed to amplify a fragment of the 18s gene for *B. conradae*, *Hepatozoon* sp., and *Sarcocystis* sp., and a fragment of the B1 gene for *T. gondii*. Two samples were positive for *Hepatozoon* sp. and eight for *Sarcocystis canis*, with other results pending. This study will help to understand the *B. conradae* transmission cycle and the prevalence of other protozoan parasites that represent a risk to domestic animals and humans.

Research Grant: INTERACT-One Health
Student Support: Unknown

Effects of retaining feed on hopper pellet mycobiome and microbiome

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Rodents are essential to the advancement of biomedical research. However, feed, a cost associated with maintaining rodent colonies, can be a limiting factor for some research institutions. Reducing food waste has economic, labor, and environmental advantages for research institutions. While some institutions dump food during cage changes, others use a strategy of "topping off" food which reduces waste. It is unknown if the latter results in changes in environmental microbiome/mycobiome that may be detrimental to rodent health or alter research results. The objective of this study is to determine if food left in mouse hoppers past cage changes maintains a stable microbiome/mycobiome. C57BL/6J mice were fed either 5008 (non-irradiated food) or 5053 (irradiated food) *ad libitum* and housed in Thoren shoebox cages on individually ventilated or static racks with 3 to 5 mice per cage. Half of the cages had their food replaced at a two-week time point, while the other half followed a topping off strategy. Feed samples were collected on days 0, 7, 14, 21, and 28. One to two pellets from the bottom of the hopper, closest to the mice, were collected at each time point. DNA was extracted from the samples, amplified using 16S rRNA primers and sequenced. Following sequencing, data analysis is being performed to determine if there are significant differences in the mycobiome or microbiome among the different groups. We expect to find no significant differences in the samples from mice housed in any of the above conditions. These results would indicate that dumping food from mouse hoppers is unnecessary. This would suggest that institutions can reduce husbandry costs by not dumping food at cage changes.

Research Grant: Research is funded by NIH U42 OD010918, Mutant Mouse Research and Resource Center, the University of Missouri Office of Animal Resources, and an endowment established by IDEXX-BioAnalytics
Student Support: Grant from the American Society of Laboratory Animal Practitioners Foundation

Evaluation of novel renal biomarkers as predictors of post-anesthetic AKI associated with IOH in healthy dogs

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Healthy human patients receiving elective, non-cardiac surgeries have an increased risk of developing post-operative acute kidney injury (AKI) when they sustain intra-operative hypotension (IOH) with a mean arterial pressure (MAP) less than 50 mmHg for more than 10 minutes (Tang 2019) and less than 60 mmHg for more than 20 minutes (Sun 2015). Patients who develop AKI are more likely to develop long-term complications, such as chronic kidney disease (Rewa 2014), and have an increased mortality within 30 days of surgery (Kheterpal 2009). However, the development of AKI in association with IOH is poorly understood in veterinary patients. The objective of this study is to correlate the severity and duration of IOH with biomarkers measured pre and post-operatively in healthy dogs anesthetized for spay or neuter procedures with the Iowa State University College of Veterinary Medicine Summer Community Outreach Surgery Program. Blood and urine samples collected pre and post-operatively were analyzed to evaluate the following biomarkers: serum creatinine (sCr), blood symmetric dimethylarginine (SDMA), cystatin C (CysC), neutrophil-gelatinase-associated lipocalin (NGAL), gamma glutamyltransferase (GGT), urine specific gravity, and urine protein:creatinine ratio. It is hypothesized that increased severity and duration of IOH is associated with higher risk of AKI. Additionally, one or more of the renal biomarkers will provide early recognition of acute renal injury. Identifying and understanding anesthetic risks such as IOH severity and duration that result in post-anesthetic AKI can improve perioperative care and guide intervention and post-operative care and potentially decrease morbidity/mortality.

Research Grant: Veterinary Clinical Sciences Research Incentive Grant
Student Support: Boehringer Ingelheim

Parasite survey of Atlantic Canadian bats

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Atlantic Canada provides essential habitat for numerous bat species and populations. However, the culminating threat of various anthropogenic factors and infectious diseases has placed many of these species at risk. While surveillance and conservation efforts for North American bats have increased in recent years, the diversity and distribution of endoparasitic species have never been investigated within Atlantic Canadian bat populations. As the first endoparasite survey of bats ever completed in this region, this study aimed to determine the diversity and distribution of nematodes, trematodes, and cestodes within Atlantic Canadian bats. In order to identify and quantify helminth diversity within these bat populations, we performed necropsies on frozen bat carcasses collected between 2016 and 2022, as well as fresh specimens that became available throughout the summer of 2022 (n = 19). Parasites were recovered using small-scale organ parasite recovery procedures, which included placing the organs in saline and teasing them apart under a stereomicroscope. Organs sampled included: lungs, heart, trachea, esophagus, nasal turbinates, gastrointestinal tract, liver, kidneys, and urinary bladder. Preliminary findings include gastrointestinal trematodes found in 16 of the 19 bats examined, a nematode found in the nasal turbinates of one Newfoundland Little Brown Bat (*Myotis lucifugus*), and a trematode of the urinary bladder found in one New Brunswick Big Brown Bat (*Eptesicus fuscus*). These specimens have been preserved and will be identified as precisely as possible through morphological identification and PCR.

Research Grant: Canadian Wildlife Health Cooperative, Atlantic Region
Student Support: Canadian Wildlife Health Cooperative, Atlantic Region; AVC VetSRA Program

The effect of cannabidiol as adjunctive therapy for refractory idiopathic epilepsy in dogs

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Idiopathic epilepsy (IE) is the most common neurological condition in dogs, affecting up to 5.7% of the canine population. It is a chronic condition characterized by epileptic seizures of unknown origin. Standard treatment for seizures is the use of antiepileptic drugs (AEDs); however, these drugs are ineffective in controlling seizure activity in up to 30% of dogs. Cannabidiol (CBD) has been proposed as a potential adjunct treatment to AED therapy due to its anti-convulsant properties and reportedly minimal side effects. The primary aim of this study was to determine the impact of CBD on seizure activity in dogs with IE. Secondary aims included determining CBD plasma concentrations after being administered oral CBD, along with assessing the impact of CBD on concurrent AED concentrations. To be included, dogs were required to have a presumptive diagnosis of IE, with two or more seizures per month while receiving routine AED treatment. Out of the 502 patients screened, 61 met the inclusion criteria and were enrolled. In this double-blinded crossover study, dogs were randomly assigned to be given a CBD-infused oil (4.5 mg/kg BID PO) or placebo oil for 12 weeks, and then, after a 4 week washout period, given the opposite oil for an additional 12 weeks. Seizure activity, biochemical panels, plasma CBD concentrations, plasma AED levels, and adverse effects were recorded and will be compared between treatment groups. Although results from this study are not yet available, CBD as an adjunct therapy to standard AED therapy may be a safe and effective treatment for dogs with IE.

Research Grant: American Kennel Club Canine Health Foundation (AKC CHF)
Student Support: AKC CHF and McGrath Research Group

Direct threat assessment of African Swine Fever virus competent ticks, *Ornithodoros* spp., in Texas

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African Swine Fever virus (ASFv) is a re-emerging global swine disease that, if introduced to the U.S., would cause severe economic consequences. The widespread presence of feral hogs in addition to the presence of competent *Ornithodoros* spp. ticks, specifically *Ornithodoros turicata*, foster a greater risk of ASFv establishment, especially in Texas. The specific aim of this study was to determine the potential distribution of *Ornithodoros* spp. ticks in Texas, particularly along the U.S.-Mexico border and near large-scale swine operations. A systematic literature review and field collections were conducted to identify *O. turicata* localities. Ticks obtained from field studies were identified using standard morphological keys. Tick identifications were confirmed molecularly through amplification and sequencing of a 16S rRNA gene fragment. Ecological niche modeling was used to determine the suite of bioclimatic variables associated with the presence of *O. turicata*. Six variables of importance were identified: precipitation seasonality, the maximum temperature of the warmest month, the minimum temperature of the coldest month, the mean temperature of the wettest quarter, the mean temperature of the warmest quarter, and the mean temperature of the coldest quarter. Then, a map was constructed of the potential distribution of this species which stretched from southern California to Texas with an allopatric population in Florida. The majority of Texas with the exclusion of the easternmost quarter of the state appears to be highly suitable for this species. Establishing the current and projected distribution of *O. turicata* is essential to understanding the potential sylvatic cycle of ASFv and creating long-term surveillance zones.

Research Grant: Department of Homeland Security, Cross-Border Threat Screening and Supply Chain Defense, Center of Excellence at Texas A&M University
Student Support: Boehringer Ingelheim Veterinary Research Scholars Program, Texas A&M University

Development of multiple diagnostic molecular assay for *Anaplasma phagocytophilum* in cattle

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Molecular-based diagnostic testing such as PCR is a vital tool within veterinary medicine. It is capable of assessing animal and human pathogens, while also being efficient and cost-effective. PCR technology can diagnose common tick-borne diseases such as babesiosis, anaplasmosis, Crimean-Congo hemorrhagic fever, and Encephalitis virus. Higher prevalence of tick-borne diseases have become a growing concern for cattle farmers due to their significant economic impact. Although antibiotics and vaccines have helped reduce certain diseases, reinfection still poses a threat. Costly serological diagnostic approaches for screening also discourages farmers from using such preventative methods. Development of a molecular diagnostic tool catered for detection of tick-borne cattle diseases can help farmers minimize production losses and maintain herd health. Our lab designed and created a singleplex diagnostic test using gene-specific primers and probes that detect for the presence of *Anaplasma phagocytophilum*. We conducted the singleplex following a PCR on positive and negative cattle blood samples for *A. phagocytophilum*. Gel electrophoresis confirmed the singleplex positive results were indeed *A. phagocytophilum* and not another tick species. QPCR on positive cattle blood samples helped to evaluate the sensitivity of the gene-specific primers and probes. Using this data, our lab hopes to further develop the singleplex test for *A. phagocytophilum* into a multiplex test. The multiplex test would be a rapid single test that has the ability to identify *A. phagocytophilum* along with other common cattle tick species such as *Borrelia burgdorferi* and *Babesia bovis*.

Research Grant: Research Grant: United States Department of Agriculture (USDA)
Student Support: Student Support: CVM Veterinary Summer Research Program

Application of molecular diagnostics to investigate the prevalence of *Tritrichomonas* infection in cats

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Tritrichomonas is a protozoan parasite that is an important cause of chronic large-bowel diarrhea in domestic cats. Currently, the prevalence of *Tritrichomonas* has only been studied once in Northern California and found to be around 4%, however, no studies have focused on cats in shelter settings. Cats within multi-cat environments are at the highest risk for infection because many cats are often asymptomatic and shed trophozoites before being diagnosed, making the management of this disease difficult. Diagnosing infections is also challenging because the only readily available testing methods, fecal smears and InPouch fecal cultures, have diagnosis rates of around 14% and 55%, respectively, and no molecular test has been developed for clinical use. The aims of this study were to determine the prevalence of *Tritrichomonas* infections in cats from high-risk environments with the use of PCR and to optimize a diagnostic molecular test, both of which would improve the overall management of these infections. The hypothesis of this study was that molecular detection of *Tritrichomonas* would provide more sensitive detection as compared to fecal culture and that with this detection, the prevalence of infections would be greater in this area than previously determined. As of now, a total of 18 fecal samples and fecal cultures have been collected from 18 shelter-derived cats between the ages of 1 month to 2 years old, 14 of which being diarrhetic, by trained fosters. Both the fecal samples and fecal cultures were processed for DNA extraction with a QiaAmp DNeasy Blood & Tissue Kit, then run under nested PCR assays in two rounds to look for amplification products that aligned with the positive bovine *Tritrichomonas foetus* control.

Research Grant: Center for Companion Animal Health, UC Davis School of Veterinary Medicine
Student Support: Boehringer Ingelheim Veterinary Scholar Fellowship

Effective dose 50 of dexmedetomidine-midazolam for sedation of Buff Orpington hens

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Chickens are increasingly popular pets, leading to an increased need for routine veterinary care. Sedation in chickens has not been well investigated. Combination of several sedative drugs potentiates the sedative effect of each drug while decreasing individual drug adverse effects. This study determined the effective dose 50 (ED_{50}) of intramuscular midazolam and dexmedetomidine to induce sedation for clinical procedures. Twenty, 23-week-old, Buff Orpington hens were used in this randomized, non-blinded, clinical trial using an Up-and-Down study design. The first random chicken received 60 μg/kg of dexmedetomidine and 2.5 mg/kg midazolam; doses based on another study. At 10 min after injection, sedation was scored. The score of 1 was awarded to each parameter if 1) recumbent in the carrier, 2) recumbent for 10 seconds when placed on right lateral, 3) successful right jugular venipuncture, 4) tolerated ventrodorsal positioning for sham radiograph, and 5) tolerated right lateral position for sham radiograph. Conversely, negative responses were awarded 0. Animal was classified as "sedated" if total score of 3 or higher, and "not sedated" if 2 or lower. If the chicken was "sedated", the subsequent animal received 50% less of each drug, but if "not sedated", the doses were increased by 50%. A total of 4 contradictive responses between 2 sequential animals (crossovers) were identified. No side effects were noted. The ED_{50} of dexmedetomidine was 17 μg/kg and the midazolam was 0.64 mg/kg. The effective dose for sedation of 99% of population was predicted to be 89 μg/kg dexmedetomidine and 2.7 mg/kg for midazolam.

Research Grant: The Oklahoma State University Debbie and Wayne Bell Professorship in Veterinary Clinical Sciences and Department of Veterinary Clinical Sciences
Student Support: Oklahoma State University College of Veterinary Medicine

Investigating meloxicam inhibition of COX enzymes in Ball pythons (*Python regius*)

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Appropriate analgesia and anti-inflammatory drugs are a vital element in medical care. Despite reptiles significantly increasing in popularity as household pets, the information regarding non-steroidal anti-inflammatory drug (NSAID) pharmacology in snakes remains scarce. Elucidating cyclooxygenase inhibition by NSAIDs in snakes such as *Python regius* (*P. regius*) is a key step to improving and ensuring proper analgesic/anti-inflammatory treatment, especially given how frequently NSAIDS, such as meloxicam, are prescribed in private practice. Meloxicam has been studied in other species such as humans, dogs, cats, iguanas, and birds. We looked at the phylogeny and sequence resemblance between those species. *P. bivittatus* is the closest species to *P. regius* for which the COX genes sequences are publicly available. Sequence identity between python and human sequences are 72% for COX-1 and 79% for COX-2. COX isoforms were shown to be appreciably conserved among the vertebrate species studied, supporting the use of meloxicam to provide analgesia in reptiles such as *P. regius*. Our next step is to establish the meloxicam pharmacological inhibition characteristics in *P. regius* COXs. This will be done using a colorimetric enzymatic assay kit. COX enzymes will be isolated from 8 snake tissues and an inhibition dose response with meloxicam (and other NSAIDs) will be established in the tissue with the highest COX-1 and COX-2 expression. This will allow us to evaluate inhibitory constants on those two enzymes in snakes for the first time. Replication of these studies in different species within the class Reptilia will better direct the scientific and medical community in providing effective analgesic treatment for all reptiles.

Research Grant: None
Student Support: Western University of Health Sciences Office for Research, College of Veterinary Medicine

Characterization of cardiac phenotype in a novel cAMP reporter mouse

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Cyclic AMP (cAMP) is an important second messenger for intracellular signaling. In the heart, cAMP is involved in control of heart rate and contractility. To investigate cAMP signaling in the heart, a new cardiac-specific cAMP-encoded reporter (*CAMPER*) mouse was developed, which reports cAMP signaling with a fluorescence resonance energy transfer (FRET)-based biosensor. The sensor changes fluorescence upon cAMP binding. However, binding of the *CAMPER* sensor to cytosolic cAMP may cause buffering of this second messenger, which could impact cardiac function. Therefore, we hypothesize that buffering of cytosolic cAMP may lead to a baseline cardiac phenotype in the *CAMPER* mice. This is important to characterize, as this novel mouse model has the potential for use in various studies focused on cardiovascular disease. To test our hypothesis, we used echocardiogram (echo) and electrocardiogram (ECG) to assess cardiac function and electrophysiology of the *CAMPER* mouse hearts and compared these parameters to wild-type control mice (C57Bl6). Using M-Mode echo in the short-axis view, we measured wall thickness, cardiac output, ejection fraction, fractional shortening, stoke volume, and diastolic and systolic volumes of the left ventricle. Using the long-axis view, we measured diastolic and systolic area of the left ventricle. Using ECG, we measured the heart rate, PR, QR, and QT intervals, and the QTc of the CAMPER mouse hearts. Additionally, we conducted a stress test during the ECG using the beta-adrenergic agonist isoproterenol. Using these methods, we were able to characterize the electrophysiology and cardiac function of the *CAMPER* mouse heart, which can then be used to inform future studies with this novel mouse model.

Research Grant: NIH R01 HL111600
Student Support: NIH T35 Training Grant T35 OD010956

Evaluating infectivity of Spondweni virus and Zika virus in male reproductive cells

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Spondweni virus (SPOV; *Flaviviridae*) is a zoonotic arbovirus closely related to Zika virus (ZIKV). SPOV and ZIKV are transmitted through the bite of infected mosquitoes and cause fever, nausea, headache, and myalgia in humans. Similar symptoms and serological cross-reactivity result in misdiagnosis of these viruses. ZIKV can be sexually transmitted in human and mouse models and is reported to cause pathological damage in the male reproductive tract. ZIKV is detectable in ZIKV-infected patients' semen samples and infects sperm cells. In mice, the virus causes persistent infection of the epididymis, resulting in cell death and testicular atrophy. Despite genetic similarity to ZIKV, SPOV has a lower rate of sexual transmission in mouse models. Although research was conducted investigating which cell types in the male reproductive tract ZIKV infects, little is known on what cell types SPOV infects. We hypothesized that ZIKV replicates in male reproductive tract cells better than SPOV. GC-1 murine spermatogonia cells were selected and we predicted that infection of these cells with ZIKV will generate higher titers than SPOV. A growth curve at a MOI of 0.01 was performed and timepoints were collected for five days. Virus titers were quantified via plaque assays using Vero cells. In the murine spermatogonia cells, SPOV reached significantly higher titers compared to ZIKV. Our results suggest that there are differences in which cells SPOV and ZIKV infect. Further growth curves will be performed using different male reproductive cells to further assess viral infectivity. This investigation helps identify cells involved in sexual transmission which could potentially be used to target and prevent the spread of ZIKV.

Research Grant: National Institute of Health R21 AI142504
Student Support: NIH T35OD011887

Comparison of Embryonic and Endometrial Gene Expression of the Day 11 and Day 13 Post-Ovulation Mare

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Equine embryonic mobility is essential for the maternal recognition of pregnancy (MRP), and it is regulated by the conceptus's own derived prostaglandin secretion. Our objective was to compare prostaglandin-related genes (PRG) of embryonic and endometrial tissues in early pregnancy. We hypothesized that prostaglandin related-genes would be differently expressed in embryonic and endometrial samples of Day 11 compared to Day 13 post-ovulation. Mares were examined daily with transrectal palpation and ultrasound from estrus until the day (D) of ovulation (D0), and randomly assigned to the non-pregnant (NP) (n = 7) group or the pregnant (Preg) (n = 6) group. Mares assigned to Preg were bred using artificial insemination. On Day 11 or Day 13 post-ovulation, a uterine lavage was performed to retrieve the conceptus, and an endometrial biopsy was collected. Total RNA was extracted from the embryonic and endometrial samples and evaluated for expression of fourteen prostaglandin-related genes using Real-time PCR. Mean threshold cycle (Cq) was determined and then normalized to the reference gene (*GAPDH* and *ACTB*) (ΔCT). The endometrial expression of *PTGES* was higher in D11 Preg compared to D11 NP ($P < 0.04$), but not different in D13. Earlier embryos (D11) had higher expression of the following genes compared to D13: *PTGS2, PTGES, CBR1, mPGES2, PTGIS, PTGER4, PTGFS,* and *HPGD* ($P < 0.05$). However, *CREB, PTGFR,* and *SLCO2A1* were upregulated on D13 compared to D11 ($P < 0.05$). Understanding the embryonic and endometrial prostaglandin regulation during maternal recognition of pregnancy will help develop future therapies to ameliorate pregnancy loss in the horse.

Research Grant: Harry M. Zweig Memorial Fund for Equine Research
Student Support: The Cornell University College of Veterinary Medicine

Cardiac disease in small dogs: results from The Dog Aging Project

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For dogs, both age and size are important predictors of disease risk and mortality; smaller dogs tend to live longer than larger dogs. However, when individual cardiac diseases were grouped into and considered as one category, initial results from The Dog Aging Project via owner survey show higher reported prevalence of cardiac diagnoses with age in smaller dogs when compared to larger dogs. We analyzed specific diagnoses of cardiac disease within their respective eleven categories: arrythmia, cardiomyopathy, congestive heart failure, endocarditis, hypertension, murmur, pericardial effusion, pulmonary hypertension, pulmonic stenosis, subaortic stenosis, and valve disease. The hypothesis of this study is that purebred dogs of extreme size (both small and giant) have increased risk of developing a cardiac disease and that individual cardiac pathologies are dependent on size. Other studies investigating canine growth rates and lifespan have revealed correlations between increasing breed size and risk of developing cardiac pathology with increased risk of cardiac disease and associated mortality reported at both extremes of the body size spectrum in dogs. This study uses data from an online owner-reported Health and Life Experience Survey (HLES) providing a large cohort of 13,618 purebred dogs with 734 purebred dogs reported to have cardiac disease. We reevaluated this dataset at the level of individual diagnoses of eleven specific cardiac diseases of purebred dogs to better understand the bimodal distribution of cardiac diseases by size and the likely contribution of these diagnoses to morbidity and mortality risk by size and breed.

Research Grant: The Dog Aging Project is supported by U19 grant AG057377 from the National Institute on Aging, a part of the National Institutes of Health, and by private donations
Student Support: Cummings School of Veterinary Medicine at Tufts University

Validation of a species-specific probe-based qPCR for detection of *Setaria yehi* in Alaskan moose (*Alces alces*)

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Moose (*Alces alces*) are culturally and ecologically important animals across North America, with communities in rural Alaska relying on moose as a major source of food. Recently, wildlife biologists in Alaska are concerned about emerging cases of peritonitis in moose due to *Setaria yehi*, a mosquito-borne filarial nematode. Currently, detection of *S. yehi* infection is performed by modified Knott's test for detection and identification of circulating microfilariae (mf), or post-mortem examination of cervids for detection of *S. yehi* adults in the peritoneal cavity. The aim of this project is to test and validate a novel qPCR for detection of *S. yehi* in blood samples of Alaskan moose. Species-specific primers targeting a 78-base pair fragment of the cytochrome oxidase c subunit 1 (*cox1*) of *S. yehi* and a species-specific probe were designed. During protocol optimization, qPCR had a detection threshold of 1x10-6 ng/μL, and efficiency of 93.6%. A total of 166 blood samples were collected from moose kept at the Kenai Moose Research Center, and wild moose captured on the Kenai Peninsula, southern Alaska, from 2019 to 2022. Matching blood aliquots were tested by Knott's test and subjected to DNA extraction for subsequent qPCR. Quantitatively, blood samples had an average *S. yehi* microfilaremia of 472.2 mf/mL (0-14,490 mf/mL). Qualitatively, 32.53% (n = 54) of samples tested positive in each of the tests, and 37.35% (n = 62) when both tests were combined. Kappa statistic shows very good agreement between the results from Knott's test and qPCR (*kappa* = 0.90). The validation of this test allows for faster, less labor-intensive diagnosis and epidemiological surveillance for this emerging parasite in moose populations and other cervid hosts.

Research Grant: Verocai Parasitology Lab, Department of Veterinary Pathobiology
Student Support: Boehringer Ingelheim VSP Texas A&M University School of Veterinary Medicine

PGC1α overexpression preserves cardio-metabolic and skeletal muscle function in a type 2 diabetic mouse model

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Type 2 diabetes (T2D) is a common metabolic disorder in cats that is characterized by hyperglycemia due to insulin resistance and/or insufficient insulin production. Reoccurring hyperglycemia and insulin malfunction are tied to damage or failure of differing organs such as kidneys, nerves, vasculature, and skeletal muscle (American Diabetes Association, 2007). Skeletal muscle contains oxidative and glycolytic fibers; however, oxidative fibers are more insulin sensitive and resistant to fatigue. Overexpression of peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1α) is known to increase insulin-sensitive oxidative muscle fibers, but what remains unknown is whether it is effective at preventing cardiometabolic disease and skeletal muscle dysfunction of T2D. Our hypothesis is that overexpression of PGC1α will improve muscle performance by preventing fatigability, preserve glucose homeostasis, and protect against kidney dysfunction in a mouse model of T2D. Overexpression of adult PGC1α mice was obtained by crossing the MCK-PGC1α transgenic mice onto the db/db (obese T2D) background. Four mouse groups (lean control, obese control, lean PGC1α and obese PGC1α overexpression) were used to assess glucose homeostasis (plasma glucose, HbA1c, IGTT), muscle function (in vivo plantarflexion of gastrocnemius muscle), and fluid dynamics (via metabolic cages). Overexpression of PGC1α improves glucose homeostasis, decreases muscle fatigability, conserves fluid dynamics, improves blood glucose levels and renal function in the T2D models restoring them to a normal physiological state. Altogether, this data suggests that PGC1α is a novel therapeutic target for T2D and potentially other metabolic diseases.

Research Grant: NIA 5K01AG064121, NIGMS P30GM122744, OCAST HR21-045-1
Student Support: Oklahoma State University, Niblack Research Scholars

Detection of *Leptospira* diversity in the environment through enrichment culture and metagenomic sequencing

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Leptospirosis, caused by the spirochete bacteria, *Leptospira*, is a life-threatening disease in humans and animals and is one of the most widespread global zoonosis. Contaminated soil and water are the major source of transmission. In this study, we explored the usefulness of next generation sequencing when compared to traditional culture-based methods and PCR to detect *Leptospira* in water and soil samples. Direct PCR on DNA extracted from water and soil samples were positive for *Leptospira* genus specific *23S* and *16S* ribosomal gene targets but was negative for *lipl32* gene which is present in pathogenic *Leptospira*. *Leptospira* enrichment cultures followed by PCR and sequencing detected pathogenic and nonpathogenic *Leptospira* in soil and water samples. The pathogenic and intermediate groups of *Leptospira* were more prevalent in soil samples tested. The enrichment cultures sequenced displayed a large diversity in pathogenic *Leptospira* species. We detected the presence of 11 pathogenic species from the soil sample and 13 pathogenic species from the water sample. We propose that metagenomic sequencing on enrichment can be an ideal method in detecting the abundance and diversity of various *Leptospira* spp. in environmental samples. Soil samples in proximity to water may be an ideal niche for *Leptospira* growth and survival thus soil may be an appropriate sample of choice for testing.

Research Grant: UT College of Veterinary Medicine intramural funds
Student Support: UTCVM intramural grant

Application of eDNA metabarcoding to assess fish biodiversity in a legacy PAH contaminated stream

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Environmental DNA (eDNA) metabarcoding has emerged as a quick and cost-effective tool for monitoring biodiversity in aquatic ecosystems. The high throughput nature of this technology and improved sample efficiency makes eDNA an ideal alternative to traditional, more labor- and time-intensive environmental survey methods. eDNA metabarcoding can be used to simultaneously evaluate the effects of environmental stressors on the biodiversity of a wide variety of taxa and thus obtain proxy indicators of environmental and wildlife health. Polycyclic aromatic hydrocarbons (PAHs), a byproduct of fuel combustion, industrial manufacturing, coal-tar creosote operations, etc., are persistent carcinogens frequently found at contaminated sites. To evaluate the utility of eDNA metabarcoding in assessing PAH exposure effects on fish biodiversity, a study was conducted of the Little Scioto River near Marion, OH, a known creosote contamination site dating back to the 1800s. Extensive chemical monitoring and a series of traditional biological surveys, in addition to several remediation efforts by the US EPA, have taken place at this site over the past several decades. The objective of this study was to use eDNA metabarcoding to evaluate fish biodiversity of an upstream reference site and compare this with biodiversity at the PAH remediation site, as well as a downstream site to assess the impact of PAH contamination and the efficacy of remediation efforts 15 years later. Triplicate water samples were pooled from each site and filtered through cellulose nitrate filters. After filtration, eDNA was extracted from these filters, and samples were sequenced using a 12S MiFish primer metabarcoding approach to compare fish biodiversity among sites.

Research Grant: None
Student Support: NIH T35 Training Grant OD010977

Using machine learning models to predict age in cats using biochemical markers

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The application of machine learning (ML) models is expanding in many fields, including veterinary medicine. MLs' ability to identify patterns within large data sets provides the opportunity to enhance health by recognizing relevant features with efficiency. Access to reliable information about a patient, such as age, is one of the challenges faced by veterinary professionals. Increasing the predictability of age will aid clinicians in diagnosis, treatment, and ultimately improve patient outcomes. We explored whether ML models can predict age using chemistry profiles and complete blood counts (CBCs) from the domestic cat. We also examined the relationship between these blood panel values and age. Small animal chemistry profiles and CBCs were gathered retrospectively from the Scott-Ritchey Research Center's cat colony at Auburn University's College of Veterinary Medicine. The cats (n = 107), ranging from 6 weeks to 10 years of age, included in the study were deemed healthy in their medical records after physical examination by a veterinarian and had a small animal blood chemistry performed. Statistical analyses and ML models (lasso and elastic-net regularized generalized linear models, support vector regression, k-nearest neighbor, random forest, gaussian process regression) were implemented with the programming language R. We found that random forest best predicts age. The age of 67% of cats can be predicted within one year accuracy. Our findings support the clinical application of ML models to predict age from feline blood chemistry panels. Further investigation can expand age prediction capabilities to other species and explore the influence of disease on biological age.

Research Grant: Boehringer Ingelheim Veterinary Scholars Program, Auburn start-up funds.
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Seeking intestinal inflammatory biomarkers in equine feces as a diagnostic modality for colitis

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There are few reliable diagnostic modalities for large colon inflammation in horses. Fecal biomarkers have been utilized to quantify intestinal inflammation in humans but not assessed in horses. The objective of this study was to validate commercially available enzyme-linked immunosorbent assay (ELISA) kits for the detection of the inflammatory biomarkers myeloperoxidase (MPO) and calprotectin (CP) in equine feces. ELISA kits validated for detection of MPO and CP in equine serum were used. Seventeen fecal samples were each processed to produce a supernatant that was then analyzed along with a paired serum sample. Assay validation steps included intra- and inter-assay variability, dilution linearity, spike recovery, and sample type correlation. Intra-assay coefficients of variation were 10.4 - 31.4% for CP and 0.8% - 34.7% for MPO. Inter-assay coefficients of variation were 54.8 - 62.5% for CP and 19.9 - 147.3% for MPO. Sample dilution resulted in linear measurements for MPO ($P = 0.001$) but not CP ($P = 0.27$). Spiking of fecal samples resulted in percent recovery of 64.2 $\pm$ 66.8% for CP and 360.5 $\pm$ 107.8% for MPO. There was a significant difference between serum and fecal samples for both CP ($P = 0.03$) and MPO ($P < 0.001$). There was no significant difference for fecal CP, fecal MPO, or serum CP for comparisons between sick and healthy horses, but sick horses had a higher serum MPO ($P < 0.001$). Limitations of the study include a small sample size and lack of a gold-standard test for comparison. However, these results demonstrate that the current commercially available ELISA kits for MPO and CP cannot be used reliably with equine feces and therefore this approach is not a valid diagnostic modality for equine colitis.

Research Grant: 598 AQHF 105599
Student Support: T35 OD011145

Mobile gait analysis: evaluating mobile applications for kinematic analysis in dogs

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The use of kinematic gait analysis provides veterinarians with a way to objectively assess joint motion in dogs with orthopedic disease. With the increased accessibility of mobile kinematic applications, a comparison with more professional gait analysis software is needed. The objective of this study was to compare kinematic measurements obtained by two different mobile applications (M1:OnForm and M2:Dartfish Express) to those obtained using a computerized software application (K1:Kinovea). We hypothesized that mobile application values would be comparable to the computerized software. Data was obtained from 5 dogs and evaluated by 3 investigators. Thoracic and pelvic limb kinematic data was obtained from dogs during a trot and walk in a defined collection space with markers applied to the skin. Optical video was recorded at 60 Hz on an iPhone camera. For all dogs, maximum joint extension and flexion as well as overall joint range-of-motion were recorded in triplicate for all major appendicular joints using each program. Comparisons were performed with an ANOVA and a Tukey test. All tests were two-sided with $P < 0.05$. Preliminary results from 4 dogs recorded at a trot revealed significant differences between joint angles measured by M1, M2, and K1. No differences were found between tarsal angles. In all other joints, significant differences were found between M1 or M2 as compared to K1. Differences between M1 and M2 were only found in the stifle. In most dogs, smaller measurements were obtained from mobile apps. Overall, these preliminary results demonstrate differences in joint angles obtained from mobile applications as compared to computerized software. Further research is warranted.

Research Grant: None.
Student Support: Dr. Natalie Rabiner, alum of the University of Missouri College of Veterinary Medicine.

Translational study of metabolite signatures in trauma: identifying novel therapeutic targets using lipidomics

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Traumatic injuries account for a large proportion of presentations for care in both human and veterinary patients. Many trauma patients experience a complicated recovery leading to co-morbidities and long-term hospitalization. In humans and dogs, traumatic injury causes a profound activation of the innate and adaptive immune responses. Oxylipin metabolites are known to be immunologically active, playing a role in pro-inflammatory, anti-inflammatory, and resolving activity. Investigation of a suite of small molecules from the oxylipin category of metabolites in patients with traumatic injury may elucidate their role with regard to bone trauma, healing, and disease processes relating to traumatic injury. Initial investigation has been completed in 11 human trauma patients, and this study seeks to examine canine trauma patients as well as elective surgical patients to compare outcomes across species. Blood samples from 11 canine, and 11 human patients with traumatic cruciate ligament rupture presenting for surgical repair are taken pre- and post-repair. Bone marrow is sampled from the affected bone at the time of surgery. Samples will be analyzed by ultra-high-performance liquid chromatography-tandem mass spectroscopy (UPLC-MS/MS). Lipidomic analysis consisting of principal component analysis (PCA) and random forest will be performed. It is expected that the oxylipin component of the plasma metabolome after repair of long bone fractures will reveal relevant clinical biomarkers to improve patient care and outcomes. Comparison of canine and human lipidome responses will be made to further evaluate canine trauma as a natural model for improving trauma outcomes.

Research Grant: College of Veterinary Medicine and Biomedical Sciences College Research Council Grant 2022
Student Support: Alex Woken, Olivia Uzan, Jennifer Baughman, and Ashia Ellison

The effect of NSAIDs on concentrations of cytokines and growth factors in PRP and APS preparations in horses

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Autologous blood-based therapies, such as platelet-rich plasma (PRP) and autologous protein solution (APS), are frequently used intra-articularly and intra-lesionally for management of musculoskeletal injury in the horse. These substances have been shown to promote healing through their immunomodulatory and anabolic properties by concentrating leukocytes and platelets that release cytokines and growth factors after activation. These same horses with musculoskeletal injury are often also managed with non-steroidal anti-inflammatories (NSAIDs) to decrease inflammation and pain. It is possible that the administration of NSAIDs prior to obtaining blood samples for processing may result in a less effective product due to the documented effects of NSAIDs on platelet function. NSAIDs have been shown in both horses and humans to affect the concentrations of cytokines and growth factors in blood-derived autologous substances such as PRP. However, there is no peer reviewed literature evaluating the effects of administration of NSAIDs on PRP and APS in the horse. Therefore, the objective of this study is to determine the effects of commonly used NSAIDs, phenylbutazone and firocoxib, on the concentrations of growth factors and cytokines in PRP and APS during and after NSAID administration. We hypothesize that the administration of firocoxib and phenylbutazone will decrease the concentrations of clinically important anti-inflammatory cytokines and growth factors in PRP and APS preparations, and that these concentrations will return to normal values after a one-week washout period. This study will help to guide practitioners in optimal timing for obtaining and processing blood derived products after NSAID use in horses.

Research Grant: Zoetis, the Raymond Firestone Trust Research Grant, Boehringer-Ingelheim, and the University of Pennsylvania
Student Support: NIH T35 Training Grant – 5T35OD010919-25

Characterization of genes involved in host specificity of *Salmonella enterica* serovars Typhimurium and Typhi

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Typhoid fever is a disease specific to humans caused by infection with the bacteria *Salmonella enterica* serovar Typhi. Due to the strict host-specificity of the bacteria, the mouse models available to study typhoid fever are limited to humanized mice, which are expensive and time consuming to develop, or the use of the related bacterium *Salmonella enterica* serovar Typhimurium (*S*. Tm) that results primarily in gastroenteritis. This study aims to characterize the function of select genes present in *S*. Tm and absent in *S*. Typhi to better understand the involvement of these genes in host specificity. We generated *S*. Tm strains with deletions for one or more genes in the *ripABC* operon (*STM3117-3121*) and used an *in vitro* assay to assess their relative ability to resist the antimicrobial compound itaconate, which is generated by host macrophages. Preliminary results show no change in growth between *S*. Tm wildtype (WT) and *S*. Tm Δ*STM3117*, suggesting that this gene may not play a critical role in itaconate resistance. Additionally, we conducted a competitive index study, coinfecting mice with equal amounts of WT and Δ*STM3117* mutant *S.* Tm. The spleen, liver, cecum contents, and feces were collected at day four post infection and CFUs counted for each strain. The ratio of *S*. Tm Δ*STM3117* to WT CFUs was 0.379, 0.273, 0.820, and 0.520 for spleen, liver, cecum, and feces, respectively. These results suggests that the gene *STM3117* may still provide an advantage for the infection and replication of *S*. Tm in the mouse. By better understanding the function of genes implicated in Salmonella host-specificity, we hope to contribute to the development of a typhoid fever model through alterations in the host-pathogen interface.

Research Grant: NIH grant R01AI112949
Student Support: University of California-Davis SVM Endowment Funds

IgY and vitamin D3 Combo prevent S. aureus internalization by HC11 cells

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Staphylococcus aureus is a ubiquitous gram-positive opportunistic pathogen of the mammary tissue. Infection by Staphylococcus aureus within the mammary tissue causes inflammation leading to mastitis. Therefore, the bacteria are shed in the milk and hinder its production. The most cost-effective treatment to date is slaughter. This study aims at exploring an alternative method of controlling mastitis in-vitro by determining the treatment efficacy of combined IgY (IgY) and vitamin D3 (C) on HC11 mouse mammary epithelial cells. Ten treatment concentration combinations were evaluated: Negative control, Positive control, IgY 5μg/mL, IgY 5μg/mL C 20nM, IgY 5μg/mL C 50nM, IgY 5μg/mL C 80nM, IgY 10μg/mL, IgY 10μg/mL C 20nM, IgY 10μg/mL C 50nM, and IgY 10μg/mL C 80nM. The most effective treatments in internalization reduction were IgY 5μg/mL C 50nM and IgY 10μg/mL C 80nM (97%), and IgY 10μg/mL C50nM (96%). These findings provide data on future non-antibiotic treatments for mastitis.

Research Grant: DHHS/HRSA D34 HP00001-35-00 and NIH/NIMHD RCMI U54 MD007585
Student Support: : Boehringer Ingelheim Veterinary Service Program

Role of Osteocalcin in Glucose Homeostasis During Diabetes Mellitus

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Osteocalcin is a peptide hormone produced by bone osteoblast cells that contributes to bone mineralization. In addition to bone function, osteocalcin can stimulate insulin secretion from pancreatic β-cells. Furthermore, diabetic patients develop osteoporosis at a higher rate than healthy ones suggesting a role for osteocalcin in the etiology of these two diseases. Nevertheless, how pancreatic β-cells respond to osteocalcin under hyperglycemia remains unclear. The goal of the study was to quantify insulin secretion from β-cells in response to osteocalcin under elevated glucose conditions. We also obtained insight into its mechanism of action by performing real-time intracellular calcium recordings in single cells and RT-qPCR analysis to examine the expression of genes involved in the insulin secretion pathway. We hypothesize that insulin secretion will be decreased/or absent in the presence of osteocalcin under elevated glucose conditions, along with downregulation of one or more genes involved in the insulin secretion pathway. The results showed that 1000pg/mL osteocalcin stimulated intracellular calcium increases in INS-1 and RINm5F cells and under hyperglycemic conditions, insulin secretion was decreased. It was also shown that under low concentrations of osteocalcin, gene expression decreased during hyperglycemic conditions, while under high concentrations of osteocalcin gene expression decreased during both normoglycemic and hyperglycemic conditions. Overall, our results show that osteocalcin plays a vital role in glucose homeostasis and our lab will work further to deduce the mechanism of action.

Research Grant: None
Student Support: NIH T35 Training Grant

Effects of prostatic extracellular vesicles (EVs) on canine frozen-thawed spermatozoa function and viability

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Reproductive success after artificial insemination (AI) with frozen-thawed (FT) semen is highly variable in dogs. Even the most effective freezing methods result in impaired sperm function which negatively impacts fertility. Cryoinjuries to sperm include damaged plasma membranes, increased membrane fluidity, and premature acrosome exocytosis which results in reduced longevity and viability. It has been shown that addition of prostatic fluid (PF) to FT canine sperm had a positive effect on motility and resulted in larger litter sizes and improved conception rates after AI. Extracellular vesicles (EVs) are nanoparticles secreted by all cells that mediate cell communication via their cargo. EVs produced by prostatic cells may play a physiological role in canine sperm function. In this study our specific aim was to reverse the effects of cryoinjury to FT sperm with the addition of EVs isolated from PF collected from young dogs. Frozen-thawed semen from 3 young and 3 old dogs were extended in media without EVs (Tx 1), with EVs (Tx 2) and with PC12 (Positive Control) and incubated at 37°C. Samples were removed at 0, 1, 3, 6 and 24h, assessed for sperm motility using microscopy/CASA, and sperm viability (PI), acrosome integrity (FITC-PNA), and membrane fluidity (MC540) using flow cytometry. To assess sperm function, a perivitelline membrane (PV) binding assay was developed for canine sperm. We found that the addition of EVs to FT canine semen reduced both membrane fluidity and the proportion of sperm that bound to the PV membrane. In conclusion, the addition of PF EVs to FT canine semen improved longevity and function. Therefore, addition a of PF EVs may improve the reproductive performance of FT canine semen.

Research Grant: Center for Companion Animal Studies
Student Support: Colorado State University Veterinary Summer Scholars Program

The recombinant zona pellucida vaccine induces ovarian shutdown and leukocyte infiltration in jennies - prelim

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A: Even though zona pellucida (ZP) vaccines have been successfully used as a contraceptive method in over of 80 species , the mechanism of action of this vaccine remains unknown. Primarily it was believed that ZP vaccines would avoid sperm ZP binding and fertilization; however, these vaccines have been associated with ovarian dysfunction. This study aimed to assess the effects of recombinant zona pellucida (reZP) vaccines on ovarian dysfunction and histology in jennies. M: Fifteen reproductively sound jennies randomly assigned to control (n = 3) and treatment (n = 12) received three treatments 35 days apart (Treatment; 250μg ZP3 and 250μg ZP4) or a placebo (Control; Lactated ringers) and were monitored weekly by transrectal ultrasound. A left flank ovariectomy was performed in the treated jennies once no follicles ≥10mm were observed for ≥three consecutive weeks, with concurrent ovariectomy of controls. Ovaries were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5μm thick, and stained with hematoxylin and eosin for histological evaluation. Two sections from each ovary were evaluated in nine jennies (Treated, n = 7; Control, n = 2) for the presence of follicles and inflammatory cells. R: Jennies treated with reZP stopped cycling 146 ± 9.9 days after the first vaccination. Leukocyte infiltration was observed in the treated group in secondary (4 jennies) and early tertiary (1 jenny) follicles. Only primordial follicles were observed in the other two treated jennies. Late tertiary follicles and no inflammatory cells were observed in the controls. C: Preliminary results suggested the reZP vaccine induced ovarian shutdown in jennies through an immune response and subsequent inhibition of follicular growth.

Research Grant: The research conducted was funded by an internal grant through the Center for Conservation Medicine and Ecosystem Health (RUSVM 43003-2020)
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Developing a multiplexed immunofluorescence assay for COVID-19 and tuberculosis infected rhesus macaque lungs

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Despite decades of ongoing research, single cell data within the microenvironment of granulomatous inflammatory reactions to *Mycobacterium tuberculosis* (Mtb) remain largely a mystery. Similarly, because of its recent emergence and rapid mutation, SARS-CoV-2 is still a major pandemic around the globe and remains a highly studied pathogen. To continue seeking novel information about these diseases, new modalities must be created and validated. With relatively new and improving methods like multiplexing and tissue-based cyclic immunofluorescence, single slides of formalin-fixed paraffin-embedded tissue can be stained with dozens of markers at a time to elucidate new information on regional transcriptomics, immunophenotypes, and cellular interactions. Because of its success in cancer research, adapting this imaging method for Mtb and SARS-CoV-2 infections in lab animals could be an integral step in continuing to develop and test vaccines and therapeutics for these pandemic diseases. We developed and validated a 15-marker multiplexed immunofluorescence panel to immunophenotype immune cells in lung, spleen, and lymph node of rhesus macaques as a function of geospatial proximity to pathological regions of interest. This panel includes anatomical markers (alpha-SMA and pan-CK), immune cell markers (CD4, CD8a, CD11b, FOXP3, CD20, CD206, CD68, CD16, IDO-1, PD-1, and NCAM), and pathogen markers (Mtb and SARS-N). The slides were imaged with a Pannoramic MIDI II scanner (Epredia) and evaluated using HALO Software (Indica Labs). This panel will be used to evaluate lung specimens from rhesus macaques experimentally infected with either Mtb or SARS-CoV-2 to understand vaccine responses and drug efficacy for these infectious diseases.

Research Grant: NIH R01 (Rengarajan/Aldridge) and Martinot Co-I on NHP TB Granulomas
Student Support: NIH T35 Grant

A retrospective evaluation of oral clonazepam premedication on capture stress in captive zoo animals

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Clonazepam is a benzodiazepine that is commonly used to treat panic disorders, anxiety, and seizures in humans. In a variety of animals, clonazepam may be used as an oral sedative, but the use of this drug as a premedication for zoo animals prior to injectable anesthesia is not well documented in the literature. The objective of this retrospective case control study was to determine if oral premedication with clonazepam resulted in noticeable sedation prior to darting or injection for anesthesia. It was hypothesized that a sedation strategy using oral clonazepam would result in improved outcomes in darting and injection by causing sedation via level of calmness noted prior to induction of anesthesia. Oral clonazepam was recorded in 33 different anesthetic events in 11 species with 38 species matched controls. All cases and controls used were based upon the presence of a documented activity level of the animal in the record prior to darting or injection. Of those patients treated with clonazepam, 30.3% (10/33) were considered calm based on the scoring system whereas 29% of untreated animals were considered calm. There was no significant difference in activity between the clonazepam treated and untreated groups ($P > 0.99$). It was concluded that although the results of this study do not demonstrate significance, this is likely due to the limitations of the retrospective study design, not the efficacy of clonazepam. Further prospective studies need to be conducted to properly assess the pharmacodynamics of clonazepam on captive zoological species.

Research Grant: Research Grant: None
Student Support: Student Support: National Institutes of Health T35 Training Grant

Effect of panobinostat on NETs formation and apoptosis in canine neutrophils

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It is known that cancer cells affect and are affected by their surrounding microenvironment and alterations can have system-wide consequences related to coagulation and inflammation. One example is the overzealous formation of neutrophil extracellular traps (NETs) from activated neutrophils which may facilitate cancer progression and metastasis. Panobinostat, a histone deacetylase inhibitor, is an adjunctive anti-cancer therapy used in people to reduce tumor progression by promoting histone acetylation, thereby, reducing oncogene expression in cancer cells. NETs represent a potential therapeutic target for cancer therapy, however, the effects of panobinostat on NETosis remain unclear. Therefore, we hypothesize that, in a dose-dependent manner, panobinostat modulates *in vitro* NETosis in activated canine neutrophils by decreasing histone citrullination and promoting apoptosis. Isolated neutrophils from 6 healthy dogs were pre-treated with either 0, 2.5, 5, 10nM panobinostat before activation with either 100nM phorbal myristate acetate (PMA) or 1.9nM A23187. PMA and buffered-treated cells served as controls. After activation, nucleic acids were stained using SYTO green and SYTOX orange followed by fluorescence microscopy to quantify NETs. Panobinostat pre-treatment inhibited PMA (P = 0.0002) and A23187 (P = 0.0015) induced NET formation in a dose-dependent manner. Significant modulation was found with 10nM panobinostat pre-treatment for PMA (P = 0.007) and A23187 activation (P = 0.007). To further delineate the effects of panobinostat on histone citrullination and apoptosis, Western blot and flow cytometry will be utilized. Our current results demonstrate panobinostat is a potent inhibitor of NETosis in canine neutrophils.

Research Grant: Center for Companion Animal Health, UC Davis School of Veterinary Medicine
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Creation and validation of PCR primer sets to amplify two T cell receptor genes in research chicken lines

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The adaptive immune system is the one of, and arguably the most important, defense an organism utilizes to fight invading pathogens and cancers. A critical portion of adaptive immunity are T cells; cells capable of analyzing foreign antigens and allow antigen-specific immune response. With the wide diversity of known and unknown pathogens T cells must in turn diversify themselves, specifically, the receptor regions on the T cell. Two methods to diversity T cell receptors (TCR); from within the individual and from generation to generation, are gene recombination (VDJ recombination) and the TCR germline. VDJ recombination occurs within somatic cells themselves, giving rise to multiple variations in TCR to interact with as many pathogens as possible. TCR germline diversity creates evolutionary pathways in future generations; however, environmental pressures select specific TCR that are viable for said individual's environment, in this case, able to control specific pathogens. As we develop treatments and controls for pathogens and cancers it is essential to understand what TCR genes have high affinity to associated antigens. For the purpose of identifying TCR diversity between individuals and within a population polymerase chain reaction (PCR) is an affordable and practical technique to replicate DNA. In this project, we will be developing PCR amplicons to be used in a next gen sequencing assay, identifying all potential variations in the variable (V) and joining (J) sequences within the TCR Alpha and Beta genes in 3 inbred chicken lines.

Research Grant: None
Student Support: $8500 stipend was supported by USDA ARS NBAF AGREEMENT NO: ARS 59-3022-1-003

Exploring antimicrobial peptides as novel mRNA therapeutics for bovine trichomoniasis

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Bovine pathogen *Tritrichomonas foetus* (Tf) causes great loss of life and profit on beef farms, and no FDA-approved treatment exists. To address this, a novel mRNA therapy for trichomoniasis is proposed based on transfection of bovine cells with mRNA of antimicrobial peptides (AMPs) that may reduce Tf viability. Our objectives were to 1) test the effect of synthetic AMPs on Tf viability, 2) confirm transfection of bovine cells with mRNA, and 3) test the effect of supernatants and lysates of AMP mRNA-transfected cells on Tf viability. Tf (200,000/ml) were treated with 20 µM BMAP-28 or D-hecate for 0, 0.5, 2, 6, 12, and 24 h and assessed for viability at each point by examining motility using microscopy, metabolism via CellTiter-Glo 2.0 viability assay, and recovery from AMP treatments. Bovine kidney (BK) cells were treated with 1 µg/well of GFP or nanoluciferase mRNA in a 24-well plate to confirm transfection and subsequently with 0.5 µg/cm^2 of truncated BMAP-28 (Syn-1) or D-hecate mRNA in T25s for 24 h to generate supernatants and lysates. Tf (200,000/ml) were treated for 0, 0.5, 2, 6, 12, and 24 h with the products and assessed for viability. Marked, rapid, and sustained decrease in Tf viability showed upon exposure to synthetic BMAP-28, but not synthetic D-hecate nor products of AMP-transfected cells. Lack of D-hecate effects may be due to inhibition by FBS in media and lack of transfection product effects may be due to low AMP expression. Future work should confirm effects of BMAP-28 on Tf viability and optimize use of D-hecate and AMP mRNA-transfected cell products. This research could lead to development of a trichomoniasis therapy by utilizing AMP mRNA on bovine reproductive epithelium for treatment or prevention.

Research Grant: NIH T35 OD010432
Student Support: NIH T35 OD010432

Using infrared thermography to detect teat tissue changes after machine milking in dairy cows

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Mastitis is among the costliest diseases affecting dairy cows, partly due to its permanent reduction of the quantity and quality of milk produced. Most mastitis cases involve pathogenic organisms entering the cow's mammary gland through the teat canal. The teat canal has natural defenses against these pathogens that can be disrupted during milk harvesting. Some of these disruptions of the teat tissue morphology, also known as short-term changes (STCs), can be diagnosed through visual inspection. Infrared thermography (IRT) has previously been shown to produce precise and consistent measurements of skin temperatures on cows' hind teats. We hypothesized that STCs of the teat tissue could be identified using IRT; which, could lead to an automated monitoring system. One investigator recorded the presence or absence of STCs and obtained thermographic images of both hind teats from 147 cows before and after machine milking. Average teat skin temperatures were determined at the proximal, middle, and distal aspects. The teat skin temperatures were higher post-milking, potentially due to friction with the milking unit. Receiver operator characteristic curve analyses for the difference (pre-milking minus post-milking value) and the relative change (compared with the pre-milking value) in average temperatures yielded area under the curve values of ≤ 0.53. Our results show that average teat temperatures pre-milking and post-milking as assessed with IRT were unable to discriminate between the presence or absence of STCs of the teat tissue.

Research Grant: Cornell Initiative for Digital Agriculture (CIDA), Cornell
Student Support: NIH T35 OD010941 and Cornell University College of Veterinary Medicine

What is a veterinary desert? The first steps toward a definition

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The concept of a desert describes a mismatch between need and access to, or availability of, resources. Interest in understanding limited access to care in veterinary medicine is growing. Yet, the concept of veterinary deserts remains ill-defined. This project is the first step in a larger study that aims to define and frame healthcare deserts in a veterinary setting using the San Diego Humane Society's service area to formulate, pilot, and refine the definition of veterinary desert for publication. We performed a systematic literature review targeting publications from 2000-2021 relating to limited accessibility to food, education, and human and veterinary medicine. We also examined non-peer-reviewed resources relating to access to care in veterinary medicine. Publications were categorized according to concepts and key words relating to deserts and social disparities. Common themes identified included low income, inferior transportation access, reduced access to veterinary care education, and high demand for and short supply of veterinary professionals. Based on these results, our working definition of a veterinary desert is "a geographic area with a greater than average number of low-income individuals with pets who are unable to receive accessible, affordable, and adequate veterinary care due to financial, educational, or demographic limitations". The next step is to pilot and refine this definition of a veterinary desert by applying it to communities in San Diego. The final definition will provide a standardized method for researchers and care providers to identify communities in need of increased access to quality veterinary care and provide targeted efforts to alleviate social disparities amongst pet owners.

Research Grant: Western University of Health Sciences College of Veterinary Medicine/San Diego Humane Society-Residency Program in Shelter Medicine resident research funds to Bunke
Student Support: Western University of Health Sciences Summer Research Fellowship Program

Immune responses to radiation and myeloid cell-targeted therapy in spontaneous canine sinonasal carcinoma

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Sinonasal carcinoma (SC) is an aggressive cancer in humans and dogs. We are conducting a canine SC trial to test our hypothesis that stereotactic body radiotherapy (SBRT) and myeloid cell-targeted drugs (propranolol, losartan) will reduce immunosuppression in the tumor microenvironment, leading to better outcomes. Six canine SC patients were randomized into SBRT or SBRT + propranolol/losartan (SBRT-PL) treatment. Tumor biopsies and nasal lavage samples were collected 2-weeks post-SBRT. Immunohistochemical (IHC) quantification of tumor infiltrating immune cells and flow cytometric analysis of immune cells collected via nasal lavage was performed. Two dogs (n = 1/group) had tumor IHC and nasal lavage data available. The immune cell densities of the tumor treated with SBRT were 7.33% macrophages, 3.97% regulatory T cells (TRegs), 1.56% T cells, and 0.04% B cells; the tumor treated with SBRT-PL was 14.16% macrophages, 1.07% TRegs, 0.78% T cells, and 0.03% B cells. The percentages of immune cells collected via nasal lavage for the dog treated with SBRT were 94.25% neutrophils, 0.25% macrophages, 2.46% monocytes, 0% Tregs, 0.87% CD8 T cells, 0.01% CD4 T cells; for the dog treated with SBRT-PL, 49.50% neutrophils, 47.6% macrophages, 0.33% monocytes, 0% TRegs, 0.06% CD8 T cells, 0.37% CD4 T cells. The SBRT-PL-treated tumor had a greater density of macrophages and lower densities of TRegs and T cells compared to the SBRT-treated tumor. The dog treated with SBRT-PL had a lower percentage of neutrophils and a greater percentage of macrophages in the lavage sample compared to the dog treated with SBRT. This trial is ongoing, more results are being collected to study the immunomodulating effects of this combination for treatment of SC.

Research Grant: College Research Council, CSU Research Program; Colorado Head and Neck Cancer SPORE (P50 CA261605); Developmental Research Program; CU-CSU Joint Pilot Projects: Companion Animals in Cancer Research
Student Support: NIH T35 Training Grant T35OD015130

Evaluating the survivability of *Mannheimia haemolytica* on potential fomites exposed to low-level UV-light

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Mannheimia haemolytica is a commensal organism of the bovine respiratory tract commonly isolated from cattle with clinical respiratory disease. There are sparse data on the environmental survivability of *M. haemolytica* and no current information evaluating the effects of ultraviolet A (UVA) and ultraviolet B (UVB) light on the environmental survivability of *M. haemolytica*. The objective of this study was to evaluate the survivability of *M. haemolytica* on metal, wood, and plastic in controlled microenvironments exposed to one hour of UVA/UVB light. Microenvironments were constructed at approximately 69°F (20.6°C) using plastic containers, a UVA/UVB lamp, and UV-light sensors. Microenvironments were monitored for temperature, humidity, and UVA/UVB light exposure. Stainless steel, wood, and polypropylene spheres were inoculated with *M. haemolytica* and cultured for the presence of bacteria at 0 m, 30 m, 1 h, 2 h, and 4 h after inoculation. The UVA/UVB light exposed spheres were exposed to approximately 143 μmol m^{-2} s^{-1} of UV-light over the course of the one-hour post-inoculation while control spheres were kept in a UVA/UVB light free microenvironment. The presence of *M. haemolytica* was confirmed using visual identification of bacterial colonies on blood agar plates after incubation in 5% CO_2 for 24 hours. In both UVA/UVB light exposed and control microenvironments, cultures were positive for *M. haemolytica* at all time points. These results demonstrate that one hour of low-level UVA/UVB light exposure may not impact the environmental survivability of *M. haemolytica.*

Research Grant: The project was funded by internal funds from the Texas A&M University System.
Student Support: Boehringer Ingelheim VSP, Texas A&M School of Veterinary Medicine & Biomedical Sciences

Visualization of bartonellae via RNAscope Z-probe in situ hybridization

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Bartonellae are gram negative, intracellular, zoonotic, alphaproteobacteria that cause persistent erythrocytic infection in many hosts. Some diseases, including feline endomyocarditis and left ventricular endocardial fibrosis (FEMC-LVEF), are suspected to be associated with bartonellae infections, but verifying causation is difficult due to the paucibacillary nature of these lesions and the high rate of infection in seemingly healthy animals. Current tests are unable to visualize the small amounts of bacteria within normal or diseased tissues. RNAscope Z-probe in situ hybridization technology enables visualization of single sequences within cells through hybridization and amplification. With this study, a Z-probe to enhance detection of bartonellae in situ will be identified and validated in both pluribacillary and paucibacillary tissues. Three probes were designed, one each to target the *23S rRNA*, *porin* and *rpoB* genes of *B. henselae*. Each was tested to determine their sensitivity and specificity on cell pellets infected with either *B. henselae*, *Ehrlichia canis*, *Brucella melitensis*, or *Rickettsia rickettsii*. The 23S rRNA probe was the most sensitive, with the most intense staining and identifying the greatest number of foci. While the 23S rRNA probe cross reacted with B. melitensis, the bartonellae porin and rpoB probes were more specific. The probes were then tested on formalin-fixed, paraffin-embedded tissues to determine effectiveness in situ. The 23S rRNA probe was also the most sensitive probe when assessing tissues. The use of RNAscope technology will improve the pathological diagnosis of bartonellosis and enable insight into better understanding the pathogenesis of paucibacillary bartonellosis.

Research Grant: Morris Animal Foundation
Student Support: Georgia Veterinary Scholars Program; Boehringer Ingelheim, UGA College of Veterinary Medicine

Viability of *Melissococcus plutonius*, the causative agent of European foulbrood, on beekeeping materials

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European foulbrood (EFB) is a significant bacterial disease affecting honey bee larvae. The causative agent, *Melissococcus plutonius*, causes severe infections which weaken the colony and may lead to collapse. The economic losses associated with treatment, labor, and decreased pollination during EFB outbreaks are estimated to exceed $22 million. Current treatment recommendations, focused on disease control and preventing transmission to other hives, are limited by a lack of information regarding the viability of *M. plutonius* in the environment. Oxytetracycline hydrochloride (OTC), a bacteriostatic antibiotic, has been used for the treatment of EFB since the 1950's, and the development of resistance is a concern. The aim of this study is to evaluate the viability of *M. plutonius* on common surfaces involved in beekeeping. Materials used in the study include wax foundation, wood, steel, honey, and cloth. These materials are commonly transferred between hives, and the associated risk of EFB transmission is not well understood. These substrates were inoculated with two known strains of *M. plutonius*: a regional "atypical" strain isolated from a sick hive, and a "typical" ATCC 35311 strain. To evaluate viability over time, cultures were performed at seven time points, including a freeze/thaw cycle. We anticipate that the atypical, more virulent, strain of *M. plutonius* will remain viable longer than the typical strain. We predict survival will be longer on porous surfaces, such as wood and cloth. Understanding the viability of *M. plutonius* on these surfaces will inform biosecurity, treatment, and prevention recommendations to reduce morbidity and mortality by EFB.

Research Grant: None
Student Support: Boehringer Ingelheim and the Graduate School at Michigan State University

OO-MY! Seeking novel anti-oomycete treatments to aid patients with life-threatening pythiosis

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Pythium insidiosum is the major cause of human and animal pythiosis, a historic disease with emerging importance. *P. insidiosum* is an oomycete that is found in the plants and soil associated with aquatic environments. It enters its host through ingestion of contaminated water or breaches in the skin. The primary clinical signs of *P. insidiosum* infection tend to be gastrointestinal or cutaneous depending on the route of zoospore entry. In animals, pythiosis can lead to severe clinical signs that result in amputation of an affected limb or euthanasia. Treatment options are limited because *P. insidiosum* is not a fungus and therefore generally unresponsive to clinically available antifungal drugs. Immunotherapy, which involves injection of the patient with an extract of *P. insidiosum*, has shown some success. Pythiosis cases have been reported across the globe from Thailand to India, Brazil, Australia, and the United States. In the U.S., most cases occurred in animals in the southeastern states of Texas, Florida, Louisiana, Alabama, and Mississippi. However, pythiosis cases were documented in northern states during summer months, suggesting that *P. insidiosum* may be adapting to other climates. This potential for more-widespread emergence of *P. insidiosum* prompts further evaluation of current experimental resources to develop anti-oomycete treatments. Resources include genome sequences, libraries of chemical compounds to screenfor anti-Pythium activity, and examination of approaches used to control oomycete diseases in agriculture. Leveraging these resources will aid in the development of new treatment approaches for pythiosis and ideally result in a better prognosis and longer life for infected humans and animals.

Research Grant: None
Student Support: Office of the Director, NIH, T35 OD011145

Development of novel *in vitro* cultivation methods for *Ancylostoma caninum* stages

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Ancylostoma caninum, an intestinal hookworm nematode of dogs, is demonstrating anthelmintic resistance. Hence, *in vitro* studies are needed to evaluate new drugs. The study hypothesis was that heat-inactivated bacteria would support the development of *in vitro* stages of *A. caninum*. The study aim also included different culture conditions to evaluate the development of *A. caninum* from eggs through to infective larval stages. Study conditions included using Slide-A-Lyzer Dialysis Cassette, cell culture flasks, varying concentrations of amphotericin B, Super Optimal Broth, low melt agar with porcine-derived gelatin, and antibiotic-antimycotic solutions in the presence and absence of heat-inactivated *E. coli*. Cultures were observed microscopically for larvae development, movement, and viability. Larvae do develop in the presence of heat-inactivated *E. coli*. In addition, dialysis cassettes larvae were more active than flask cultures. In the substrate support media, larvae were predominantly found in the air pockets. These results suggest that the larvae will migrate towards areas of greater gas exchange. Furthermore, the optimal larvae temperature development was 27°*f*. The low antimycotics concentration cultures experienced increased fungal growth, resulting in larvae trapping and death whereas the higher amphotericin B concentrations suppressed the growth of the fungus without affecting the development of *A. caninum*. These outcomes provide information on the supplements and conditions for *in vitro* cultivation of *A. caninum*.

Research Grant: Duncan Alexander Research Funding
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Generating reference intervals for marine species in Species360 Zoological Information Management System

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Hematology is an essential diagnostic tool in veterinary medicine. To be fully utilized, reference intervals calculated from healthy species representatives are required for comparison to patient results. These intervals may vary significantly by species, and an independent study must be conducted for each one. While much attention has been devoted to reference intervals for traditional pets and production animals, many nontraditional species are still lacking reference data. This study aims to use historical data from multiple Species360 member aquariums, a global collaboration, to produce reference intervals for several aquatic species. Data is entered into ZIMS electronic medical records, and when sufficient results are collected, a global reference interval can be calculated based on the American Society for Veterinary Clinical Pathologist guidelines. This reference data can then be used in practice by veterinarians and animal care staff. Over the course of the project, novel global reference intervals or basic statistics were created for various species of pinnipeds, cetaceans, and elasmobranchs, as well as multiple local reference intervals for participating institutions.

Research Grant: None
Student Support: Van Sloun Foundation

Histological microanatomy of the broad-snouted caiman female cloaca

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Details of the female cloaca and male phallus interactions during crocodilian reproduction leading to fertilization are unclear. Evidence of female cryptic choice regulating crocodilian reproduction makes the study of female cloaca gross and microanatomy vital in understanding copulatory function. Previous research shows that the intromitted and inflated male broad-snouted caiman (*Caiman latirostris*) phallic glans physically interacts with the female uroproctodeal fold and compresses the female clitoris while the inseminating glans tip projects toward the vagina openings. Therefore, we histologically studied these structures to infer biomechanical properties and functions during copulation. Further, we digitally 3D reconstructed the cloaca gross anatomy from MRI scanning to understand deep tissue architectures. These results move toward a better understanding of crocodilian reproduction; knowledge that is crucial for conservation efforts in captivity and the wild.

Research Grant: None
Student Support: Stephens College

Pharmacokinetics of transdermal flunixin meglumine in North American bullfrogs (*Lithobates catesbeianus*)

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Amphibians are commonly maintained under human care and routinely require analgesia for painful conditions or procedures. Despite this, evidence-based data regarding optimal amphibian analgesics remains scarce. A novel pharmacokinetic study of commercially available transdermal flunixin meglumine in marine toads (*Rhinella marina*) demonstrated promise with rapid absorption and presumed therapeutic plasma concentrations, but investigation in other amphibian species, particularly frogs, has not been performed. The objective of this study was to assess the pharmacokinetics of transdermal flunixin meglumine in North American bullfrogs (*Lithobates catesbeianus*). Twenty-one clinically healthy adult bullfrogs (9 male, 12 female) were enrolled. Frogs were removed from water, weighed, and administered topical flunixin meglumine (3.3 mg/kg) on a dried area of the dorsum under manual restraint. Frogs were maintained out of water for four hours and randomly assigned to two of the following venipuncture timepoints: 1, 2, 4, 8, 12, and 24 hours, with seven samples collected per time point. Blood was collected from the right (first sample) or left (second sample) popliteal sinus, centrifuged, and the plasma separated and frozen. Plasma samples were then individually analyzed by ultra-performance liquid chromatography-tandem mass spectrometry to quantify flunixin meglumine concentrations. All frogs tolerated drug administration with no mortality. Pharmacokinetic data is pending. Results of this study will guide analgesic management of amphibians, specifically frog species.

Research Grant: None
Student Support: AVMA/AVMF 2nd Opportunity Research Scholarship and Triangle Community Foundation

Role of Hypothalamic PACAP and its Cognate PAC1 Receptor in Regulating Energy Balance via Homeostatic Circuits

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Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) and its PACAP-specific (PAC1) receptor are reported to be involved in regulating energy homeostasis. Previous studies have shown that PACAP neurons can depolarize proopiomelanocortin (POMC) neurons within the arcuate nucleus (ARC) of the hypothalamus. In vivo, direct PACAP administration into the ARC inhibits food intake and increases energy expenditure. Given this, we will test the hypothesis that PACAP inhibits orexigenic neuropeptide Y (NPY) neurons within the ARC, by activating PAC1 receptors and KATP channels. The last arm of this project will examine how in vivo ablation of PACAP neurons in the hypothalamic ventromedial nucleus (VMN) affects both energy intake and energy expenditure. We further posit that VMN PACAP neurons will inhibit NPY neurons since they act in a reciprocal fashion to anorexigenic POMC neurons, and that a knockdown of its PAC1 receptor will negate the effect in these NPY neurons. Moreover, we predict that complete ablation of PACAP neurons will cause aberrations in normal energy intake and expenditure. We found that both bath application of PACAP1-38 as well as photostimulation of VMN PACAP neurons causes a significant inhibition of NPY neurons due to direct stimulation of the PAC1 receptors and KATP channels. The inhibitory effect of PACAP was markedly blunted upon PAC1 receptor knockdown. We further found that ablation of these VMN PACAP neurons causes an increase in both energy intake and body weight, coupled with a more transient decrease in energy expenditure. Overall, the findings demonstrate that anorexigenic PACAP neurons exert an inhibitory effect on NPY neurons due to direct stimulation of PAC1 receptors and KATP channels.

Research Grant: PHS Grant DA024314 and intramural funding from Western University of 742 Health Sciences
Student Support: Boehringer Ingelheim

Evaluation of a Swine Outreach Program directed to Small Producers and Non-Swine Veterinarians

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The emergence and rapid appearance of Foreign Animal Diseases (FADs) are a constant threat to animal and human health. The swine industry specifically is under constant threat of introduction of diseases including African Swine Fever (ASF), Foot and Mouth Disease, and Classical Swine Fever. Even though several national and local efforts occurred in the past years to support swine producers in preventing and detecting these diseases, the focus has been mostly on large commercial production, but not in small-scale production. The main objective of this study was to use data collected from small producer outreach sessions to identify potential knowledge gaps within this population and to assess the effectiveness of the program in educating about FADs and biosecurity concepts. A total of 31 participants attended five sessions, the average (± SD) age of participants was 43.36 (19.62), and the mean number of pigs owned by participants was 51.2 (126.61). Approximately 48% of participants reported having a veterinarian-client relationship, but only called the veterinarian as needed. A total of 97% of participants indicated they had heard of ASF, however, only 29% of participants felt that they understood important aspects of the disease. This is a concern for the US swine industry because it indicates that there could be potential delays in early identification of the disease and timely implementation of control measures in an outbreak situation. After the seminar, 71% of participants reported that they understood a lot about ASF, which was reflected on knowledge-based questions. These initial results indicate a possible positive impact of the outreach program in the small producer and non-swine veterinarian community.

Research Grant: Ohio Pork Council
Student Support: Epperson Summer Research Fund

Effects of long-term cannabidiol administration in dogs

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Interest in the use of cannabidiol (CBD) to treat epilepsy, inflammation, anxiety, and other conditions in veterinary patients is increasing. However, the long-term tolerability of CBD supplementation in dogs remains unknown. Based on previous research, chronic administration of CBD is expected to cause mild gastrointestinal events, as well as an elevation in alkaline phosphatase (ALP). Cannabidiol in a carrier oil was administered at a 0 (control, just carrier oil), 5 and 10 mg/kg doses to 18 dogs (n = 6) in a complete randomized design to determine the effects of long-term CBD administration (ongoing study). Monthly physical and blood exams were conducted to monitor dog health. Adverse events including abnormal fecal scores were recorded. Liver enzymes data were analyzed as repeated measures over time using the GLIMMIX procedure in SAS (v 9.4). Dogs receiving the 10 mg/kg dose had higher ALP values than the placebo, and the ALP values of the 5 mg/kg CBD were similar to the extremes ($P < 0.05$). Other blood parameters were within normal reference ranges and the dogs appeared clinically healthy. Additionally, all treatment groups had abnormal fecal scores, with dogs dosed with 10 mg/kg CBD having the most episodes of loose stool. The elevation of serum ALP values in dogs administered CBD without changes in other liver enzymes may indicate that no observable hepatocellular damage is occurring. However, further studies investigating hepatocellular integrity in response to CBD administration are needed to fully understand CBD's impact on hepatocellular activity.

Research Grant: Unknown
Student Support: Unknown

Comparative Respiratory Disease Diagnosis with Sequential Thoracic Ultrasound and Clinical Signs in Calves

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Bovine Respiratory Disease (BRD) impacts dairy profitability and animal welfare. Historically, diagnosis and treatment have been based on clinical respiratory signs (CRS). Thoracic ultrasonography (TUS) offers a supplemental diagnostic tool for BRD with purported enhanced sensitivity and specificity. This study investigated the quality of CRS performance relative to TUS throughout BRD progression. Sixty Holstein dairy heifers were enrolled (18-25 d old) on two dairies in central WA. On-farm treatment records were collected, and calves were assessed weekly to 11-wks of age for CRS and consolidation via TUS. Calves were categorized weekly as non-diseased (Unconsolidated or Recovered) or diseased (Pre-, Onset, or Chronic consolidation) based on the sequence of TUS findings. Receiver Operating Characteristic curves compared CRS in non-diseased versus diseased calves using TUS as a gold standard qualifier and the sum of CRS as the unknown qualifier. Results indicated that lobar consolidation at Onset had the strongest alignment with CRS (AUC = 0.77). Pre- (AUC = 0.67) and Chronic consolidation (AUC = 0.66) showed weaker alignment indicating limitations for using CRS for preventive treatment or evaluations of treatment success. Of the calves that were treated for BRD, 41% (7/17) were treated after the onset of consolidation. Overall, 58% (15/26) of diseased calves recovered with or without treatment. Although a single TUS assessment did not provide a comprehensive overview of lung health, TUS did improve detection of untreated BRD suggesting that TUS might help improve treatment timing and retreatment options. Further research aims to assess associations between nasal pathogens and severity of disease based on TUS and CRS.

Research Grant: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2019-68008-29897 & 2021-68014-34144 from the USDA National Institute of Food and Agriculture
Student Support: Washington State University College of Veterinary Medicine Summer Research Fellowship

Evaluating the effects of histotripsy-treated canine osteosarcoma on monocyte phenotype

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Osteosarcoma (OS) is the most common primary bone tumor diagnosed in dogs. Surgical resection of primary tumors followed by adjuvant chemotherapy has been shown to prolong the median survival time (MST) in dogs with OS, but the MST for canine OS has not greatly improved in the last three decades, with most patients still succumbing to metastatic disease. Substantial research efforts have thus sought to improve survival times in canine OS, with the integration of immunotherapy recently procuring newfound interest. Histotripsy, a focused ultrasound tumor ablation technique, is currently being investigated for its immunomodulatory potential in canine OS. Histotripsy delivers high-intensity pulses via custom ultrasound transducers capable of inducing acoustic cavitation within targeted tissue. This acoustic cavitation mechanically emulsifies targeted tissue without the production of heat, thereby preserving vital tumor antigens for immune recognition. Subsequent antitumor immune responses could act to delay or inhibit metastatic progression, thereby improving prognoses for canine OS patients. This study aimed to characterize the immunomodulatory effects of histotripsy-treated D17 canine OS cells co-cultured with primary canine monocytes. Using flow cytometry analysis, CD80 expression in primary canine monocytes exposed to histotripsy-treated OS cells was lower than controls while CD62L expression was negligible. Ongoing work includes co-culturing DH82, a canine macrophage-like cell line, with histotripsy-treated OS cells for further immunophenotypic analysis. Collectively, our data provide the foundation to design follow-up studies to further evaluate the use of histotripsy as an immunotherapeutic for canine OS.

Research Grant: American Kennel Club Canine Health Foundation
Student Support: NIH T35OD011887

Furosemide-induced dilation of pulmonary veins as a prophylactic for exercise-induced pulmonary hemorrhage

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Horses undergoing intense exercise are often diagnosed with exercised-induced pulmonary hemorrhage (EIPH). As a preventative, veterinarians routinely administer furosemide (Lasix™), prior to exercise. The use of Lasix™ is controversial since its mechanism of action is incompletely understood. Our research is aimed at elucidating pulmonary mechanisms by which furosemide is protective against EIPH. We hypothesized that furosemide induces dilation of pulmonary veins, potentially through inhibition of the Na+, K+, Cl- cotransporter, NKCC1. Pulmonary veins (2-4mm diameter) were isolated from the caudodorsal (CD) and cranioventral (CV) right lung lobe regions of eight horses. Each vessel was subjected to wire myography to assess dilation to furosemide (1e-6 to 1e-3 [logM]). As hypothesized, furosemide induced dilation of equine pulmonary veins taken from both lung lobe regions (87.15 ± 4.07% relaxation at 1e-3 [logM]). qPCR was used to determine mRNA expression of NKCC1. The CV portion of the lung had 73.8-fold greater mRNA compared to the CD portion of the lung as well as kidney (positive control). In future, histology will be used to determine the protein location of the NKCC1 transporter in horse lung veins. In conclusion, preliminary findings indicate furosemide dilates isolated equine pulmonary veins *in vitro*, and that NKCC1 is expressed in pulmonary tissue. These data are the 1st ever to demonstrate furosemide has a direct effect on equine pulmonary vasculature, and that NKCC1 mRNA exists in the lung. Given NKCC1 mRNA level is highest in the CV portion of the lung, this suggests NKCC1 inhibition is a plausible pulmonary vein-mediated mechanism underlying the efficacy of furosemide for prophylaxis of EIPH in horses.

Research Grant: Research Grant: CVM COR Grant
Student Support: Student Support: Boehringer Ingelheim

Isolation of Human Monoclonal Antibodies Targeting Conserved Protein Antigens to *Streptococcus*

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Streptococcus is a genus of gram-positive bacteria that is responsible for numerous diseases worldwide. Group A *Streptococcus* (GAS) infections account for an estimated 500,000 deaths per year and are implicated in the development of chronic autoimmune sequelae. Yet, a safe and effective commercial vaccine for GAS infection does not exist. Factors impeding vaccine development include, strain diversity, antigenic variation, differences in geographical distribution of serotypes, and the unnerving potential of GAS antigens to trigger autoimmune sequelae. *Streptococcus pneumoniae* is a leading infectious pathogen, causing pneumonia, bacteremia, meningitis, acute otitis media, and nearly one million deaths worldwide each year. Unlike GAS, there is widespread use of polysaccharide-based vaccines for *pneumococcal* infection. However challenges still exist, serotype coverage and limited vaccine efficacy against some vaccine-included serotypes have led to increased incidence of colonization and infection of non-vaccine serotypes, as well as an increase in drug and multi-drug antibiotic resistance. Monoclonal antibodies (mAbs) targeting conserved antigens have been shown to be an effective tool to address treatment limitations. The structural determinants mediating the serotype breadth and protective efficacy of broadly reactive human mAbs that prevent and treat *pneumococcal* and GAS infections need to be further elucidated. This study will isolate new human mAbs and determine the serotype breadth and protective efficacy of human mAbs targeting conserved protein antigens. The study will also express and purify proteins to define the epitopes mediating the protective efficacy of the human mAbs.

Research Grant: NIH T35 OD 010433 Georgia Veterinary Scholars Summer Research Program
Student Support: Student Support: NIH Office of Research Infrastructure Programs, Grant Number 5T35OD010433-14

Pilot investigation of *Mycoplasma bovis* in Mississippi beef cattle populations using culture and qPCR

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Mycoplasma bovis contributes to Bovine Respiratory Disease Complex (BRDC), which has significant welfare and economic impacts on cattle production. The aim of this research was to describe growth characteristics of *M. bovis* isolates and evaluate respiratory shedding in populations of beef cattle at risk for BRDC. Known *M. bovis* isolates were transferred from -80°C to pleuropneumonia-like organism (PPLO) agar, incubated at 37°C with 5% CO_2, and monitored for growth. To develop selective media for field use, PPLO plates containing ceftiofur (200, 100, 50, 25, and 12.5 μg/ml) were prepared. *Pasteurellaceae* were transferred from -80°C to each media preparation, incubated at 37°C with 5% CO_2, and observed for growth. To assess *M. bovis* respiratory shedding, three populations (n = 5, n = 9, n = 5) of recently weaned beef calves were sampled using double guarded deep nasopharyngeal swabs (NPS), which were streaked onto selective media. Colonies with typical *Mycoplasma*-like morphology were confirmed by *M. bovis* specific PCR. DNA was also extracted directly from NPS for quantitative real-time PCR (qPCR), with C_q < 31 considered positive. Known *M. bovis* isolates were first evident in culture at 48-120 hours after plating. Growth of *Pasteurellaceae* was inhibited at all concentrations of antibiotic tested and PPLO with 100 μg/ml ceftiofur was chosen for field use. The prevalence of *M. bovis* across all populations was 42% as determined by culture (20%-80%; $P > 0.05$, Fisher's Exact test) and 58% as determined by qPCR (0%-89%; $P < 0.05$ Fisher's Exact test). In this study, *M. bovis* growth was faster than previously described, and prevalence of calves shedding *M. bovis* significantly varied among populations when measured by qPCR but not culture.

Research Grant: None
Student Support: NIH T35 ODO10432

Methionine sulfoxide reductases (MSRs) and Fe-S cluster biogenesis

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Iron-sulfur (Fe-S) clusters are protein cofactors that facilitate essential cellular processes. A deficiency in Fe-S proteins or Fe-S cluster biogenesis is linked to several disorders, ranging from cancer to ataxias. Reactive oxygen species (ROS) are highly reactive molecules formed by O2 reduction. Methionine side chains are particularly susceptible to oxidation by ROS. Notably, several Fe-S biogenesis enzymes contain higher than average methionine content. The eukaryote, *S. cerevisiae*, has been a model for the study of Fe-S biogenesis. Previous work in yeast established that strains deleted for the enzymes that repair oxidized methionines (methionine sulfoxide reductases, MSRs) show an upregulation of genes involved in iron regulation and a decrease in Fe-S protein activity (Sideri et al. 2009 *Microbiology* 115:612). We hypothesize that the mitochondrial Fe-S biogenesis machinery is susceptible to methionine oxidation, which disrupts the early stages of Fe-S cluster biogenesis. To test this model, we aim first to establish a role for the mitochondrial MSR in Fe-S cluster formation. Yeast have two MSR enzymes: Mxr1 and Mxr2. Mxr1 is localized to the cytoplasm, while Mxr2 is dual localized to the cytoplasm and the mitochondria depending on start codon usage. A methionine-to-isoleucine mutation in Mxr2 (Mxr2-M1L) disrupts mitochondrial Mxr2 production but maintains cytoplasmic activity. My goal is to establish if Fe levels and Fe-S protein activities are altered in cells lacking mitochondrial Mxr2 activity. If a disruption in Fe-S biogenesis can be linked to an inability to repair methionine oxidation, a new focus for the prevention of the damage to Fe-S proteins may emerge.

Research Grant: NIH R01 GM105958
Student Support: None

QMRA of fluoroquinolone-resistant *Campylobacter jejuni* infections associated with unpasteurized milk in the US

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The consumption of unpasteurized milk has increased in popularity in the United States over the past 30 years. *Campylobacter jejuni* is ubiquitous in the dairy environment and is shed in the feces of cattle. It is commonly found in unpasteurized milk and is the leading cause of foodborne illness in the US. Fluoroquinolone-resistant (FqR) *C. jejuni* has been isolated from various sources including unpasteurized milk, and it is of growing public health concern. Fluoroquinolones are commonly prescribed as a first measure for humans suffering from diarrhea, which is typical with a *C. jejuni* infection. In this study, a quantitative microbial risk assessment (QMRA) was developed to determine how many US consumers per year will develop a FqR *C. jejuni* infection as a result of unpasteurized milk consumption using a dose-response model. The QMRA model included steps involved in the raw milk chain from production to consumption. Model modules included milk collection and storage in the milking bulk tank, bottling milk for retail sale, storing the bottles on-farm, transporting the bottles to a retail store, and storing the bottles in the retail store until the time of consumer purchase and consumption. The estimated median number of illness cases due to consumption of raw milk contaminated by FqR *C. jejuni* was 223,383 annually in the US. The factor most strongly correlated with development of an infection was the initial concentration of FqR *C. jejuni* in the milking bulk tank. Strategies to reduce the concentration of FqR *C. jejuni* in the milking bulk tank involve good milking practices and overall dairy farm hygiene, such as ensuring clean bedding for lactating cows and spraying milking units clean before each cow is milked.

Research Grant: USDA NIFA SCRI [2019-51181-30016]; USDA NIFA Hatch [7000433]; Foundation for Food and Agriculture Research [CA18-SS-0000000206]
Student Support: NIH T35 OD010941 Cornell University College of Veterinary Medicine

Characterization and surveillance of *Leptospira* lineages in inland northwestern US

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Bacteria in the genus *Leptospira* include 10 pathogenic species and 250 pathogenic serovars which impact the health of both humans and domestic animals with a global distribution including the United States. Leptospires are transmitted through direct or indirect contact with urine from infected animals with bacteria persisting in contaminated soil and water for weeks to months. In this study, we aim to characterize the lineages of *Leptospira* spp. detected in domestic animals through the Washington Animal Disease Diagnostic Laboratory (WADDL) and through surveillance in wild rodent populations sampled in Washington and Idaho as part of an ongoing Hantavirus surveillance study. We will test the hypothesis that one or more pathogenic *Leptospira* spp. circulate in local synanthropic rodent populations and contribute to illness of companion animals and livestock. Wild rodents were trapped from agricultural sites such as farms and crop lands and in natural areas. Samples are screened by polymerase chain reaction and positive samples will be genetically characterized by multi-locus sequence typing (MLST). We will report MLST lineages for 7 clinical samples of leptospirosis positive urine and tissue as well as nucleic acids from dogs, cattle, a moose and a miniature donkey, and kidney samples from 24 wild rodents including deer mice and chipmunks. Population genetic characterization of circulating leptospires in wild rodents and domestic species will inform on the source of *Leptospira* in animals in the inland northwestern US, including wild rodents that may serve as pathogen reservoirs.

Research Grant: None
Student Support: Washington State University College of Veterinary Medicine Summer Research Fellowship Program

Correlations between periodontal disease and systemic health using dental radiographs and clinical pathology

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Periodontal disease is one of the most common diseases among canines and felines. It is induced by the dental microbial biofilm along with an inflammatory response, which destroys the supporting structure of teeth. As in humans, the prevalence of periodontal disease increases with age in dogs and cats, and it is known to occur more frequently in smaller dogs. As the main entrance to the gastrointestinal tract, it has been speculated that the oral cavity has some association with systemic health in both humans and animals. Suggested systemic associations in humans and dogs include, but are not limited to, cardiovascular disease, diabetes, kidney disease, auto-immune diseases, anemia, and obesity. Our previous study found anemia (low RBC, HCT, HGB), decreased creatinine, and thrombocytosis to be common in canine dental cases that required tooth extractions. Subjects in this study were canine and feline patients who were presented for dental procedures in 2021-2022 at the Veterinary Medical Teaching Hospital at Tuskegee University College of Veterinary Medicine. Here, we retrospectively quantified the severity of periodontal disease by totaling the periodontal disease score of each tooth per patient based on the intraoral radiographs, and then compared with the clinical pathology data (complete blood cell counts and serum chemistry) of each patient to investigate any indications that severity of the periodontal disease affected systemic health differently. Oral health plays an important role in systemic health and contributes to a good quality of life in dogs, cats and humans.

Research Grant: DHHS/HRSA D34HP00001-35-00 and NIH/NIMHD RCMI grant # U54MD007585
Student Support: DHHS/HRSA D34HP00001-35-00

Maternal dietary deficiencies during neurodevelopment result in sex differences after ischemic stroke in mice

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The incidence of ischemic stroke is increasing in middle-aged adults and nutrition is a modifiable risk factor for stroke. Maternal nutrition is essential for offspring neurodevelopment and deficiencies often lead to health issues. Specifically, B-vitamins, such as folic acid and choline, are important nutrients during pregnancy for the neurodevelopment of the offspring, including the closure of the neural tube *in utero*. However, the role of maternal nutritional deficiencies on offspring brain function after birth is not well defined, especially after ischemic stroke. Our study aimed to investigate the role of maternal deficiencies in folic acid and choline on offspring stroke outcomes. Female mice were maintained on either a control diet or deficient diet before pregnancy and during pregnancy and lactation. When female and male offspring were 10-months of age, ischemic stroke was induced via photothrombosis targeting the sensorimotor cortex. Stroke outcome was assessed by measuring motor function in living animals and ischemic damage volume, apoptosis, and neuroinflammation in the brain postmortem. No significant difference was observed between maternal dietary groups in offspring motor function; however, males and females differed in their performance. Maternal diet significantly impacted ischemic damage volume. Interestingly, offspring from deficient mothers showed significantly reduced active caspase-3 cell counts within the ischemic damage region. We are in the process of investigating neuroinflammation in damaged brain tissue. Preliminary conclusions indicate that maternal dietary deficiencies do not impact offspring motor function following ischemic stroke but do play a role in other ischemic stroke outcomes.

Research Grant: American Heart Association 20AIREA35050015
Student Support: Boehringer Ingelheim Veterinary Scholars Program and Federal Work Study

Accuracy of canine vertebrae pedicle pin placement using an augmented reality neuronavigational device

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Standard of care for canine thoracolumbar vertebral fractures requires stabilization using plates, pins, or screws. Placement of implants is challenging and often deviates from the planned trajectory when relying on anatomical landmarks as a reference. This study aims to assess the accuracy of an augmented reality (AR) neuronavigational device in placement of pedicle pins in canine vertebral bodies. In this study, 4 canine cadavers were used. CT scans were performed on all cadavers. For control sites, the neurosurgeon placed 10 right-sided pedicle pins from T9-L5 based on a pre-planned trajectory from the 2D transverse CT images. 3D models were reconstructed from the CT scans to include pre-planned trajectory and imported into an AR device. For AR-assisted sites, the neurosurgeon placed 10 left-sided pedicle pins from T9-L5 while wearing the AR device. Post-operative CT scans will compare deviation of the pin placement from the pre-planned trajectories in the X, Y, Z axis. We hypothesize that AR-assisted neuronavigation will improve the degree of accuracy and precision of pedicle pin placement compared to the traditional approach.

Research Grant: None
Student Support: Student Support: Boehringer Ingelheim Veterinary Scholars Program

Detection of IgM and IgG antibodies against *Cytauxzoon felis* in cat serum from experimental infections

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Cytauxzoon felis, the causative agent of cytauxzoonosis, is one of the most lethal tick-borne parasites of domestic and wild felids in North America. A lack of specific prevention strategies and rapid, patient-side diagnostic tests remain significant road blocks to developing control methods and prompt treatment to improve clinical outcome of infected cats. The aim of this study was to determine IgM and IgG antibody levels in cats inoculated with sporozoites of *C. felis* or infested with *C. felis*-infected *Amblyomma americanum* adult ticks that had been acquisition-fed as nymphs on a cytauxzoonosis survivor cat. An ELISA designed for plasma-based samples was adapted and optimized to detect IgG and IgM antibodies from feline serum-based samples. Serum samples were collected from sporozoite-inoculated and tick-infested cats approximately every three days and will be processed to evaluate levels of IgM and IgG in response to infection. Absorbance values will be analyzed in response to known positive and negative samples. We expect results will most likely reveal the appearance of IgM first, since it is the first antibody normally produced during the adaptive immune response, followed by IgG at later time points.

Research Grant: Oklahoma State University, College of Veterinary Medicine.
Student Support: Boehringer Ingelheim Veterinary Scholars Program to J.E.H.

Mercury and Lead Exposure in Eastern Whooping Cranes: Cause for Concern?

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The endangered whooping crane (*Grus americana*) is at risk for exposure to Hg, Pb, and other environmental contaminants which may pose a critical threat to its recovery. Previous research involving the reintroduced Eastern Migratory Population (EMP) suggests at least infrequent exposure of cranes to Hg and mortality associated with Pb. The factors underlying the unsustainable population dynamics of the EMP (established in WI beginning in 2001) remain unclear, but both Hg and Pb may have deleterious impacts on wild bird survival and reproduction. This study was intended to assess exposure to Hg and Pb among cranes, especially during their breeding and chick rearing seasons. We expected 5% or greater EMP birds to have elevated concentrations in blood or feathers. For each of the 16 cranes captured, trained personnel sampled up to 12mL of blood and six non-flight contour feathers. Personnel also collected unhatched eggs from wild whooping crane pairs that were determined to be infertile or dead. To date, we have collected and tested 14 blood, 9 feather, and 12 egg samples. All blood, feather, and egg samples were subject to inductively coupled plasma mass spectrometry (ICP/MS) for Hg and Pb level determination. The mean ± SD concentration of Hg in blood and feather samples was 60.17 ± 31.11 and 0.78 ± 0.49 ppb, respectively. The mean ± SD concentration of Pb in blood was 10.50 ± 5.68 ppb and in feathers was 0.06 ± 0.07. The results from the egg samples are pending. Thus far, no results suggest heavy metal exposure has occurred at harmful levels within the EMP. The complete study will provide a more thorough assessment of the threat of heavy metals to this fragile population of whooping cranes than previous research.

Research Grant: ICF Conservation Impact Fund
Student Support: Boehringer Ingelheim Veterinary Scholars Program BIVSP-UW-Madison

Effective Dose 50 of Single Drug Protocol for Sedation of Buff Orpington Hens

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Chickens are common pets. To reduce stress and to allow for non-painful procedures, sedation is commonly necessary; however, there is a surprisingly low number of sedation studies in chickens. We sought to determine the effective dose 50 (ED_{50}) of single agent sedation (midazolam, dexmedetomidine, and alfaxalone). Twenty, juvenile, Buff Orpington hens were used in this study. A randomized, non-blinded, clinical trial using an Up-and-Down study design was performed. At ten minutes after intramuscular drug administration (midazolam 1 mg/kg, dexmedetomidine 100 μg/kg, alfaxalone 1 mg/kg), the sedation was scored using five different criteria: recumbent in the cage, stayed on right lateral for 10 seconds, allowed for right jugular venipuncture, allowed for ventro-dorsal sham radiographic positioning, and allowed for right lateral sham radiographic positioning. If the criteria were present the animal received the score of 1 for each. Conversely, if absent, the score of 0 was awarded. If total score was ≤ 2, it was classified as "not sedated", while score of ≥ 3 was classified as "sedated". If the first bird was "sedated", the dose was decreased by 50% on the next random animal. If "not sedated", the dose was increased by 50%. At least four crossover events (i.e., contradictive responses between 2 sequential animals) were achieved per drug. We concluded that the ED_{50} for midazolam was 2.51 mg/kg and the ED_{50} for dexmedetomidine is 61 μg/kg. Hyperesthesia was detected at 28.9 mg/kg of alfaxalone. Due to this adverse effect, alfaxalone ED_{50} was discontinued. This study provides initial data for multi-drug studies.

Research Grant: The Oklahoma State University Debbie and Wayne Bell Professorship in Veterinary Clinical Sciences and Department of Veterinary Clinical Sciences
Student Support: OSU College of Veterinary Medicine and Department of Veterinary Clinical Sciences

Antibacterial activity characterization of *Staphylococcus chromogenes* isolates originating from dairy cattle

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Staphylococcus chromogenes is commonly identified among samples collected from bovine milk. Previous work has identified a potential protective effect of *S. chromogenes* when identified on the teat end, potentially mediated through the production of antimicrobial peptides, known as bacteriocins. No previous work has evaluated the antibacterial activity or presence of bacteriocin associated genes among a large collection of *S. chromogenes* isolates. The objective of this study was to identify and characterize *S. chromogenes* isolates that can inhibit in vitro growth of *Staphylococcus aureus*. A banked collection of *S. chromogenes* isolates was used, including isolates originated from quarter level milk samples (n = 112), bulk tank samples (n = 131), teat swabs (n = 131), and used bedding (n = 4). Selected isolates were plated on Columbia Blood Agar (CBA) and incubated for 24hr at 37 °C. Isolate concentration was standardized to 0.5 MacFarland Standard and a single center streak was plated on 2 CBA plates. After 24 hr of incubation at 37 °C, the agar was flipped and all *S. chromogenes* center streaks were crossed streaked against two different *S. aureus* strains. After 24 hours of additional incubation, phenotypic growth inhibition was categorized as complete inhibition, partial inhibition, or no inhibition. Overall, 11(2.91%) isolates displayed partial growth inhibition against one strain of *S. aureus* and 2(0.53%) isolates displayed complete inhibition against both *S. aureus* strains. Future work will include PCR detection and prevalence determination of bacteriocin associated genes among this collection of *S. chromogenes* isolates. While phenotypic antibacterial activity has been identified among tested isolates, it is rare.

Research Grant: USDA National Institute of Food and Agriculture, Animal Health project 7001296
Student Support: USDA National Institute of Food and Agriculture, Animal Health project 7001296

Ticked-off plants: exploring the relationship between ticks and invasive plant species

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Nationally and throughout Illinois there has been an increase of tick-borne disease in humans and livestock. In recent literature, one risk factor for tick abundance may be the presence of invasive plant species and their effects on microclimate. Invasive plant species are non-native plants that disrupt an area's natural vegetative balance. These plants are typically unintentionally spread through foot traffic or the inclusion of non-native plant species in home gardens. This study sought to find the relationship between invasive plant species, microclimate, and tick abundance. Tick abundance was determined using a standard dragging procedure in which a one-square meter white cotton sheet was pulled across the detritus of three 10-ft transects within each experimental plot. Plots were classified based on abundance of invasive plant species. Tick drags were performed at Dixon Springs Agricultural Center from April to December 2021. Plots were classified as uninvaded (n = 3) and invaded by *Alliaria petiolata* (Garlic Mustard, n = 1), *Microstegium vimineum* (Japanese Stilt Grass, n = 3), and *Lonicera maackii* (Amur Honeysuckle, n = 3). Collected ticks were identified for developmental stage, species, and will later be tested for pathogens. To collect microclimate data, dataloggers (HOBO), were placed at 0.3 and 1 meter above ground at each transect to record temperature and relative humidity at 30-min intervals from April to December. Descriptive and multivariable statistical analysis will be conducted to assess the relationship between tick population, invasive plant species, and microclimate. This study will lead to innovative approaches to tick-borne disease prevention through the control of invasive plants species.

Research Grant: ACES Dudley-Smith Initiative DSynergy Program
Student Support: Office of the Director, NIH, T35 OD011145

Use of 3D models for pre-procedural intrahepatic portosystemic shunt planning: 2D imaging versus 3D modeling

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Computed tomography angiography (CTA) is used for diagnosis of intrahepatic portosystemic shunts (IHPSS). When planning for transcatheter intervention, caudal vena cava (CVC) measurements are made on 2-dimensional (2D) images to aid in stent selection. Objectives were to generate 3D IHPSS models and compare CVC dimensions taken from 3D models to 2D images. We hypothesized 3D models can be generated for IHPSS, and CVC measurements would not differ significantly between 2D images and 3D models. CTA datasets were from dogs with IHPSS at University of Georgia Veterinary Teaching Hospital from 2016-2022. Materialise Mimics 25.0 and 3-matic 17.0 were used for 3D modeling. CVC diameters were measured in 2D coronal and axial planes 20mm cranial and caudal from the shunt ostium, and compared to 3D diameters calculated from axial plane area along contour lines from the model. Length was measured on 2D coronal plane between midpoints of cranial and caudal diameter, and compared to 3D length measured in Mimics. Data are presented as mean(SD). 3D models were generated for 32 IHPSS (15 right-, 12 left-, and 5 central-divisional). 2D coronal and axial diameter measurements were 16.1mm(5.2) and 15.0mm(4.2) cranial; 14.3mm(4.3) and 14.0mm(3.6) caudal. 3D diameter measurements were 15.3mm(4.4) cranial and 14.0mm(3.6) caudal. 2D length was 60.5mm(8.1) and 3D was 59.9mm(7.2). There were no significant differences between 2D coronal and 3D diameters (cranial $P = 0.5235$, caudal $P = 0.7986$), nor 2D and 3D length ($P = 0.7678$). 3D IHPSS models can be generated using vascular modeling software, with consistent measurements to 2D planar imaging, thus making 3D models useful as accurate representations of vascular disease in pre-procedural planning.

Research Grant: None
Student Support: Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Anticoagulant effect (heparin, EDTA, acid citrate dextrose solution A) on hematology of Buff Orpington hens

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Appropriate blood collection and storage is a crucial component of medicine (human and animal) as analysis of relevant components can provide valuable diagnostic criterion (e.g., cell morphology and quantification), especially regarding disease status. Stopping the coagulation pathway is a critical step leading up to the proper evaluation of blood and is easily obtained by use of commercially available anticoagulants. However, there are important gaps in our knowledge regarding the effects of common anticoagulants (e.g., ethylenediaminetetraacetic acid [EDTA], lithium heparin, acid citrate dextrose solution A [ACD-A]) and their individual effects within differing species. Chickens have become increasingly popular as pets and therefore routine diagnostics have also increased in prevalence. The most common anticoagulant used in mammalian species is EDTA. Human medicine commonly uses blood tubes with ACD-A. In avian and exotic medicine, blood tubes with lithium heparin or EDTA are commonly utilized. The goal of this study was to determine what anticoagulant (EDTA, heparin, ACD-A) best resembles the hematological results of the freshly drawn blood (criterion standard) in chickens. The four hematological parameters measured were packed cell volume (PCV), total solids, RBC morphology (specifically polychromasia which was graded from 1-3), and WBC relative and absolute values. Blood smears and PCV tubes were taken from freshly drawn blood then transferred to EDTA, lithium heparin, and ACD-A vials in randomized order. From those vials, PCV tubes and blood smears were also made. Samples and statistical analysis are ongoing. The results of this study will provide valuable information for veterinarians working with chickens.

Research Grant: Oklahoma State University Debbie and Wayne Bell Professorship in Veterinary Clinical Sciences
Student Support: OSU Debbie and Wayne Bell Professorship in Veterinary Clinical Sciences, NIA-5K01AG064121

If you give a mouse a house: assessing the well-being of mice provided with colored intracage shelters

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Laboratory mice will interact with intracage shelters when these are provided. However, even though mice spend a significant amount of time in their shelters, our pilot studies suggest that markers associated with chronic stress are elevated. Chronic stress in laboratory rodents is a welfare issue that can alter behavior and physiology, introducing uncontrolled variables and reducing comparability to human disease. In this study, mice were given access to clear, red, yellow, or blue intracage shelters. The control group was not provided an intracage shelter. Once the mice acclimated to the shelters, anxiety was measured using the open field test. A blood sample was also collected to compare serum corticosterone (ELISA) and complete blood count (neutrophil:lymphocyte ratio) between groups. Behavioral and physiological assessments were combined to determine how the provision of an intracage shelter and its color impact well-being. Comparing the no-shelter group to the groups that have shelters will assess the impact of intracage shelter presence on stress whereas comparing the groups with intracage shelters by color will assess the impact of light intensity on stress. As the mice will be acclimating to the shelters until late July, there is no data to report yet. Cage-side observations have shown more mice using the intracage shelters as the acclimation period progresses, with a current average of 30% of mice using the shelters when observed. Therefore, we are hopeful this project may help guide the recommendations for the use of intracage shelters for the enrichment of laboratory rodents.

Research Grant: None
Student Support: Boehringer Ingelheim Animal Health and Purdue University College of Veterinary Medicine

Nonspecific Stimulation of Mucosal Immunity by a Bacterial Vector for Protection Against COVID-19 Disease

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The Δ *capB* strain of *Francisella tularensis* is a highly attenuated bacterium that has been used as a vector for producing several vaccines against Tier 1 select agents *Bacillus anthracis*, *Yersinia pestis*, *Burkholderia pseudomallei*, and *Francisella tularensis*. Previous studies involving the Δ *capB* vector indicated that this vector by itself afforded some non-specific protection against severe COVID-19 disease. The aim of this study was to test the hypothesis that intranasal administration of the Δ *capB* vector would stimulate a non-specific mucosal immune response that and provide respiratory resistance to a variety of pathogens, including SARS-CoV-2. Golden Syrian hamsters were inoculated intranasally with Δ *capB* at various time intervals prior to intranasal challenge with SARS-CoV-2. Efficacy in providing protection was based on measurements weight loss, virus shedding, virus titers in lung and turbinates, and lung pathology. Plaque assays were conducted on daily oral swabs and samples of nasal turbinates and lung tissue upon necropsy to assess viral load. Significant reduction in viral titers of Δ *capB*-treated hamsters was not demonstrated, but treatment with this vector seven days prior to challenge significantly reduced weight loss, thereby offering non-specific immune protection against severe disease. The data suggests that successful non-specific stimulation of mucosal immunity may provide the respiratory system with resistance against a variety of pathogens and has the potential to be utilized in future production of inexpensive intranasal vaccinations.

Research Grant: Animal Models Core
Student Support: Supported by NIH Grant Number T35 OD015130

Systematic assessment of percent lung involvement and gross pulmonary lesions in feedlot mortalities

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Feedyard mortality is a major loss, and a more in-depth understanding of pulmonary pathology may lead to improved prevention and treatment plans. The objectives of this observational study were to systematically assess feedyard mortalities to describe the percentage of lung involvement and lesion characteristics among different diagnoses and characterize differences in pulmonary lesions based on treatment timing and animal demographics. Percent lung consolidation for each pulmonary lobe was assessed and an equation based off previous literature was used to determine total lung involvement. Investigators were blinded to treatment history at the time of necropsy. A total of 129 mortalities were included in the study with the primary pulmonary lesions of interest including bronchopneumonia (BRD) (n = 42), acute interstitial pneumonia (AIP) (n = 20), and bronchopneumonia with a secondary interstitial pattern (BIP) (n = 67). Both BRD and BIP had an average of 91% total lung consolidation, while AIP only had 78%. The highest percentage of cases with abscesses was BIP at 57%, compared to 20% and 36% of AIP and BRD. More BRD cases (79%) had pleuritis compared to only 40% and 52% of AIP and BIP cases. Respectively, average days from first treatment to death were 16 days, 22 days, and 36 days for BRD, BIP, and AIP. Average days on feed at death for BRD, BIP, and AIP were 83, 108, and 110, respectively. Diagnoses differed in timing with BRD observed earlier with more frequent abscesses and pleuritis compared to AIP. These patterns can better help health caretakers detect and treat cattle early in the feeding phase.

Research Grant: Innovative Livestock Services, Legacy Animal Nutrition, Beef Cattle Institute, and Foundation for Food and Agricultural Research
Student Support: Elanco Animal Health

Association between cerebrospinal fluid biomarkers of Alzheimer's disease and cognitive performance in vervets

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Alzheimer's disease (AD) presents with distinct changes to cerebrospinal fluid (CSF) composition as well as cognitive decline. Determining the relationship between changes in CSF constituents and cognition can be difficult in clinical settings. Vervet monkeys (*Chlorocebus aethiops sabaeus*) are well-established models of early AD-like neuropathology, and thus provide opportunities to examine this relationship. We evaluated cognitive performance and CSF markers of AD in a sample of twenty known aged female vervets (ages: 10-29y, mean = 20.1y), representative of middle-aged to elderly humans, living in large social groups at Wake Forest University. Our first goal was to determine the impact of age on two AD-related CSF biomarker ratios- phosphorylated tau to beta amyloid 1-42 (pTau181:Aβ42) and Aβ42 to Aβ40 (Aβ42:Aβ40). We next examined the correlations between CSF biomarkers and cognitive performance in a maze test of executive function (Wake Forest Maze Test) and an assessment of working memory (Delayed Response Task), while controlling for the effects of age. We found that pTau181:Aβ42 significantly increased with age ($P < 0.05$), whereas Aβ42:Aβ40 was unrelated to age ($P > 0.80$). Partial Pearson correlations did not reveal significant relationships between contemporaneous CSF biomarker values and measures of cognitive performance. These preliminary results indicate that vervets may recapitulate aging-related changes in CSF biomarkers that are relevant to AD. However, in this relatively small cross-sectional study we did not find evidence of an age-independent relationship between cognitive function and CSF Aβ and p-tau peptides. Longitudinal analyses are required to determine the temporal associations between these variables.

Research Grant: P40OD010965, UL1TR001420, R24AG073199
Student Support: NIH T35 Training Grant T35OD010946

A novel method of health monitoring in laboratory zebrafish

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Zebrafish (*Danio rerio*) are useful in scientific research due to their close genetic similarity to the human genome and fast reproductive lifecycle. Their increased use in scientific research calls for improved methods of monitoring their health, as current methods involve multiple types of testing including submission of whole fish to identify various pathogens. This study aims to create a novel sampling technique by exposing nitrocellulose filters to sump water over the course of twelve weeks. The filter was compared against other known testing methods of swabbing biofilm from the sump and passing sump water through a vacuum filter. It was hypothesized that the nitrocellulose filter would identify more pathogens over time, reducing the need for multiple testing methods. Weekly PCR testing was conducted to detect *Mycobacterium chelonae,* zebrafish picornavirus, *Myxidium streisingeri*, *Mycobacterium fortuitium*, and *Pseudoloma neurophilia*. Nitrocellulose filters were the most consistent in identifying pathogens every week as their sensitivity to identify pathogens increased over time. The vacuum filter was also a consistent and sensitive method, but to a lesser degree than the nitrocellulose filters over time. Sump tank swab samples were the least sensitive in pathogen detection as its positive pathogen identification results were inconsistent. In preliminary results, none of the methods have been able to detect *P. neurophilia*. This suggests that the nitrocellulose filters may be a useful method of monitoring the health of laboratory zebrafish colonies for most of the agents tested.

Research Grant: None
Student Support: Boehringer Ingelheim Animal Health and Purdue University College of Veterinary Medicine

Synergistic activity of single- and double-stranded viral RNA causes fatal inflammation

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Severe inflammation in patients with coronavirus disease 19 (COVID19) is a major determinant of poor clinical outcomes. However, the immunological basis for fatal inflammation caused by human CoVs is not well understood. CoVs are single stranded RNA (ssRNA) viruses and make double stranded RNA (dsRNA) intermediate during replication. Most studies have evaluated the individual roles of ssRNA and dsRNA in antiviral and inflammatory responses. However, the combined effect of ssRNAs and dsRNAs in host immune response to virus infections is not well understood. We hypothesized that ssRNA plus dsRNA stimulation will likely have an additive effect on inflammatory cytokine production, thus causing cytokine storm. In our experiment, we stimulated bone marrow macrophages (BMMs) with ssRNA (R837) and dsRNA (PolyIC) mimics and measured inflammatory/antiviral cytokines using ELISA/qPCR. Our results show that combined stimulation of BMMs with R837 and PIC had synergistic effect that resulted in > 10 fold increase in inflammatory cytokine production compared to R837- or PolyIC-alone. Prior stimulation with R837 reduced PolyIC-induced antiviral interferon (IFN) and interferon-stimulated gene (ISG) response. Since MAPK (P38, JNK, and MEK) and NFkB pathways are central to viral RNA-induced inflammation, we postulated that one of these pathways will play key role in synergistic inflammation. Using BMMS treated with two inhibitors for each pathway, we found that NFKB>MEK1/2>P38 MAPK pathways all played a significant role in inflammatory cytokine production. Our results demonstrate that targeting one or more of these pathways will moderate cytokine storm and protect the host from fatal inflammation caused by CoVs and other emerging viruses.

Research Grant: NIH P20 GM103648 (CoBRE- Oklahoma Center for Respiratory and Infectious Diseases) and Oklahoma State University Start-up funds to RC
Student Support: Summer Research Internship Program at Oklahoma State University

Can PRRS Resilient fetuses be identified using gene expression assays?

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Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) infections are detrimental to swine both as a respiratory disease in piglets and a reproductive disease in sows. Economic losses are due to abortion in the third trimester of pregnancy as well as the birth of congenitally infected piglets. who may be stillborn, or weak, slow growers, potentially dying prior to weaning. Mortality rates and viral load vary between fetuses of the same litter, with some fetuses being uninfected and others having wide-ranging viral loads. Our current work has focused on outcomes for fetuses with a high viral load comparing viable (HV_VIA) to meconium stained (HV_MECb) fetuses. Viable fetuses are "Resilient" and expected to survive despite high viral loads. Meconium-stained fetuses are in distress and are unlikely to survive and thus are "Susceptible". The primary purpose of our experiment is to determine what causes the loss of viability (HV-VIA vs HV-MECb), what changes in gene expression occur between Resilient vs Susceptible fetuses. Our goal is to determine if there is a predictive tissue and set of genes that differentiate these fetuses. In this study, 30 pregnant gilts were infected with PRRSV at day 85 of gestation. After 21 days post infection, the sows were euthanized and the fetuses were grouped based on the viral load in the thymus as well as clinical signs. Purified RNA samples were prepared from the fetal heart, liver and kidney. These samples were analyzed in a Qubit for RNA concentration and overall quality (RIN #). Expression of 60 genes will now be analyzed using NanoString transcriptomics to evaluate five pathways hypothesized to predicate fetal resistance or susceptibility.

Research Grant: USDA ARS NBAF AGREEMENT NO: ARS 59-3022-1-003
Student Support: USDA ARS NBAF AGREEMENT NO: ARS 59-3022-1-003

Spatio-temporal Changes in Avian Cholera Outbreaks in the United States and its Association with the Weather

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Avian Cholera, caused by the bacteria *Pasteurella multocida*, is a respiratory and septicemic disease of domestic and wild avian species. Disease control is challenged by a lack of effective vaccines, identifying reservoirs of the disease agent, and predicting drivers of outbreaks. We analyzed publicly available outbreak reports to explore the spatio-temporal distribution of avian cholera outbreaks in the US.[1] Reports of sightings of 24 species of birds from each county in the US between January 2002 and May 2022 were extracted from eBird. Daily precipitation, temperature, and humidity data for each county were also extracted. Exploratory mapping and logistic regression were conducted to determine the association between temperature, precipitation, and the distribution of avian cholera within the US. Over the study period, there were 263 reported cases of suspected or confirmed avian cholera events with a median number of 241 birds affected per event. Snow geese (*Anser caerulescens*) and American Coots (*Fulica americana*) were the most common avian species present during outbreaks and appeared in 144 and 131 events, respectively. We hypothesize that avian cholera is associated with higher temperatures. The visualization of these spatial and temporal trends in avian cholera could be used to target surveillance in regions and during times where vulnerable species are most at risk. [1] Retrieved May, 31, 2022, from the Wildlife Health Information Sharing Partnership event reporting system on-line database, https://whispers.usgs.gov

Research Grant: Morris Animal Foundation
Student Support: College of Veterinary Medicine, Purdue University

A preliminary comparison of acepromazine and trazodone for the reduction of hospital-associated stress in dogs

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Several factors contribute to stress experienced by dogs in the hospital environment, including separation from the owner, a novel environment, restraint, and confinement. The stress response can be beneficial for acute stressors, but prolonged or exaggerated stress response may lead to adverse effects on immune, gastrointestinal, and healing function. Therefore, identifying and reducing stress is critical to the health and welfare of veterinary patients. To evaluate the stress response, cortisol can be used to measure the hypothalamic-pituitary-adrenal axis response, heart rate variability can be used to measure the sympatho-adreno-medullary axis response, and certain behaviors can indicate stress in dogs. Acepromazine and trazodone are medications commonly used to treat hospital-associated stress, but previous studies have produced mixed results on the efficacy of these drugs for this purpose. The objective of this study is to evaluate the stress reduction effects of acepromazine and trazodone compared to a negative control in dogs in a hospital setting. In this blinded pilot study, healthy adult dogs were observed in a hospital environment. Salivary cortisol samples, heart rate monitoring to assess heart rate variability, and a video recording with subsequent behavioral assessment were evaluated before and after the administration of acepromazine (0.01 mg/kg IM), trazodone (4 mg/kg), or a placebo. We hypothesized that dogs receiving acepromazine or trazodone would show a decrease in salivary cortisol, a decrease in stress behaviors, and an increase in heart rate variability compared to control dogs after treatment administrations and there would be no difference between the response to the two medications.

Research Grant: FY22 CVM Seed Grant
Student Support: Iowa State University College of Veterinary Medicine Summer Scholar Research Program

What type of inhibitory interneuron loss correlates with seizure frequency?

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Temporal lobe epilepsy is a common seizure disorder occurring across multiple species. Medical professionals, both human and veterinary, face difficulty in understanding and treating this disorder. The pathology of temporal lobe epilepsy is most consistent and severe in the hippocampus, particularly the dentate gyrus. A study in a mouse model evaluated multiple pathological abnormalities in the dentate gyrus and the only one that correlated with seizure frequency was a loss of granule cell layer inhibitory GABAergic neurons. One major GABAergic neuron type expresses Nitric Oxide Synthase (NOS) and is significantly reduced in the ventral hippocampus where seizures tend to initiate. We hypothesize that a loss of NOS interneurons causes temporal lobe epilepsy, and the degree of NOS neuron loss in the granule cell layer will correlate with seizure frequency. Pilocarpine-induced temporal lobe epileptic rats were recorded 24/7 for one month with EEG telemetry. Seizures were counted and seizure frequency calculated. 8 rats evaluated so far had 253-1478 seizures in one month. Seizure frequency (seizures/hour) ranged from 0.3-2.0 with an average of 1.0 ± 0.6 (s.d.). Rats were then perfused and hippocampi were isolated, sectioned, and processed for immunocytochemistry. The number of NOS neurons in the granule cell layer of the dentate gyrus will be estimated. The number of interneurons and seizure frequency will be compared to determine potential correlation. Increased frequency of seizures in rats with decreased numbers of NOS neurons would support our hypothesis. If results show no correlation, other kinds of granule cell layer interneurons will be counted and compared with seizure frequency.

Research Grant: NIH R01 NS107290
Student Support: NIH T35 OD010989

Evidence for optogenetic control of manual dexterity in a macaque model

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Neuropathologies involving manual dexterity are prevalent in human cases and well- represented in non-human primate models, but the means to manipulate and test manual dexterity in non-human primates in the face of neural lesions is still being refined. Currently, optogenetics have proven very successful in small animal models such as rodents and flies at precisely controlling behavior. However, there have been varied successes in the translation of this powerful neurostimulation technique to a non-human primate model. While the neuronal similarities between humans, mice, and flies can be applied to a certain extent, non-human primates represent an important missing link in the translation of novel neurological treatments to human clinical cases. The aim of this study is a proof of concept of the use of certain opsins in the macaque cortex as a means of manipulating neuronal activity. First, we verified the efficacy of C1V1, a previously injected opsin, in exciting neuronal activity in the left frontal cortex. Our recordings demonstrated visually responsive neurons with significant excitatory activity when a green laser was applied. In separate cortical columns, we injected SwiChr++, an opsin designed to inhibit neuronal activity when blue light is applied, and resume regular activity when a red light is applied. The results of the recordings after incubation of that vector will help validate the use of different opsin types to manipulate neuronal activity. The next steps will include a SwiChr++ injection to a larger area in the motor cortex in order to affect a basic reach-and-grab task and checking peripheral nerve effect with NCV testing. The ultimate goal is clinical application to neuropathologies such as epilepsy.

Research Grant: Unknown
Student Support: NIH T35 OD010919, Boehringer-Ingelheim, and the University of Pennsylvania

Evaluation of clinical decision making in equine colic

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Existing at the intersection of technical data and experiential bias, clinical decision making requires veterinary professionals to make quick and effective choices based on previous diagnoses and present diagnostics. Experience and training in the veterinary field impacts how and why veterinarians reach diagnoses and treatment plans and the confidence with which they make decisions about cases. The purpose of this preliminary study is to create an online survey to evaluate clinical decision making in cases of equine colic. The survey gathers data on participant age, sex, experience, and training. Participants are then asked to read and analyze three separate cases of equine colic from the University of Georgia's Veterinary Teaching Hospital. They are given history and a baseline physical examination and then asked to choose the first three additional diagnostics they would perform. After being given the results of each diagnostic, participants rate confidence in ability to diagnose lesion type and location and assign a treatment response. After this, participants are given all diagnostic test results, confirmed diagnoses, and treatment. Participants then have an opportunity to reflect on the diagnostic value of each test. This survey will be disseminated to preliminary study groups to integrate feedback on the questions and cases, ultimately serving as the backbone for prospective studies on clinical decision making. Preliminary survey results are pending.

Research Grant: None
Student Support: UGA-Chanin Foundation

Mucin 4 is a marker of bronchitis and bronchiolitis in influenza A virus infection

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Influenza A virus (IAV) causes significant morbidity and similar pathology in the human and swine host. IAV infects epithelial cells in the respiratory tract of mammals causing a necrotizing bronchitis and bronchiolitis. Protecting these surfaces are glycoproteins called mucins which are major constituents of mucus. Mucins can act as serum biomarkers for neoplastic progression and more recently respiratory infections. Transmembrane mucins can inhibit microbial invasion, serve as decoy receptors, and activate signal transduction pathways. Mucin 4 (MUC4) is a transmembrane mucin that consists of an alpha subunit responsible for surface protection and intracellular beta subunit involved in signal transduction to repress apoptosis and stimulate epithelial proliferation. This study was designed to determine the expression and potential role of MUC4 during IAV infection. We used immunohistochemistry in combination with a machine learning image analysis to quantify differential expression of MUC4 subunits in IAV-infected and uninfected porcine lung. There was elevated expression of the alpha and beta subunit of MUC4 in the mainstem bronchi of IAV infected swine when compared to controls. MUC4 expression was only observed in bronchioles with necrotizing bronchiolitis. These data suggest that MUC4 levels in serum may indicate IAV infection and with refinement, MUC4 concentration and expression could act as a proxy for disease severity. Understanding how increased expression and altered sialic acid structures of MUC4 during IAV infection or other respiratory disease will facilitate control strategies by elucidating mechanisms associated with resistance or enhanced susceptibility to IAV.

Research Grant: 5030-32000-231-000D
Student Support: USDA ARS NBAF AGREEMENT NO: ARS 59-3022-1-003

Canine urine mycobiome after consuming a plant-based diet for a year

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Nutrition impacts microbial communities residing in the urinary tract and can therefore influence clinical outcomes. Whereas the canine microbiome has been studied in great detail, the mycobiome is a field that remains underexplored. To characterize the canine urine mycobiome in response to long-term feeding with a plant-based diet, we studied a cohort of 15 dogs for a year following a transition from a meat- to a plant-based diet. Urine samples collected through cystocentesis at baseline and endpoint (~365 days apart) were analyzed for fungal community content via ITS2 amplification and next-generation sequencing. Mycobiome community composition significantly differed after a year of plant-based diet consumption. At a broad level, the fungal families Cladosporiaceaea and Aspergillaceae significantly decreased and increased, respectively, in relative community abundance. More specifically, high relative abundances of the genera Cladosporium with a meat-based diet and Penicillium with a plant-based diet, respectively, were observed. Interestingly, Penicillium spp. derived metabolites possess antimicrobial properties while some lineages classified as Cladosporium spp. are considered significant allergens, suggesting that diet type (i.e., meat- vs. plant-based nutrition) may influence host-mycobiome interactions of relevance to clinical health outcomes in dogs . Our data provides the first indications of a urine mycobiome response to diet change in domestic canines and suggests that nutrition, perhaps with environmentally sustainable plant-based formulations, as shown here, may serve as a prophylactic approach to targeted modulation of urinary tract health in dogs and possibly other companion animals.

Research Grant: Boehringer Ingelheim
Student Support: WesternU CVM Veterinary Summer Research Program

Preliminary investigation of exercise-induced pulmonary hemorrhage in draft pulling horses in Atlantic Canada

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Exercise-induced pulmonary hemorrhage (EIPH) is a well-documented disorder occurring in horses undergoing high-intensity exercise. EIPH occurs when capillaries in the lungs rupture, causing varying degrees of bleeding in the lungs. While well-researched in racing horses, this disorder has not been investigated in competitive draft pulling horses or how it may affect their performance, health, and welfare. This study is a preliminary investigation into EIPH prevalence in the competitive draft pulling horse population competing on Prince Edward Island (PEI). From May-August 2022 approximately 40 study horses will undergo upper airway endoscopy approximately one-hour post-exercise at numerous competitions throughout PEI. Participating horses are selected from a volunteer population recruited at competitions, and during endoscopy each horse will be given a score for EIPH (scored 0-4), left laryngeal hemiplegia (LLH, scored 1-4), and tracheal mucus (scored 0-4). Any other upper airway abnormalities will also be noted in order to examine any associations with EIPH, as well as to note any welfare concerns. The preliminary data on 18 horses shows a sampled EIPH prevalence of 39%, with LLH at a sampled prevalence of 33%. Considering only the horses with these factors present, EIPH scores had a median of 1 (mode = 1, range = 1) while LLH had a median score of 2 (mode = 2, range = 1-4). The sampled median tracheal mucus score was 1 (mode = 1, range 0-3). Statistical analysis will be conducted to determine any associations between EIPH, LLH, and demographic and competition data. Based on the preliminary data, it appears that low-grade EIPH occurs in a subset of competitive draft pulling horses during competition.

Research Grant: PEI Department of Agriculture and Land
Student Support: AVC Veterinary Summer Research Award, Boehringer Ingelheim Veterinary Scholars Program

Effect of equine ID on hepatic metabolic gene expression in two experimental models relevant to EMS

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Little is known about function of the equine liver compared to other species, including its role in the pathophysiology of Equine Metabolic Syndrome (EMS) and insulin dysregulation (ID). Insight into the hepatic regulation of specific genes related to metabolism in response to diet and medical treatment will lead to better understanding how these variables affect the liver and its central role in insulin and glucose dynamics. qPCR for genes related to ID was performed on liver collected from equids subjected to two experimental models relevant to EMS. Liver tissue was collected from lean and obese ponies after 7 days of either low or high dietary carbohydrate feeding, and liver biopsies were collected from healthy adult light-breed horses before and after experimentally inducing ID with dexamethasone (DEX) and after treatment with AMPK agonists. GLUT1, ACACβ and PEPCK expression increased in response to DEX administration. Further treatment with DEX and one AMPK agonist (aspirin or metformin) increased expression of GLUT1, PEPCK and AMPK while PPARγ expression decreased. Combination therapy with metformin and aspirin significantly increased expression of PEPCK. After metformin treatment, PEPCK expression was increased while PPARγ was decreased. High-carbohydrate feeding resulted in decreased expression of COX2 in the liver and no significant changes in the other genes. GLUT1, PEPCK, COX2, AMPK and PPARγ should be further evaluated for their effect on the equine liver in naturally-occurring ID. Current treatment for ID involves preventative measures and management. Additional information about the pathophysiology of this condition will lead to novel therapeutic approaches and biomarkers to refine diagnosis and prognosis.

Research Grant: Grayson Jockey Club Research Foundation, The Ohio State University Equine Research Funds, Michigan State University College of Veterinary Medicine, and the Morris Animal Foundation
Student Support: NIH T35 OD010977

Identification of candidate polymorphisms for feline behavior differences

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The behavior of domestic animals has the potential to impact adoption rates, use as therapy animals, and euthanasia rates. In cats, most genetic behavior research has focused on heritability and associating factors, such as, breed and color with personality, but few studies are focused on specific genes. Here, three genes associated with behavior in humans and other species are investigated for potential polymorphisms affecting behavior in cats. *Monoamine oxidase A (MAOA)* is associated with increased aggression and disruptive behavior in humans, primates, and rats. *Dopamine receptor D4 (DRD4)* has alterations associated with novelty-seeking behavior and attention deficit hypersensitivity disorder in people, birds, and primates. *Serotonin transporter gene (SLC6A4;5-HTT)* is associated with schizophrenia and anxiety in humans, chickens, and primates. The 99 Lives cat genome sequencing dataset, which includes data from 336 cats of various breeds with different behaviors, was analyzed to identify candidate polymorphisms that may be causing differences in personalities across cat breeds. Deleterious variants in *SLC6A4*, *DRD4* and *MAOA*, as well as a variable number of tandem repeats (VNTRs) in *DRD4* exon 3, which is in humans and cats, will be analyzed by Sanger sequencing and genotyped in various breed cats, particularly those with distinctive characteristics. The most promising variants can then be examined in more cats with more in-depth behavior scoring, which could lead to understanding genetic control of feline behaviors and better management for adoption and caregiving.

Research Grant: Gilbreath-McLorn Endowment
Student Support: Kent Tomazi Memorial Research Fund in Veterinary Medicine & an IDEXX BioAnalytics endowment

Dietary advanced glycation end-products in differently processed canine therapeutic kidney diets

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Dietary advanced glycation end products (dAGEs) are by-products of the Maillard reaction, which occurs during high heat processing techniques utilized in pet food production, such as extrusion for dry kibble and retorting for wet foods. One prior study showed that these processing methods resulted in a 122-fold daily increase in consumption of dAGEs by dogs as compared with intake by humans on a metabolic body weight basis. However, this previous study only examined growth and maintenance canine diets and did not include therapeutic diets. Given that, in humans, dAGEs are linked to various diseases such as chronic kidney disease (CKD), our study aimed to specifically evaluate dAGEs in differently processed canine therapeutic kidney diets (TKDs). The objective of this study was to quantify dAGE levels, specifically carboxymethyllysine (CML) and carboxyethyllysine (CEL), in these differently processed TKDs. It was hypothesized that more processed TKDs, such as dry (kibble) and wet (loaf and stew) food, would contain higher levels of dAGEs than less processed TKDs, such as fresh and raw food. Three adult maintenance canine diets (1 dry, 1 loaf, 1 stew) representative of the pet food industry were used as controls. A total of 16 TKDs (5 dry, 5 loaf, 2 stew, 4 fresh) were purchased and stored according to the manufacturers' instructions until dAGE quantification. Each sample underwent ultra-high-performance liquid-chromatography-tandem mass spectrometry (UPLC-MS) twice to quantify CEL and CML. The results of this study are pending.

Research Grant: This project was funded through both a research grant from the Companion Animal Nutrition & Wellness Institute (CANWI) and funding from the Department of Small Animal Medicine and Surgery.
Student Support: UGA Foundation, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Targeting corticostriatal circuitry involvement in impulsivity via chemogenetics and behavioral interventions

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This study analyzed the results of suppressing the prelimbic cortex, specifically through analysis of pyramidal cell activities between the prelimbic cortex and dorsal striatum which form a part of the corticostriatal circuit. This circuitry is of interest because of the unique integration of timing and choice behaviors. This pathway was analyzed using Designer Receptors Exclusively Activated by Designer Drugs selectively targeted by Clozapine N-Oxide during intervention or choice tasks. The effects of suppression during behavioral sessions can aid in addressing effective behavioral interventions that can be utilized in individuals with a predilection for impulsive behaviors, such as those seen in addictions, obesity, or ADHD. The hypothesis for this work is that suppression of the prelimbic cortex should disrupt the central timing circuit and increase impulsive choices, further emphasizing the important role the prelimbic system plays in timing and choice behaviors. 48 Sprague-Dawley rats went through a training period followed by stereotaxic surgery to infuse an active Designer Receptor Exclusively Activated by Designer Drugs or a Sham virus into the prelimbic cortex that was later activated by Clozapine N-Oxide during behavior testing that took place in 24 operant chambers outfitted with a lever system to deliver rewards in the form of food pellets. Histology was performed on the brain using a c-Fos assay to confirm placement and expression of the virus as well as suppression of neural activity.

Research Grant: National Institutes of Health Grants R01-MH085739 and P20-GM113109
Student Support: National Institutes of Health T35 Training Grant

Virulence factors and integron-encoded antimicrobial resistance in *Salmonella enterica* isolated from poultry

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Antimicrobial resistance (AMR) is an escalating global public health concern in *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium), an enteric pathogen known for its broad range of hosts, including food producing animals and poultry, along with its significant risk to food safety. In tackling the issues regarding AMR in *S*. Typhimurium, previous studies indicated that integron-encoded *S*. Typhimurium contain increased numbers and diversity of AMR genes; however, little is known about the association of virulence factor genes with integron-encoded *S. enterica*. Thus, the aim for this study is to understand the interrelationship between integrons, AMR genes, and virulence factors among *S*. Typhimurium utilizing whole genome sequencing (WGS) methods. For this study, poultry isolates (n = 26) were sourced from veterinary diagnostic laboratories at two separate institutes. Three of the 26 isolates were initially found to contain integrons, each with a length of 1000 base-pairs. The results show that the percentage of isolates with integrons have resistance to certain drugs such as beta-lactams, phenicols, and tetracyclines (100%, 66.7%, and 100%, respectively) more than those with no integrons (8.7%, 0%, and 21.7%, respectively). Virulence factor profile was similar among most isolates; however, AMR and virulence factor genes under bacteriocin class were different between isolates with and without integrons. Furthermore, both AMR and virulence genes were found to be more localized on chromosomes than on plasmids regardless of integron presence. Overall, these results can be utilized to better predict the pathogenicity of *S*. enterica based on the presence of integron-encoded AMR genes and virulence determinants among poultry.

Research Grant: USDA Animal Health & Disease through CRC grant from CVMBS
Student Support: CVMBS Veterinary Summer Scholar Program

Period-prevalence and distribution of *Babesia* species in thrombocytopenic dogs in the upper Midwest

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Babesiosis is a tick-transmitted zoonotic disease caused by hemotropic parasites in the *Babesia* genus. It affects a wide range of vertebrate hosts including humans and dogs, and human babesiosis became a reportable disease in Wisconsin in 2001. Historically, *Babesia* spp. infection in dogs was thought to be exceedingly rare in the upper Midwest. However, new studies in both dogs and humans suggest that babesiosis prevalence may be increasing. Now there is concern that *Babesia* spp. that infect dogs can infect humans as well. The prevalence and geographical distribution of *Babesia* spp. infection in dogs in the upper Midwest is unknown and it is possible that *Babesia* spp. infections in this geographic region go undetected or are misdiagnosed as other diseases with similar clinical presentations. It is important to determine these epidemiological factors for babesiosis due to its zoonotic potential and to provide veterinary medical professionals in the upper Midwest new information regarding *Babesia* spp. exposure in dogs. In this descriptive cross-sectional study to assess the period-prevalence, we used serum and EDTA whole blood samples from 209 thrombocytopenic dogs presented to University of Wisconsin Veterinary Care (UWVC), with a clientele throughout the upper Midwest, from March 2021-2022 to screen for *Babesia* spp. through broad and species-specific PCR of whole blood and indirect fluorescent antibody (IFA) assay of serum. We hypothesize that we will detect *Babesia* spp. infection in dogs in the upper Midwest. Using this data and travel history of affected dogs, we will determine the species of *Babesia*, prevalence, and geographic location of infections in thrombocytopenic dogs in the upper Midwest.

Research Grant: American Kennel Club Canine Health Foundation Grant No. 02978-A
Student Support: National Institutes of Health (NIH) T35 OD011078-12

Vaccine opinions and practices in people and animals

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Vaccinations are important tools to prevent and reduce disease in people and animals. Millions of lives have been saved or improved through their use. Despite this, it seems that vaccine hesitancy has been on the rise over the past few decades. More recently, veterinarians have been reporting vaccine hesitancy among pet owners as well. It is currently unknown how common this is and what factors play a role in vaccine decision making. The purpose of this study is to gain a better understanding of vaccine perceptions and how attitudes and practices regarding vaccines are correlated in people and animals. To investigate this, a survey regarding attitudes toward and practice of vaccination was developed, piloted, and IRB approved. The survey is currently being conducted online using Qualtrics software. It is being distributed to adults in the general population of the U.S. using Facebook advertising in order to reach a broad section of the population. Specifically, this study aims to 1) identify factors associated with individual perceptions on core and optional vaccines; 2) identify preferred sources of vaccine information; 3) determine the association between the way people vaccinate themselves, their children, and their animals; and 4) investigate whether individual views on vaccines changed during the pandemic. Once survey data is collected, descriptive and comparative analysis will be conducted using commercial software programs. The results will help identify the determinants of vaccine decision making. This knowledge is important as it could be used to tailor educational efforts so that people can make more informed decisions about preventive healthcare for themselves, their children, their pets and their livestock.

Research Grant: None
Student Support: Dr. Thomas Mack Global Health Fund

Getting to the meat about Salmonella: does antimicrobial susceptibility depend on intended use of the animal?

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Salmonella, a food-borne bacterium, causes the human enteric disease salmonellosis. Antimicrobial resistance (AMR) complicates the treatment and prevention of bacterial disease. The purpose of this project was to assess AMR trends in *Salmonella* isolates from meat products (ground beef, beef trim, and ground turkey) that went to the human food supply versus diseased or culled livestock diverted from harvest. Intended use of livestock affects the regulatory protocols that govern antimicrobial use in animals; the strictest regulations apply to livestock going to harvest. *Salmonella* samples from beef were recovered from beef products submitted to the Food Safety and Inspection Service by Illinois meat inspectors. *Salmonella* isolates from ground turkey were collected from a previous study. *Salmonella* isolates from cattle and poultry were retrieved retrospectively from cases submitted through University of Illinois Veterinary Diagnostic Laboratory. A broth micro-dilution technique (Sensititre) was used to measure antimicrobial susceptibility of *Salmonella* isolates. The sensitivity tests provided quantitative minimum inhibitory concentration (MIC) values based on host-specific antibiotics. The MIC values were used to build four separate MIC_{90} tables, two for cattle and two for poultry. We hypothesize that *Salmonella* serovars common to poultry or cattle that are going to harvest are more susceptible to antibiotics than serovars common to their diseased or culled counterparts. We also expect to find antibiotic susceptibility levels matching expected antibiotic usage. These data will be used to understand how certain drug classes used in livestock may contribute to empirical treatment failures in human gastroenteric disease.

Research Grant: None
Student Support: Office of the Director, NIH, T35 OD011145

The effect of quaternary ammonium compounds on estrogen and progesterone in pregnant mice

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Quaternary ammonium compounds (QACs) are common substances utilized throughout various industries for their antimicrobial, antistatic, and surfactant properties. QACs can be found in household cleaners, ophthalmic solutions, swimming pools, cosmetics, and a multitude of other consumer goods including hair and laundry products. Previous studies have shown that chronic QAC exposure can have reproductive, endocrine, and immunological effects in both mice and humans. Based on these studies, it was hypothesized that mice exposed to QACs will have decreased levels of progesterone and estrogen as compared to the control group that was not exposed to QACs. In this study CD-1 mice received either a dose of 60mg/kg/day of QAC through gel food or non-dosed gel food with 24 mice per group. After being bred, the pregnant mice were euthanized at 6, 8, 10, 12, 14, and 16 days gestation and blood was collected via intracardial draw. Plasma collected from these mice at various stages of gestation was then analyzed through ELISA to determine the levels of progesterone and estrogen. Based on the known reproductive effects of QACs, we expect to show decreased levels of both progesterone and estrogen in QAC exposed mice compared to controls. Due to how ubiquitous QACs are becoming, it is important to investigate the effects these compounds on the health of both humans and animals.

Research Grant: Summer Veterinary Student Research Program
Student Support: Virginia-Maryland College of Veterinary Medicine

Anxiolytic and sedative effects of diazepam on *Buteo jamaicensis* (red-tailed hawk) in a rehabilitation setting

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Buteo jamaicensis or red-tailed hawk (RTHA) is a raptor species prevalent throughout California. Because of their proximity to urban environments, they are often presented to rehabilitation centers, which can be very stressful. These stressors cause physiological changes even in short-term captivity, potentially lengthening healing times and affecting their welfare. To improve their rehabilitation success, diazepam has been used for its sedative and anxiolytic properties. The purpose of this project is to assess the effects of diazepam by measuring corticosterone levels and viewing the behavioral changes of RTHAs in a rehabilitative setting after being dosed with diazepam. Diazepam is a benzodiazepine used in birds that produces little respiratory/cardiac depression, is reversible, and has short recovery periods. The subjects chosen will be juvenile or adult RTHAs that are not on prescribed medications that interact with diazepam and have fully recovered from their presenting illness. They will be given either diazepam or a placebo orally and then will be video recorded in an isolated cage. Their behavior will be assessed using an ethogram and blood and fecal samples will be collected to measure corticosterone. Videos will be scanned at 3-minute intervals, recording the presence or length of specific behaviors. The birds will act as their own control and the analyst will be blinded as to whether the bird was medicated. The findings will be statistically analyzed to draw correlations between behavioral changes among the subjects as well as in corticosterone levels. We hope this research will provide evidence as to whether diazepam is a successful sedative and potential anxiolytic for raptors undergoing rehabilitation.

Research Grant: None
Student Support: WesternU University Research Office's 2022 Summer Student's Fellowship Program

FGF8-induced murine joint regeneration is independent of *Prg4* expression, but is derived from the *Prg4*-lineage

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Humans and mice have limited regenerative capacity restricted to amputations of the digit tip, the distal phalanx (P3), whereas more proximal amputations, such as amputation of the middle phalanx (P2), fail to regenerate. Previous studies have shown that the introduction of exogenous BMP9 to a healing P2 amputation wound induces synovial joint regeneration. BMP9-induced joint regeneration requires the expression of Proteoglycan 4 (*Prg4*). *Prg4* encodes for the proteoglycan Lubricin which is necessary for normal joint homeostasis, but not embryonic joint development. Here, we report the identification of another potent inducer of synovial joint regeneration, FGF8. Following P2 amputation, FGF8 induces a multi-tissue regeneration response characterized by bone, articular cartilage, synovial cavity, tendon, and ligament regeneration. To test the hypothesis that the FGF8-induced joint is derived from the *Prg4*-lineage and that *Prg4* is required for this response, P2 amputations were performed on male and female neonatal $Prg4^{GFPCreERt2+/-}$;Ai9, and Prg4 null mice at postnatal day 3 (PN3), followed by implantation of FGF8 (500ng/μl) or BSA (0.1% in PBS) coated beads at PN7. Lineage tracing studies using $Prg4^{GFPCreERt2+/-}$;Ai9 mice show that the FGF8-induced *Prg4*-lineage gives rise to the regenerating chondrocytes, the regenerating tendon and ligament, as well as the synovial cavity. Unlike BMP9-induced joint regeneration, *Prg4* null mice demonstrate *Prg4* is not required for FGF8-induced joint regeneration. These data suggest that BMP9 and FGF8 can induce synovial joint regeneration at amputation wounds, but the response is driven by two distinct mechanisms to ultimately regenerate the same structures.

Research Grant: Texas A&M University
Student Support: NIH T35OD010991-17, Texas A&M School of Veterinary Medicine and Biological Sciences

Effects of COX and soluble epoxide hydrolase inhibition on histopathology and gliosis in murine arthritis

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Osteoarthritis (OA) is a pervasive and painful condition that lacks a pharmacological treatment that can both provide antinociception, as well as slow disease progression. Our previous results have shown that there are similar decreases in the development of mechanical hypersensitivity between soluble epoxide hydrolase (sEH) and cyclooxygenase (COX) inhibition. The purpose of this study was to compare the impact that extended oral treatment with inhibitors of each enzyme had on histopathology and gliosis. Arthritis was induced via injection of type IV collagenase into the left knee. Mice were then treated with a sEH inhibitor, COX inhibitor, or both, with some left untreated as a control. It was hypothesized that sEH inhibition would have a positive impact on joint pathology based on previous research showing prevention of apoptosis in chondrocytes. Furthermore, sEH and its metabolites have been shown to localize to astrocytes, suggesting that there may be a relationship between astrocyte activation in neuroinflammation and the antinociceptive properties of sEH inhibition. Histopathologic results showed a significant negative impact among mice treated with the COX inhibitor carprofen. Mice treated with an sEH inhibitor did not show significant difference from the controls. Gliosis was assessed by immunohistochemistry of the collected spinal cords using glial fibrillary acidic protein as a marker for astrocyte activation. Results of the gliosis study are pending. Future research into the clinical effectiveness of sEH inhibition in naturally occurring OA is necessary to assess true impact on disease.

Research Grant: None
Student Support: Supported by Boehringer Ingelheim and the College of Veterinary Medicine

Gut epithelial adaptations to dietary, genetic, and surgical perturbation dissected at single-cell resolution

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As a critical site for nutrient absorption, barrier defense, and hormone secretion, the gut is central to the regulation of metabolic health. These diverse functions are coordinated by specialized absorptive and secretory cell lineages of the intestinal epithelium, which continuously differentiate from crypt-based stem cells. Under different metabolic contexts, epithelial function can be modified in a maladaptive or therapeutic manner, specifically by dietary intervention, genetic perturbation, or bariatric surgery. However, the mechanisms underlying these changes remain poorly characterized. To bridge this knowledge gap, we performed single-cell RNA-seq analysis of two murine models. First, our lab recently identified miR-375 as one of the most highly expressed microRNAs (miRNAs) in stem and secretory cells of the murine intestinal epithelium. Given the established and conserved role of miRNAs in regulating intestinal homeostasis, we profiled epithelial crypts and villi isolated from wild-type and miR-375 knockout mice fed either a low- or high-fat diet. We found that the loss of miR-375 exerts diet-specific effects on epithelial lineage composition and gene expression, which correspond with *in vivo* metabolic phenotypes. Second, we studied crypts and villi from a mouse model of metabolic disease subject to bariatric surgery. Preliminary results indicate that surgery rescues high-fat diet-induced changes in gene expression, notably related to mitochondrial function. Altogether, our findings represent the first high-resolution study of gut epithelial adaptations to genetic perturbation or bariatric surgery, which further our mechanistic understanding of metabolic disease in pursuit of more effective therapeutic options.

Research Grant: ADA 1-16-ACE-47 (awarded to PS), NIH R01-DK-121995 and ADA 1-19-IBS-252 (awarded to DS), NIH-K01-DK-129367-01 (awarded to KSK)
Student Support: NIH-F30-OD-031914-01 (awarded to KKL)

Effects of reproductive hormones on sperm-neutrophil-bindings under the influence of DNase I

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After insemination, neutrophils are vital in removing excess sperm from the female reproductive tract and clearing breeding-induced bacterial contamination via Neutrophil Extracellular Traps (NETs). However, premature activation or overexuberance of NET formation can result in the sperm being trapped and failing to reach the egg. The seminal plasma of the stallion contains a large amount of DNase I that can free entangled sperm from NETs which may improve fertility. Neutrophil function is also greatly affected by steroid hormones including the reproductive hormones estrogen and progesterone, as well as the stress hormone cortisol. There is currently limited knowledge on the mechanisms behind sperm-neutrophil interactions and thus, the objective of this study is to better understand the effects of hormones and pathological hormonal imbalances on the interaction between neutrophils and sperm. The goal is to further improve the treatment of persistent breeding-induced endometritis, a common problem in mares that results in infertility, by modulating sperm-neutrophil bindings. This project focuses on the sperm-neutrophil binding rates under the influences of DNase I, progesterone and estradiol as well as therapeutics such as Dexamethasone. We hypothesize that estradiol reduces the sperm-neutrophil binding rates and thus favors fertility, whereas hormonal imbalances might increase sperm-neutrophil bindings, leading to infertility. We hope to achieve this by using time-lapse analysis, binding assays as well as quantification of DNA release by staining. Further research needs to be done to be able to integrate this knowledge into diagnostics and treatments of infertility.

Research Grant: None
Student Support: Boehringer Ingelheim and the Cornell University College of Veterinary Medicine

The pathogenesis of and immune response to SARS-CoV-2 oral challenge in K18-hACE2 mice

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The first cases of SARS-CoV-2 were reported in December of 2019 and as of this year, the worldwide mortality reached 6.34 million citizens. The virus infects its host by binding to ACE2, receptors present in the lungs, intestines, kidneys, and blood vessels, explaining the non-specific symptoms that ensue. The primary mechanism of transmission is through inhalation of infected respiratory droplets. However, it is unclear if oral transmission, specifically the fomites that linger in the environment, poses just as much of a threat as intranasal transmission. The objectives of the present study were to see if oral gavage with SARS-CoV-2 infected K18-hACE2 mice and what type of immune response would develop due to an oral infection as opposed to an intranasal infection. Both male and female mice with a low gut microbiota diversity, aged 12-16 weeks, were used in this study. Mice were challenged by oral gavage of either 5×10^5, 5×10^6, and 5×10^7 copies (quantified by qPCR) of SARS-CoV-2. Mice were necropsied at either 6- or 14-days post infection for both tissue and blood collection. We have extensively characterized this model during intranasal challenge and expect that mice, more so males, will lose weight and develop clinical signs in a dose-dependent manner. Additionally, we expect to see high viral loads and pro-inflammatory cytokines, such as IL-1β, IL-6, IL-12, and IFN-γ, in the lungs, intestines, and brain, showcasing lymphocytic histology. Such results would indicate that oral transmission is just as likely to cause disease as intranasal transmission. This in turn would suggest that gut microbiota, in both humans and animals, should be investigated more thoroughly to determine susceptibility to SARS-CoV-2.

Research Grant: NIH U42 OD010918, Mutant Mouse Research and Resource Center; additional support by the MU Division of Research, Innovation, and Impact
Student Support: IDEXX-BioAnalytics

Purification Method for Tumor Suppressor Protein INI-1

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Atypical teratoid rhabdoid tumors (AT/RT) are aggressive, rapidly growing central nervous system tumors that typically affect children. Unlike many other cancers AT/RT has a normal karyotype, except for a mutation or deletion to the Integrase Interactor 1 (INI-1) gene. Genetic abnormalities of the INI-1 protein is correlated with the development of AT/RT. INI-1 is ubiquitous in eukaryotes and is known as a core component of the chromatin remodeling SWI/SNF complex, which is important for DNA-dependent cellular processes such as transcription, DNA replication, and DNA repair. However, details of they specific role of INI-1 in these processes are lacking. To understand how AT/RT develops, a greater understanding of the structure of INI-1 would be beneficial, since protein structure aids in the elucidation of protein function. To achieve this, the use of x-ray crystallography would allow for a highly detailed 3D image of INI-1. A 3D image would allow for further investigation into its function and interactions within the SWI/SNF complex. X-ray crystallography requires protein samples to be > 95% pure and at a 5 mg/ml concentration in order to form the crystal required. The focus of this study is to establish a method of purification for INI-1 which would allow for further investigation into its structure using x-ray crystallography. Protein purification is typically achieved empirically using multiple methods of chromatography such as affinity, ion exchange, or gel filtration. Our protein contains a his-tag therefore our initial approach will utilize affinity chromatography with a Ni-NTA resin. Further methods will be explored in series in order to achieve the purity and concentration desired for x-ray crystallography.

Research Grant: NIFA Hatch OKL03167
Student Support: Oklahoma State University CVM

Economic impact and factors influencing metaphylactic choice to control BRD in feedlot cattle

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Metaphylactic antibiotic use in feeder cattle is a common practice to control disease. Proper antimicrobial stewardship is important to ensure continued antimicrobial efficacy and to protect animal welfare. The project objective is to identify characteristics of feeder cattle cohorts that benefit from metaphylaxis when economics are used to measure differences in health outcome. Cohorts (n = 12,785) from 13 feedlots were given a standard entry date, with pricing based on weight categories, and each of three metaphylactic options: no metaphylaxis, a low cost/low efficacy metaphylaxis, or a high cost/high efficacy metaphylaxis. An economic model was developed to compared net returns of each cohort across the three metaphylactic options. Logistic regression models that included covariates for entry weight, sex, average daily weight gain, number of animals in the cohort, and days on feed, with feed yard as a random effect, were used to determine the model-adjusted probability of a cohort benefiting economically from metaphylaxis. Most (72%) of cohorts did not benefit economically from metaphylaxis. Entry weight was important with the lowest weight category (181-227 kg) having the highest probability of benefiting from metaphylaxis (59%); whereas cohorts that arrive at 454-499 kg had the lowest probability (5%) of benefiting from metaphylaxis. Steers were more probable to benefit from metaphylaxis compared to heifers. Results illustrate that cattle cohort demographics influenced the probability that cohorts would benefit economically from metaphylaxis and the type of metaphylaxis utilized.

Research Grant: Foundation for Food and Agriculture; ICASATWG-0000000041
Student Support: FFAR Veterinary Student Research Fellowship

The effect of the reproductive cycle stage on equine endometrial organoids

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In order to study the physiology and pathologies of the endometrium *in vitro*, hormone-responsive equine endometrial organoids have been established, using endometrial biopsies. However, the effect of the hormonal milieu (reproductive cycle stage) during sample collection on the development and function of the endometrial organoids remains unknown. The purpose of this study was to determine whether the reproductive cycle stage (estrus versus diestrus) of the mare at the time of the biopsy influences organoid growth rate, morphology, or viability. We hypothesized that there are differences between estrus and diestrus driven organoids. Endometrial biopsies were collected during estrus and diestrus (n = 5). Single-cell suspension was generated from the tissue and was cultured in Matrigel and DMEM-based expansion medium. Organoid morphology was assessed with brightfield microscopy, the growth rate was determined by the number of days until passage, and viability was assessed by fluorescent staining with Hoechst and Propidium iodide, demonstrated as a ratio of the live cells to dead cells with ImageJ. Data was compared using a paired two-tailed T-test. There was no significant difference in days to passage 1 and 2, respectively, between estrus (P1: 4.6 ± 0.55, P2: 4.8 ± 0.45) and diestrus (P1: 5 ± 1.22, P2: 4.8 ± 0.84; $P > 0.05$). No significant difference was observed in viability% between estrus (69.04% ± 17.97%) and diestrus (65.28% ± 20.98%; $P > 0.05$). Organoids from mares in estrus and diestrus showed similar morphology. These findings show that there is no difference in organoid developmental capacity between estrus and diestrus-driven samples.

Research Grant: The Horse Foundation
Student Support: Boehringer Ingelheim

Impact of variable egg incubation regimes on hatchling American alligator external genitalia

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American alligators (*A. mississipensis*) undergo temperature-dependent sex determination; nest incubation temperature regulates gonadal differentiation. Eggs incubated at a constant 33°C produce exclusively testes/male hatchlings, at a constant 30°C results in ovaries/female hatchlings. Testicular androgens putatively cause the previously undifferentiated cliterophallus (CTP) to develop into penis, while absence results in clitoris development. However, the effect of incubation regime variations on genital morphologies requires further clarification. We characterized the morphological divergence of hatchling alligator genitalia CTP morphological features soon after hatching. We hypothesized that incubation environment variation is associated with subtle, but quantifiable variations of the hatchling CTP morphology. Alligator eggs were incubated at five temperature regimes: 30°C, 31.2°C, 33°C, 31.2 ± 0.6°C and 31.2 ± 2.8°C. CTP were dissected from 7-day post hatch alligators, digitally photographed, and images were landmarked. We analyzed landmarks using Procrustes Superimposition, Principle Components Analysis, MANOVA, and Discriminate Function Analysis. Additionally, pigmentation on the ventral aspect of the glans was measured semiquantitatively. Elevation in egg incubation temperature above 30°C results in an elongated genital shape and decreased pigmentation, regardless of gonadal sex. Thus, genital development is semi-independent of gonadal sex in alligators. The ambiguity in genital morphology at this early stage may be due to egg incubation environment variation resulting from the complexity of the alligator nest, ambient temperature fluctuations, or other environmental factors.

Research Grant: None
Student Support: Stephens College

Mouse Allergen Exposure on Single-Use Versus Multi-Use Disposable Gowns

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Disposable gowns are essential protective equipment against exposure and spread of rodent allergens. The widespread shortage of disposable gowns during the SARS CoV-2 pandemic necessitated reuse of these gowns at some institutions. Even though gowns are now readily available, there are cost saving and decreased waste benefits for continued reuse post pandemic however, this raises concern for allergen exposure. This study is designed to measure the presence of mouse allergen, Mus m1, on single-use versus multi-use disposable gowns worn during routine clinical case management, using an animal transfer station at our barrier mouse facility. We hypothesize that the reuse of disposable gowns during clinical case handling increases the risk of exposure and allergen load of Mus m1. To simulate clinical case management, a staff veterinarian opened and examined mice in 10 cages, daily, for 3 days: reflecting the daily average number of clinical cases at this facility. For the multi-use condition, disposable gowns were reused for 3 days within 1 week and for single-use, disposable gowns were used for 1 day. Two sites were sampled on each gown: an area of the right arm, and the center of the front torso. All groups had 3 samples (3 arm sites single-use, 3 torso sites single-use, 3 arm sites multi-use, 3 torso sites multi-use). Gown samples were collected by cutting a 10x10 cm square from the sample site. Samples were processed using the Multiplex Array for Indoor Allergens. Additionally, Charm PocketSwab ATP tests were performed at the same sites to detect the level of organic matter. Thus, we aim to statistically analyze the data collected to understand the potential risks of Mus m1 exposure during the reuse of disposable gowns.

Research Grant: Center for Animal Resources and Education (CARE)
Student Support: American Society of Laboratory Animal Practitioners (ASLAP)

The Role of Advanced Glycation End Products in neurodevelopment and neurodegeneration

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Advanced glycation end products (AGEs) are a group of chemical compounds produced by non-enzymatic reactions between reducing sugars and proteins, lipids, or nucleic acids. AGE introduction into the body can derive from exogenous or endogenous sources. Exogenous sources primarily include high-fat foods sweetened with sugars and fried at high temperatures, while endogenous sources include products of glycosylation pathways. Adverse effects of AGEs on tissues occur through non-receptor and receptor-mediated mechanisms. In the receptor-mediated mechanism, the interaction of AGEs with the receptor for advanced glycation end-products (RAGE) elicits several pathological conditions, most notably diabetes. However, little is known about the impact of AGE accumulation on neurodevelopment. The aim of this study was to determine the various effects of AGEs on a neuronal network containing neurons, astrocytes, and microglia, that was generated from human-induced pluripotent stem cells with a stem cell neuronal differentiation platform. Immunostaining of the neuron, microglia, and astrocyte markers indicated that increased AGE concentrations correlated with decreased neurodevelopment and neuronal populations, along with increased amyloid beta accumulation. Quantitative measurements from confocal microscope images and real-time PCR further confirmed that AGE exposure increased RAGE and inflammatory microglia marker M1 expression, activation of NLRP3/Caspase1 inflammasomes, and inflammatory cytokine IL-1b production. These results showed that increased AGE accumulation could inhibit neurodevelopment and promote neurodegeneration mediated by microglia activation.

Research Grant: Western University of Health Sciences Intramural Grant
Student Support: Boehringer Ingelheim

Investigation into the associations of quality of life, euthanasia, and the veterinary medical record

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Previous studies have found that the use of quality of life (QOL) instruments provided to owners can be a useful tool for both clients and veterinarians with end-of-life discussions and decisions. Little is known about how often QOL is documented in the veterinary electronic medical record (VEMR) and how discussions of QOL may influence the decision to euthanize. The aims of this study were to first gain new data about owners' perceptions of the experiences of their dogs' euthanasia and owners' feelings around the death of their dogs, and second, to investigate the frequency of QOL documentation in the VEMR. A novel survey was developed to capture owners' thoughts around the death of their dogs while previous responses to the Dog Aging Project's End of Life Survey instrument were used to analyze the VEMRs of 70 euthanized dogs and 70 dogs who experienced unassisted death for specific mentions of QOL, QOL instruments, and owner concerns around QOL. QOL was more likely to be mentioned in the VEMRs of euthanized dogs compared to those who experienced unassisted death as determined by logistic regression. We found no statistical association between standardized QOL instrument use and the choice of euthanasia due to a very small number of VEMRs reporting a standardized QOL instrument. We also looked at demographic factors associated with euthanasia and found that older dogs were more likely to be euthanized than die an unassisted death, but factoring in QOL it is more likely this is due to QOL discussions and not the age of the dog. QOL instruments are an underutilized tool and more veterinarians should consider using standardized QOL surveys to help owners with end of life decisions.

Research Grant: U19AG057377 from the National Institute on Aging (NIA)
Student Support: NIH T35OD010991-17, Texas A&M School of Veterinary Medicine & Biomedical Sciences

DMH1 attenuates tumor growth of chemo-resistant prostate cancer in the mouse xenograft

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Chemo-resistance is a major therapeutic problem in patients with prostate cancer (PCa). The drug docetaxel (Taxotere) has become the mainstay for patients with metastatic PCa, but chemo-resistance usually develops within a year. The molecular mechanism underlying PCa chemo-resistance is poorly understood, and effective therapies are not available. Previous studies have shown that up-regulation of bone morphogenetic protein (BMP) signaling is closely associated with PCa chemo-resistance. The purpose of this study is to define the roles of BMP signaling in PCa cell chemo-resistance and determine if BMP signaling inhibitor DMH1 can re-sensitize chemo-resistant PCa cells to docetaxel. This study employed a mouse xenograft model, where immunocompromised mice were injected with chemo-resistant PCa cells. The mice were separated into 4 groups and were treated with either saline, docetaxel, DMH1, or both docetaxel and DMH1. After the animals were sacrificed, tumor tissues from each group were weighed and examined through immunohistochemistry using Ki67 as a marker for cell proliferation. RT-PCR was performed on tumor tissues from each group to verify if DMH1 treatment down-regulated the mRNA levels of Id1/Id2/Id3, the direct target genes of BMP signaling. Results from these tests showed that there was a statistically significant decrease in tumor weight and cell proliferation along with a decrease in Id expression for the mice treated with both docetaxel and DMH1 versus the other groups. These findings support the hypothesis that BMP signaling up-regulation is implicated in PCa chemo-resistance and that DMH1 can re-sensitize chemo-resistant PCa cells to docetaxel.

Research Grant: Elsa U Pardee Foundation
Student Support: WesternU CVM Veterinary Summer Research Program

Examination of the IgG Fc receptor CD16A on canine NK cells

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Natural killer (NK) cells are lymphocytes of the innate immune system capable of killing tumor cells and virally-infected cells. Due to the potent cytotoxic capabilities of NK cells as well as the potential to be safer as an adoptive cell therapy for cancer patients than T cells, NK cells are promising candidates for the treatment of various malignancies. One means by which NK cells kill target cells is by antibody-dependent cell-mediated cytotoxicity (ADCC). This process involves the recognition of the Fc portion of immunoglobulins attached to target cells by Fc receptors on the surface of NK cells. CD16A (FcγRIIIA) is the sole IgG Fc receptor expressed by human NK cells that mediates ADCC. At this time, little is known about CD16A on dog NK cells. We were the first to produce monoclonal antibodies (mAbs) that recognize canine CD16A (cCD16A) and characterize its expression on dog leukocytes. We show that canine CD16A is expressed on NK cells and what appears to be NK T cells. CD16A is a potent activating receptor on human NK cells. Our study is focused on further examining dog NK cells and their activation by CD16A. This will be accomplished by attaching an anti-CD16A mAb to the surface of microwell plates to crosslink CD16A on transduced cells expressing cCD16A and enriched dog NK cells. The purpose of these studies is to provide a better understanding of the underlying mechanisms of ADCC by dog NK cells, and how to stimulate these cells to enhance their function and proliferation for therapeutic purposes. The use of adoptively transferred dog NK cells and tumor targeting antibodies may provide an important combination therapy to treat various types of cancer in dogs.

Research Grant: University of Minnesota Signature Programs Comparative Medicine
Student Support: NIH, Office of the Director, Award Number T35OD011118

Pathogenesis and Persistence of Lung Injury in Diabetic Mice repeatedly exposed to Ozone

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Epidemiological studies suggest that people with metabolic diseases are particularly prone to adverse health effects of air pollution. We recently reported that diabetic KKAy mice have more severe lung injury than nondiabetic C57BL/6 mice after repeated exposures to ozone, a common gaseous air pollutant. In the present study we further elucidated the pathogenesis of ozone-induced lung injury and resolution in KKAy mice as compared to C57BL/6 mice. Male mice of both strains were exposed to 0 or 1 ppm ozone for 4, 8 or 12 consecutive weekdays, 4 h/day, and euthanized 1-day postexposure (PE). Another group of these mouse strains were exposed for 12 weekdays but sacrificed 26 d PE (recovery group). Lung tissue was prepared for light microscopic, immunohistochemical and morphometric analysis. Minimal lung lesions were present in ozone exposed C57BL/6 mice. Lesions were restricted to centriacinar regions and resolved by 26 d PE. In contrast, ozone exposed KKAy mice had multifocal, necrotizing alveolitis that expanded beyond centriacini to more distal alveolar parenchyma. There was a time dependent increase in severity of alveolar histopathology that included necrosis and loss of alveolar type I epithelial cells, influx of eosinophils, fibrin accumulation, hemorrhage, proliferation of alveolar type II epithelial cells and alveolar transitional epithelial cells, and interstitial fibrosis. KKAy recovery mice had resolving yet persistent epithelial, inflammatory, and interstitial lung lesions. These findings give biological plausibility to the epidemiologic suggestion that people with diabetes are particularly susceptible to health effects of air pollution.

Research Grant: Albert C. and Lois E. Dehn Endowment in Veterinary Medicine at Michigan State University
Student Support: NIH Grant 5R25HL103156-12 to Michigan State University

Interplay between environmental exposures and *Staphylococcus aureus* infections in diabetic wound healing

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There are many known risk factors that contribute to pathogenesis of type 2 diabetes, and recent studies have shown a positive association between exposure to persistent organic pollutants and the increase in prevalence. A common comorbidity of type 2 diabetes are foot ulcers that often become infected, with the most isolated organism being *Staphylococcus aureus*. The goal of our study is to determine potential effects of exposure to organochlorine pesticides on wound formation and resolution in diabetic patients. In our study, we dosed healthy male C57BL/6J and male diabetic Tallyho mice with a mixture of the following organochlorine metabolites: dichlorodiphenyldichloroethylene (DDE), trans-nonachlor, and oxychlordane (referred to as DTO), formed pressure-induced wounds using two circular magnets on the dorsal skin causing ischemic-reperfusion injury, and measured the wounds up to 12 days post wounding. This method was repeated in another cohort with the addition of *S. aureus* infection. The results showed that wound resolution decreased in diabetic Tallyho mice that were treated with DTO. Wound areas were also significantly increased in DTO treated, *S. aureus* infected diabetic animals at day 1 post wounding/inoculation compared to uninfected vehicle and uninfected DTO treated animals. In conclusion, our study suggests that exposures to environmental pollutants have an impact on diabetic wound healing and can be used in future research for different treatment options or clinical settings to help assess patients for risk of developing foot ulcers and their ability to heal or avoid infections.

Research Grant: NIH grant R21ES030786; NIH grant P20GM103646
Student Support: NIH grant T35OD010432

Irisin: a survey in domestic animals

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Irisin is a 112-amino acid peptide hormone that is cleaved from fibronectin type III domain-containing protein 5 (FNDC5), a type I transmembrane protein abundantly found in muscle tissue. Irisin is a putative mediator of the benefits of exercise, neuroprotection, bone growth, and cardiac health. Yet, few studies have focused on irisin in domestic animals. To address this, we first conducted an in silico survey of the irisin (peptide) sequences from domestic animals. The irisin sequence is identical between humans, mice, rats, dogs, and horses. To detect the presence of irisin in tissues, total RNA and protein were extracted from skeletal muscle samples of pigs (n = 5) and ducks (n = 10). RT-PCR analysis found FNDC5 mRNA in all pig and duck skeletal muscle samples. An approximately 25 kDa band representing irisin was detected in both pig and duck skeletal muscle. Fluorescence immunohistochemistry using a rabbit monoclonal FNDC5/irisin primary antibody and a goat polyclonal anti-rabbit secondary antibody found FNDC/irisin-like immunoreactivity in the muscularis region of pig, dog and cat stomach tissue sections. We also attempted to measure circulating levels of irisin in horse, pig, and duck serum using a multispecies ELISA kit. However, the assay was not sensitive enough to detect FNDC5/irisin in the samples tested, which warrants retesting using more sensitive assays. Our results provide important information to support the presence of irisin in several domestic animals. This helps pursue irisin as a biomarker or a therapeutic agent in veterinary medicine for diseases in domestic animals.

Research Grant: Saskatchewan Agriculture Development Fund, University of Saskatchewan
Student Support: Boehringer Ingelheim, Natural Sciences and Engineering Research Council of Canada

Single cell sequencing and cytokine analyses reveal insights into equine osteoarthritis progression

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Osteoarthritis (OA) is one of the most common disorders treated in equine practice, affecting ~33% of horses in the US. OA progression is increasingly thought to be a multifactorial disease process wherein the innate immune system plays a role in perpetuating low-grade inflammation. Improved understanding of the transcriptomic response of cells in the joint in OA progression will facilitate development of targeted therapies tailored to disease stage. We hypothesized that in-depth analysis of the functional shift in cytokine production combined with single cell RNA sequencing (scRNA-seq) would identify temporal changes in cellular heterogeneity and subpopulations of immune and synovial cells within a diseased joint. We examined the early immune response using an expanded multiplex panel of cytokines and correlated that to the transcriptomic response of cells within the joint using synovial fluid collected from a horse 70 days following OA carpal chip model induction compared to normal fluid. The functional immune response was characterized using a fluorescent bead-based multiplex assay (23 cytokines) to determine synovial cytokine levels. Transcriptome wide scRNA-seq and analysis was performed on cells isolated from synovial fluid. Interestingly, preliminary findings showed differentially expressed genes in OA vs normal joints with an upregulation of CCL2 (monocyte recruitment marker) and FABP5 in osteoarthritic fluid and upregulation of MARCO (anti-inflammatory marker) in healthy synovial fluid. These findings support further horse enrollment to classify the temporal immune response and its role in the pathophysiology of OA with the overall objective of developing treatments tailored to disease stage.

Research Grant: Foundation for the Horse, USDA Animal Health and Disease Funds, CSU College Research Council, NIH/NCATS CTSA 5TL1TR002533-02, NIH 5T32OD010437-19, Carolyn Quan and Porter Bennett
Student Support: Veterinary Summer Scholars Program Grant; CVMBS Dean's Office

Effect of VTA glutamate neuron excitation and inhibition on behavior in a schizophrenia mouse model

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Schizophrenia and related developmental neuropsychiatric disorders affect about 1.1% of the world's population across all sex, ages, and backgrounds. The neural circuits impacted in schizophrenia are broad and involve multiple neurotransmitter systems in the cortex, subcortical regions, midbrain, and brainstem. Understanding the neural circuit mechanism for specific behavioral phenotypes, and targeting these circuits to rescue cognitive performance, is innovative and will advance our understanding of disease mechanisms and approaches to intervention. The aim of this study is to assess the effect of ventral tegmental area (VTA) glutamate neuron excitation and inhibition on habituation behavior in a schizophrenia mouse model (129S1/SvImJ) compared with a wild type strain (C57BL/6J). In order to achieve this objective, mice were injected with either an excitatory or inhibitory DREADD adeno-associated virus (AAV) into the VTA to target glutamate neurons. To assess habituation behavior, an olfactory habituation test was used in which mice were introduced to a novel odor three consecutive times. During each presentation, movements of the mice were recorded to assess frequency and duration of visits to the odor as a measure of their rate of habituation. Baseline results were collected from each mouse, a week after stereotaxic injections. Following expression of the DREADD virus after 21 days, compound 21 was injected, and olfactory habituation test results were collected again. Work is ongoing, and the results of the olfactory habituation test will be compared across strain, gender, and virus.

Research Grant: CBS Bridging Grant
Student Support: Kenneth F. Burns Trust

Quantification of *ex vivo* neutrophil extracellular trap formation in horses and foals

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Bacterial sepsis and systemic inflammatory response syndrome (SIRS) are associated with high morbidity and mortality in foals. Neutrophils phagocytose, degranulate, and release neutrophil extracellular traps (NETs) to eliminate pathogens. NETs are lattices of extracellular DNA, histones, and myeloperoxidase (MPO) that immobilize microbes. Excessive NET release (NETosis) contributes to increased mortality in SIRS and sepsis. Studies in humans, rodents, and dogs documented associations between increased NETosis, sepsis severity, and organ dysfunction, but the role of NETs in equine sepsis is not known. Images from our lab show that healthy horse neutrophils undergo NETosis in response to *ex vivo* exposure to bacterial antigens. However, to determine if NETosis varies with age, disease severity, and prognosis, methods to quantify equine NETs are needed. The objective of this study was to use an equine MPO ELISA assay as a first step to quantifying induced NETosis in equine neutrophils. We hypothesized that treating equine neutrophils with various antigens increases MPO release. Neutrophils from healthy horses (n = 3) and foals (n = 4) were stimulated with killed whole-cell *Staphylococcus aureus*, phorbol myristate acetate, lipopolysaccharide, or media alone. MPO release was quantified with a commercial equine MPO ELISA. Our data suggest that MPO release from equine neutrophils increases in response to *ex vivo* exposure to bacterial antigens. We suspect that this increased MPO release is associated with NETosis, but MPO release alone is not unique to NETosis. Thus, we will use this MPO ELISA to develop an equine-specific sandwich ELISA to quantify MPO-DNA complexes to confirm the observed increase in MPO release is due to NETosis.

Research Grant: None
Student Support: AVMA/AVMF 2nd Opportunity Summer Research Scholarship

Drug discovery for diabetes mellitus by targeting feline amylin

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Amyloid deposits have been detected in the majority of feline diabetic patients. These deposits originate from islet amyloid polypeptide (IAPP or amylin). Amylin is a normal satiety hormone that is produced and co-secreted with insulin by beta-cells, which are the most common cell type in the islets of Langerhans in the pancreas. However, amylin misfolding leads to the development of amyloid deposits, which have been associated with beta-cell death during the progression of diabetes. IAPP aggregation can be inhibited by several molecular entities such as silibinin and resveratrol. However, these agents have poor bioavailability and cause a variety of pharmacological effects. Currently, there is no commercially available drug treatment to stop or prevent pancreatic amyloidosis in diabetes mellitus. The goal of this project is to identify inhibitors of feline IAPP (fIAPP) fibril formation, and to demonstrate that the aggregation of fIAPP can be modulated by IAPP-interactive compounds in vitro. Three series of urea-based compounds were developed for this purpose, and their ability to reduce the formation of fibrils from IAPP was assessed in vitro using biophysical methods such as Thioflavin T (ThT) fluorescence assays, dynamic light scattering, and transmission electron microscopy (TEM). Six potent inhibitors of IAPP fibril formation were identified. This study has the potential to point toward new therapeutic strategies for type 2 diabetes in cats.

Research Grant: EveryCat Health Foundation (Winn Feline Foundation, W20-020)
Student Support: Purdue University College of Veterinary Medicine and Boehringer Ingelheim Animal Health

Leishmania major inactivation using riboflavin and ultraviolet light

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Leishmaniasis is a vector-borne, parasitic disease that currently has no licensed vaccine for use in humans. The World Health Organization estimates over 350 million people are at risk of infection and about 700,000-1million new cases and 20,000-30,000 deaths are reported annually. L. major is a cutaneous strain of the disease. This study seeks to employ Mirasol, a pathogen reduction technology, to produce a killed whole parasite vaccine. Mirasol uses riboflavin and ultraviolet light to reduce pathogen load in blood products. Based on previous work with other pathogens, we hypothesized that 1J/ml would inactivate a parasite load of up to1e6 (or 10^6) parasites/ml in media, using the Mirasol device. By exposing aliquots of L. major to increasing concentrations of energy from the Mirasol device, then checking these samples for regrowth of parasites in one week intervals, the efficacy of parasite killing by the Mirasol device may be determined. By completing a kinetic curve we have demonstrated the 1J/ml dose completely inactivated all parasites and no parasite replication has been seen for 7 days. Successful inactivation of L. major parasites could be a forerunner of producing a killed whole parasite vaccine against leishmaniasis.

Research Grant: None
Student Support: Veterinary Summer Scholars Program at Colorado State University

Using blood meal analysis to understand insect-host dynamics impacting *Culicoides*-borne diseases

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Blood meal analysis is a valuable research tool in which DNA in the blood meal of a hematophagous insect is used to identify which animals are being fed upon in nature. This tool is integral in understanding the dynamics between hosts and insect determining the vectorial capacity of vector populations and improving the effectiveness of vector control measures. *Culicoides spp.* biting midges are known vectors of a variety of pathogens that include multiple arboviruses of veterinary and public health importance. Dispersion of vector-borne diseases can have a detrimental impact on both agricultural and wildlife animal populations leading to economic and ecological hindrances. The purpose of this study was to gain knowledge on the interactions of midges and hosts and to make inferences about the vector potential of midge species present in the lower Riley County, Kansas area. To do this, host DNA was extracted from the blood meals of 166 field-collected female midges from eight species. DNA was amplified by PCR targeting mitochondrial genes followed by verification of amplicons by agarose gel electrophoresis and Sanger sequencing. NCBI BLAST was used to match host sequences to a species at the ≥95% identity match level, allowing for the identification of host choice and patterns in biting habits based upon location and species. The viability of the sequencing results based upon the size and degradation of the sample collected was also evaluated to improve our understanding of the sensitivity of detection of this method. The results and conclusion of this study will be further discussed and presented at the symposium.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Clinical and pathologic features of feline infectious peritonitis (FIP) in senior cats

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Feline infectious peritonitis (FIP) has a reported bimodal age distribution with a second peak incidence in senior cats. However, variability in disease manifestation, comorbidities, and higher ranked differentials make diagnosis in senior cats challenging. Despite this, clinical, clinicopathologic, gross, and histological features of FIP in senior cats have not been described. The aim of this study is to characterize these features in FIP+ senior cats compared to FIP+ younger cats. We hypothesized that FIP+ senior cats would have unique characteristics to aid in diagnosis. Search of the OSU-CVM Applied Pathology archives identified 503 FIP suspects for review. Cases were considered FIP+ if pathognomonic lesions were noted in the pathology report, resulting in 233 confirmed cases to date. Reports were analyzed for signalment, presence of effusion, effusion volume/locations, and affected tissues. Kittens were most often affected (n = 117), followed by young adults (n = 80), mature adults (n = 21), seniors (n = 10), and unknown-age cats (n = 5). Trends in senior cats were similar to those in other age groups and included over-representation of males and mixed-breed cats with enrichment in the population by purebred cats. The majority of both senior (n = 8/10, 80%) and younger cats (n = 148/223, 66%) had effusions, with unicavitary and peritoneal effusion predominating. On gross evaluation, the liver was the most commonly affected organ in both senior (n = 6/10, 60%) and younger (n = 101/223, 45%) cats. Gross renal involvement (n = 94/223, 42%) and icterus (n = 87/223, 39%) was more common in younger cats than in seniors (both n = 2/10, 20%). Investigation into clinical, clinicopathologic, and histologic features of FIP in senior cats is ongoing.

Research Grant: Megan Schreeg, Ohio State University Start-up Funds
Student Support: Dr. William M. Busey Endowed Summer Research Fund

The Epithelial-Mesenchymal Transition regulates the expression of CD73 in human breast cancer cell lines

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The epithelial-to-mesenchymal transition (EMT), which conveys epithelial (E) carcinoma cells to quasi-mesenchymal (qM) states, enables them to metastasize and acquire resistance to several chemotherapeutic regimens. In addition, we have recently demonstrated that the EMT program also confers resistance to immunotherapies. Specifically, qM tumors recruit immunosuppressive cells to their tumor microenvironment and are refractory to immunotherapy, while E tumors are sensitive. Furthermore, qM tumors express higher levels of CD73, (an ectoenzyme that generates immunosuppressive adenosine), relative to their E counterparts. Strikingly, abrogation of CD73 from qM carcinoma cells sensitizes otherwise refractory qM tumors completely to immunotherapy. Thus, while CD73 drives resistance of qM tumors to immunotherapy, the regulatory networks that control the expression of CD73 itself in qM but not E breast cancer cells are not well defined. Thus, the objective of this study was to determine whether activation of the EMT program results in a concomitant expression of CD73 in breast cancer cells. MCF7Ras cells were induced to undergo an EMT by doxycycline induced expression of EMT-transcription factors. Induction of an EMT lead to the loss of E-cadherin and gain of Vimentin. Most importantly, activation of the EMT program also resulted in elevated expression of CD73 as determined by western blotting and flow cytometry. This increase was also observed on cells that had undergone a partial EMT. Our results demonstrate a causal relationship between the EMT program and CD73 expression. Thus, targeting the CD73 signaling pathway in qM carcinoma cells may represent an attractive strategy to potentiate the efficacy of immunotherapies.

Research Grant: None
Student Support: Cornell University College of Veterinary Medicine

Monitoring Hemangiosarcoma Treatment Success with A Peripheral Blood Test

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Hemangiosarcoma (HSA) is a highly malignant neoplasm of the blood vessel-forming cells that is commonly found in canine species. To date, there are limited ways to monitor therapeutic efficacy beyond conventional staging based on physical examination findings, bloodwork, and imaging, which can lag behind disease progression. The current study examines the value of a novel test in monitoring tumor attrition and recurrence in HSA patients undergoing therapy. The Shine-On Suspicion (SOS) test is a flow cytometry and machine learning-based blood test that has been previously shown to detect HSA, or the risk of developing HSA in dogs with high sensitivity and specificity. The current study consists of 24 dogs diagnosed with histologically confirmed HSA following surgical resection of the tumor. Six dogs received standard chemotherapy following surgery. Eighteen dogs received experimental therapy with a bispecific ligand targeted toxin (eBAT), with or without standard chemotherapy. Blood samples were taken at various time points after surgery to undergo the SOS test. The test assigns a risk category to each sample based on previously established machine learning algorithms. A projection of these risk categories over time will be composed of for every patient. By comparing the risk category projection of each post-operative HSA patient over time and their corresponding clinical development, this study will determine whether the SOS test has the potential to be used for monitoring remission, predicting relapse, and potentially implementing alternative modalities that could improve HSA treatment success.

Research Grant: 02234-MOU and 02806-MOU from the AKC Canine Health Foundations, additional support from the Animal Cancer Care and Research Program, University of Minnesota
Student Support: College of Veterinary Medicine and Skadron Family Fund, University of Minnesota

The role of flea infestation in clinical manifestations of upper respiratory infection in household cats

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Flea infestation induces physiological stress in cats by causing irritation, allergic dermatitis, anemia, and acting as an infectious disease transmission vector for pathogens such as *Bartonella* and *Rickettsia*. In laboratory setting, research has shown that *Ctenocephalides felis* can act as a vector for Feline Calicivirus to infect healthy cats that later displayed upper respiratory infection (URI) signs. However, the possibility of *C. felis* for transmitting any URI pathogens under natural settings remains unclear. This study aims to investigate the role of flea infestation in upper respiratory health in household-owned cats. We hypothesize that flea infestation is associated with worse URI symptoms. In this study, 94 cats from 57 residential homes in Florida, USA, were enrolled. Homes were categorized into high, low, and no flea burden groups based on the flea comb count from each cat as well as the number of fleas trapped in the home overnight (High N = 41; Low N = 40, No N = 13). Feline upper respiratory health was assessed by using Maddie's Fund URI score scale. Nasal and oropharyngeal swabs were collected from each cat for feline upper respiratory pathogen PCR testing, which could be used as a confounder to explain the difference in severity of their URI status (*Chlamydia*, Feline Calicivirus, Feline Herpes virus, Influenza A, *Mycoplasma*, and SARS-CoV-2). By comparing the percentage of cats with different upper respiratory health outcomes among the three groups of different flea burdens, this study will provide insights on identifying vector-borne URI pathogens and the necessity of keeping cats on flea preventative for upper respiratory health.

Research Grant: Elanco Animal Health
Student Support: Elanco Animal Health

Oral administration of firocoxib in swine and evaluation of pain using infrared thermography

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Currently, pigs in commercial production farms are not provided with routine pain management for surgical procedures such as castration and tail docking, which raises significant concerns for animal welfare. This study investigated the oral use of a common NSAID, firocoxib, given to the sow and delivered transmammary to piglets, to alleviate pain and stress associated with these procedures. Infrared thermography (IRT) is a non-invasive method used to assess physiological processes such as inflammation and infection. IRT data analysis was used to determine which dose of transmammary-delivered firocoxib (3.0, 4.0, 5.0, 6.0 mg/kg) was most effective at alleviating pain associated with castration and tail docking procedures in piglets. Fourteen sows nursing ten viable piglets (5 male and 5 female) were either provided an oral dose of firocoxib (at one of the above doses; FIRO) or no analgesic drug (control; CON). IRT images were taken of the surgical sites and surrounding tissue of each piglet 24 hours prior to processing (surgical castration and tail docking), and at 1, 7, 24, 36, and 48h post-processing. Preliminary results showed that piglets in the CON treatment group had significantly higher tail docking site temperatures (i.e., more inflammation) than piglets in the 3.0, 4.0, and 5.0 mg/kg FIRO groups ($P < 0.05$), with a trend found in the 6.0mg/kg FIRO group ($P = 0.06$). Piglets in the CON treatment group also had significantly higher castration site temperatures than piglets in the 3.0, 4.0, 5.0, and 6.0mg/kg FIRO treatment groups ($P < .0001$). The long-term aim of this study is to improve food animal health and welfare through minimally invasive drug administration and novel pain evaluation techniques.

Research Grant: USDA, NIFA, AFRI Grant #2021-67015-34084
Student Support: National Institute of Health T35 Training Grant

Evaluation of the pathogenesis of osteochondrosis in the elbow joint of swine using MRI

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Osteochondrosis dissecans (OCD) is a developmental orthopedic disorder that is characterized by formation of osteochondral flaps/fragments in developing joints that causes pain, locking of the joints and disability. Despite being the 2nd and 3rd most affected site in boys and girls, the elbow joint has received only a limited amount of study compared to the knee joint in both children and animal models. Indeed, it remains to be determined whether the pathogenesis of OCD is shared across the elbow and stifle joint predilection sites. Thus, the aim of this study was to assess the pathogenesis of OCD in the elbow joint by evaluating the development and progression of naturally occurring OCD precursor lesions in the distal humeral epiphyseal growth cartilage in juvenile pigs using MRI. Four domestic pigs underwent in vivo MRI of the right and left elbows at 4, 6, 8, 10 and 12 weeks of age to obtain high resolution 3D DESS sequences to be applied to a quantitative multi-slice, multi-echo T2 mapping sequence to calculate median T2 relaxation times in the epiphyseal cartilage. After the last MRI session, pigs were euthanized, and their distal humeri were harvested for histologic evaluation. MRI revealed multifocal delay of the endochondral ossification primarily involving the humeral trochlea, consistent with osteochondrosis manifesta lesions in all four pigs. Data analysis is ongoing to describe the temporal and spatial features of lesion emergence and progression or resolution. MRI findings will be validated using the results of histologic evaluation of the harvested distal humeral specimens. Our preliminary findings suggest that the pathogenesis of OCD is shared among the distal humeral and femoral predilection sites.

Research Grant: NIH/NIAMS R56 AR078209
Student Support: NIH, Office of the Director, Award number T35OD011118

Environmental modulators of DNA methylation in cranial neural crest cell biology and orofacial cleft etiology

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Orofacial clefts (OFCs) rank as the second most common birth defect in the United States and result from complex gene-environment interactions. Inadequate understanding of causative factors, and environmental influences in particular, impedes the development of prevention strategies. DNA methylation is an environmentally malleable epigenetic mechanism that has been implicated in OFC etiology. The Lipinski Lab has generated preliminary data demonstrating that in utero exposure to a DNA methylation inhibitor or selectively disrupting DNA methylation in the cranial neural crest cell (cNCC) mesenchyme, a multipotent stem cell population that forms most of the connective tissue of the head and face during embryo development, causes cleft palate in mice by attenuating tissue outgrowth in the developing palatal shelves. Based on these observations, we investigated the impact of five DNA methylation-modulators on global DNA methylation andproliferation of murine cNCCs in vitro utilizing 5-mC ELISAand EdU as respective endpoints. We hypothesize that all chemicals tested will decrease global DNA methylation;however, those previously demonstrated to increase OFC risk will be associated with a decrease in cNCC proliferation, while those that possess no known relationship will have no effect. These studies will establish a tractable in vitro model that can be used to identify dietary and environmental modulators of DNA methylation that specifically alter cNCC biology and inform novel prevention strategies for OFCs.

Research Grant: Research Grant: National Institutes of Health (NIH) T35 OD011078-12
Student Support: Student Support: Morris Animal Foundation

Effect of Age and Contraceptive Type on Female Reproductive Tracts of Servals

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Servals are small felids in African savannas and are considered Least Concern. Servals are common in private and zoo populations, both populations have breeding individuals within them. But the management and handling are drastically different between the two groups. Servals have been neglected from being examined in depth specifically in reproductive care and effects, including cystic endometrial hyperplasia, pyometra, infertility and cancer leading to death. With the similarities to domestic cats, reproductive care has been extrapolated from domestic cat models, and serval-specific information is lacking. For example, for contraceptive effects on the reproductive tracts have not been specifically evaluated in servals. To answer these questions, we will be examining reproductive tracts gathered over the last 31 years as part of the of the Association of Zoo and Aquaria Reproductive Health Surveillance Program, grossly and microscopically identifying the characteristics of normal reproductive cycle and the presence of reproductive lesions. We will use ImagePro to measure different factors to make an objective measurement of those lesions, and test for significance; we will be looking at risk factors that including housing with/near males, age, and contraceptive method. With all the information gathered, we will use Spearman's correlation test to look at the effect of age and contraception type on the occurrence and type of lesion present within serval's reproductive tracts. We hypothesize that reproductive pathology increases with age and the use of contraceptives within female servals. We expect these results to inform serval owners and population managers on the best management techniques for this species.

Research Grant: Research is also supported the Association of Zoos and Aquaria Reproductive Management Center
Student Support: Student funding provided by NIH Grant 5T35OD016477-20 to Michigan State University

The role of GP5-specific antibodies in neutralization of porcine reproductive and respiratory syndrome virus

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PRRSV is a virus that causes great economic loss to global pig production. Studies have shown that passive transfer of PRRSV-specific neutralizing antibodies(NAbs) can protect pigs from PRRSV infections. However, the widely used PRRSV modified live virus (MLV) vaccines only provide limited protection despite production of PRRSV-specific antibodies. NAbs in vaccinated pigs are generated alongside antibodies to viral glycoprotein 5 (GP5), a major envelope protein. These observations prompted us to assess the correlation between GP5-specific epitopes and NAbs generated upon vaccination. Sera were collected at 64 days post-vaccination (d pv) from pigs vaccinated with two MLV vaccines containing viruses of lineage L1 and L5, respectively. Antibody binding response to homologous/heterologous virus strains, as well as a full length L5 specific GP5 protein and peptides corresponding to GP5 ectodomains of specific viral sub-lineages were evaluated by ELISA. Similar levels of antibody responses were observed in the L5 virus variant and the full-length L5-derived recombinant GP5 protein. Stronger ELISA readings to L5-specific peptides were observed in the L5-vaccinated pigs in comparison to sera from L1-vaccinated pigs. Notably, higher levels of antibody response to L5-specific GP5 peptides were found in both vaccinated groups, compared to those to L1A/L1D-specific GP5 peptides, while recognition of L1C-specific GP5 peptides was much lower in both vaccinated groups. Also, more experiments are underway to evaluate serum neutralizing activity against homologous/heterologous strains of PRRSV. Outcomes of this study will elucidate the role of GP5-specific antibodies in neutralizing PRRSV and further benefit development of vaccines.

Research Grant: Swine Disease Eradication Center, the USDA National Institute of Food and Agriculture, and by the joint NIFA-NSF-NIH-BBSRC Ecology and Evolution of Infectious Disease award 2019-67015-29918
Student Support: unknown

miR-291-293 as serum biomarkers for pluripotent embryonal carcinoma in a mouse testicular cancer model

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Testicular germ cells tumors (TGCTs) are the most common solid malignancy diagnosed in young men in the US. Current diagnostics for TGCTs include conventional serum protein markers, but these lack the sensitivity and specificity needed to serve as accurate markers of malignancy across all histologic TGCT subtypes. microRNAs (miRNAs) are small non-coding regulatory RNAs expressed by almost all cells in the body and can be used as biomarkers of many different diseases. In humans, miRNAs in the miR-371-373 cluster are highly expressed in the serum of TGCT patients and outperform existing serum protein markers in TGCT detection. We previously developed a genetically engineered mouse model featuring spontaneous, malignant mixed germ cell tumors consisting of pluripotent embryonal carcinoma (EC) and differentiated teratoma. Here, we report that miRNAs in the mouse miR-291-293 cluster, homologs of human miR-371-373, can be used as biomarkers for malignant TGCTs in mice. miR-291-293 were detectable in the serum of mice with malignant TGCTs but not in control non-tumor-bearing mice. Interestingly, treatment with the differentiation-inducing agent thioridazine extended the survival of mice with malignant TGCTs by eliminating the tumor-propagating EC cells within the tumors, and this greatly reduced serum miR-291-293 levels, suggesting that EC cells specifically express and secrete these miRNAs. Quantification of serum miR-291-293 levels will be a useful tool to track disease progression and response to experimental therapeutics in our mouse model, and these findings also pave the way for future studies aimed at determining functional roles for miR-291-293 in tumor pathogenesis.

Research Grant: None
Student Support: NCI F30CA247458

Surveillance of Enteric Bacteria and Antimicrobial Resistance in the Food Chain in Iowa

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To address the concerns regarding antimicrobial resistance (AMR), the United States Food and Drug Administration (FDA) developed a program known as the National Antimicrobial Resistance Monitoring System (NARMS). Under this program, enteric bacteria that are monitored and identified in meat products such as chicken, pork, turkey and beef include *Campylobacter, Enterococcus*, non-typhoidal *Salmonella*, and *Escherichia coli*. Since 2017, our laboratory at Iowa State University has collaborated with FDA to collect and test meat samples from FDA-designated grocery stores located in Iowa while following the NARMS Retail Meat Surveillance Protocol. Compared to the data collected from January 2017 to March 2020, *E. coli* prevalence slightly increased from 53.8% to 56.4% and *Enterococcus* prevalence slightly decreased from 79.5% to 77.1%. The prevalence rate of *Salmonella* increased from 4.9% to 11.7% in the meat products that were tested. For the chicken and turkey samples, the *Campylobacter* prevalence increased from 11.1% to 15.5%. When evaluating the data from 2017-2019 isolates, the minimum inhibitory concentration (MIC) results showed that carbapenems were highly active against *Salmonella* and *E. coli*. Aminoglycosides were found to be active against *Campylobacter jejuni*, but not *Campylobacter coli*. Both *Campylobacter* species were tested consistently susceptible to macrolides, lincosamides and phenicols. These findings provide important insights into the magnitude and extent of enteric bacteria and AMR in the food chain in Iowa.

Research Grant: FDA NARMS
Student Support: Foundation for Food and Agriculture Research (FFAR),2022 Veterinary Student Research Fellowship

Impact of tongue exercise on hypoglossal neuron survival and glial density after induced neuronal cell death

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Motor neuron diseases (e.g., amyotrophic lateral sclerosis) result in life-threatening alterations in upper airway function primarily due to degeneration of hypoglossal (XII) motor neurons, which leads to ventilator and/or feeding-tube dependence. Despite its critical importance, upper airway function has seldom been studied in motor neuron diseases; thus, effective treatments remain to be discovered. Since genetic rodent models of motor neuron loss develop global symptoms (e.g., limb dysfunction, etc.), we have developed an inducible model of only XII motor neuron death (adult male rats intralingually injected with cholera toxin B conjugated to saporin; CTB-SAP) in order to study targeted therapeutic interventions to enhance the functional capacity of spared XII motor neurons to improve functional outcomes. We hypothesize that deficits in upper airway function are reversed by tongue exercise that slows XII motor neuron death and decreases glial density in CTB-SAP rats. To test our hypothesis, we studied XII motor neuron survival and glial density (microglia and astrocytes) using immunohistochemistry in control and CTB-SAP rats +/- tongue exercise; n = 5/group. Confocal microscopy and ImageJ will be utilized to capture and analyze images. We expect that tongue exercise will increase XII motor neuron survival and decrease glial density in exercise vs. sham exercise CTB-SAP rats, while XII motor neuron survival will be decreased and glial density will be increased in sham exercise CTB-SAP vs. controls. If successful, this work will identify a behavioral (tongue exercise) strategy for future translational studies to slow XII motor neuron death and mitigate upper airway deficits in patients with motor neuron diseases.

Research Grant: Funding provided by R01 HL153612
Student Support: Grant provided by Boehringer Ingelheim

Implantation and early placentation in the mare: the role of kisspeptins in trophoblast invasion

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The invasion of trophoblast cells and subsequent formation of endometrial cups is a crucial step in equine fetal development. Yet, much remains to be elucidated about this process in the context of kisspeptins. Equine placental invasion is similar to that of humans, in which kisspeptins and their receptor have been shown to regulate the invasion of trophoblast cells. As the cells penetrate the maternal endometrium, the response resembles that of metastatic lesions, which kisspeptins control. During this intrusive process, maternal and fetal communication is vital in order to procure a successful implantation. Consequently, in humans, a change in kisspeptin concentrations has been correlated with early complications during pregnancy. Therefore, understanding the role of kisspeptins during equine trophoblast invasion may provide a novel application in assessing compromised pregnancies. Using immunohistochemistry, samples of embryos and uterine biopsies from six pregnant mares at 30-, 36- and 40-days post fertilization, will be stained and compared to non-pregnant mare samples. This will provide information on the maternal-fetal interface: prior, during and after invasion of the endometrium. Localizing kisspeptins and their receptor, relative to the invading trophoblast cells, would establish their role in regulating invasion in the horse. The results of this study will therefore not only provide key information about equine fetal development, but provide an opportunity for clinical application as well.

Research Grant: Grayson Jockey Club Research Foundation
Student Support: USDA Animal Health & Disease Scholar

The effect of hypertension on carotid artery structure in male and female C57BL6 mice

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Hypertension results in inward hypertrophic remodeling in peripheral arteries, leading to vascular insufficiency. Reduced blood flow through carotid arteries can result in cerebral hypoperfusion and cognitive decline. The impact of biological sex on hypertension-induced vascular damage is not well understood. We tested the hypothesis that hypertensive mice would have carotid arteries with reduced lumen diameter and increased wall thickness compared to normotensive controls. Sixteen-to-eighteen-week-old male and female C57BL6 mice were implanted with angiotensin II (AngII)-filled osmotic minipumps to induce hypertension. Male mice were treated with 800ng/kg/min AngII, and female mice were treated with either 800ng/kg/min or 1200ng/kg/min AngII. Blood pressure was measured using tail-cuff plethysmography. Carotid artery structure was assessed using Masson's trichrome staining and Image J software. Systolic blood pressure was elevated in AngII-treated male mice and in 1200ng AngII-treated female mice. Preliminary data suggests AngII-hypertension may increase carotid artery wall thickness in male but not female mice. AngII-hypertension may also reduce lumen diameter and area in carotid arteries of male but not female mice. Appropriate blood flow to the brain is essential for cognitive function. Understanding sex differences in hypertension-associated vascular disease is critical for developing effective therapeutic strategies.

Research Grant: Unknown
Student Support: Unknown

Validation of owner-based sleep questionnaires using polysomnography in dogs

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Disturbances in sleep can impair cognitive and physical performance. Polysomnography (PSG) is the gold standard for evaluating sleep architecture in dogs. However, collecting data overnight requires an expensive wireless video-electroencephalography (EEG) machine and someone to keep the dog from removing the electrodes. There is a need for a more convenient tool to evaluate sleep in dogs. This study aimed to compare owner-based sleep questionnaires to PSG in dogs. Owners were asked to fill out a questionnaire about their dog's sleep. The questionnaire asked how quickly the dog falls asleep, how often vocalizing and twitching disrupt sleep, the frequency of daytime naps, and how often the dog wakes up, paces, or needs to eliminate at night. The higher the score, the more disrupted the sleep with a max score of 60. All dogs underwent PSG during an afternoon nap, and every 3-second epoch was scored as wakefulness, drowsiness, non-REM (NREM), or REM using Spike 2 (Version 7.0 CED). The time spent in and the latency to drowsiness, NREM, and REM sleep were determined. The correlation of those variables with the questionnaire scores was evaluated using linear regression. Sleep was evaluated in 31 dogs with a median age of 11.9 years (range = 1.3 - 16.2) and a median sleep questionnaire score of 8 (range = 5 - 24). The dogs spent a mean of 51.0% of time awake, 23.1% in drowsiness, 21.4% in NREM, and 4.5% in REM sleep. There was significant correlation between higher sleep questionnaire scores and longer latency to NREM sleep (P = 0.0008, r = 0.59), less time spent in NREM sleep (P = 0.0137, r = -0.45), and worse sleep efficiency (P = 0.0420, r = -0.37). In conclusion, our questionnaire can be used to evaluate sleep in dogs.

Research Grant: None
Student Support: NIH T35OD011070 Interdisciplinary Biomedical Research Training Program

Improving the reproductive success in the Black-footed ferret using new assisted reproductive techniques

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Assisted reproductive technologies (ARTs), such as semen banking and artificial insemination, have helped bring the black-booted ferret (*Mustela nigripes*, BFF) population back from the brink of extinction. At present, electroejaculation and laparoscopic artificial insemination are the most commonly used ART methods for the propagation of this endangered species. While both procedures are successful, they are invasive and require special equipment and sterile facilities. There is a clear need to build upon the foundational success of these methods and optimize the ART approach in BFFs so that these procedures can be more easily carried out in field settings. Recent ART developments in cats and dogs include transcervical insemination and collection of semen via urethral catheterization and pharmaceutical induction of ejaculation. We are utilizing domestic ferrets as a model to explore the efficacy and success rates of these ART procedures in ferret species with the aim of improving the cost efficiency, field friendliness, and success of ART in black-footed ferrets. By introducing techniques that are easier to conduct and less invasive, this work aims to advance the recovery of the black-footed ferret by more rapidly increasing the population size and genetic diversity of this North American icon.

Research Grant: American Association of Zoo Veterinarians (AAZV)
Student Support: Supported by NIH Grant Number T35 OD015130

Regulation of folate metabolism by miR-34a identifies a potential combination therapy against osteosarcoma

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Osteosarcoma (OS) is the predominant bone cancer in dogs and humans. Due to the highly metastatic nature of this disease, OS carries a poor prognosis, necessitating further research into alternate therapeutic approaches. An emerging field of research in cancer therapy is microRNA (miR) therapeutics. Preliminary work using a proteomic approach in canine OS cells exposed to exogenous human miR-34a showed reduced levels of multiple proteins involved in the folate metabolism pathway. The aim of this study is to validate this novel antifolate function of the tumor suppressive miRNA prodrug, BioRNA/miR-34a, in canine and human OS cells in vitro. This study will also evaluate the combination of miR-34a and methotrexate, another antifolate chemotherapy drug commonly used in human OS treatment, for synergistic antitumor activity. Treatment of OS cells with BioRNA/miR-34a is expected to increase intracellular mature miR-34a and downregulate several proteins involved in the folate metabolism pathway, including GGH, TS, MTHFD1, MTHFD2, and SHMTI. Through dual inhibition of the folate pathway, a critical component of one-carbon metabolism in cancer, this augmented treatment approach is expected to result in a greater inhibition of cell proliferation and an increased level of apoptosis in canine and human OS cells compared to either treatment alone. Mature miR-34a levels following prodrug exposure will be measured by quantitative real-time PCR, protein levels via western blot, and proliferation and apoptosis via bioreductive fluorometric assay and caspase activity, respectively. Data obtained from this study will provide insight into a potential alternate therapeutic approach, improving clinical outcomes for OS patients.

Research Grant: National Institutes of Health T35OD010956; National Institutes of Health K01OD026526
Student Support: Students Training in Advanced Research (STAR) Program at UC Davis

Comparison of ERG-Jet™ contact lens and DTL fiber electrodes in canine electroretinography in the UV spectrum

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Electroretinography (ERG) uses a corneal electrode to measure the electrical activity of the retina in response to light stimulus. The ERG-Jet™ contact lens and Dawson, Trick, and Litzkow (DTL) fiber are two commonly used electrodes for assessing retinal function in visible light (380-700 nm). However, the most appropriate electrode for use with ultraviolet (UV) light (< 380 nm) is unknown. It is hypothesized that dogs can detect UV light. Thus, this study aims to assess canine retinal response to UV light and compare recorded stimulus values between the ERG-Jet™ contact lens and DTL fiber electrode. We hypothesize that UV light will result in measurable canine retinal electrical activity, and the DTL fiber electrode will record higher stimulus values compared to the ERG-Jet™ contact lens, as contact lens plastic may decrease the transmission of UV light. Normal hound dogs (n = 6; age range: 2.07 to 3.04 years old) were fitted with an ERG-Jet™ or DTL electrode in one eye; UV (365 nm) and visible (470 nm) light stimuli were used to assess mixed rod-cone responses. This was repeated with the other eye/electrode. ERGs were also conducted in reverse electrode and stimulus order. ERG a- and b-wave implicit times and amplitudes were compared using multilinear regression analysis with significance set at $P < 0.05$. Results revealed that 365 nm light resulted in significantly longer a-wave implicit times and lower a-wave amplitudes compared to 470 nm. There was no significant difference between a- and b-wave implicit times or amplitudes when comparing the two electrodes. Thus, UV light results in measurable retinal electrical activity in the dog, and either the ERG-Jet™ or DTL electrode can be used for ERGs in the UV spectrum.

Research Grant: ARO W911NF-19-C-0009
Student Support: NC State University Fluoroscience Endowment

Antimicrobial susceptibility patterns of bacterial pathogens in canine urinary tract infections

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Urinary tract infection (UTI) is one of the most prevalent bacterial infections in dogs. Research on the causative bacterial pathogens and their antimicrobial susceptibility patterns are helpful for successful therapeutic outcomes. The objective of this 10-year retrospective study was to analyze the suspected UTI's in the canine population of Grenada. The diagnostic laboratory reports of canine patients from 2011 through 2021 were analyzed for the presence of bacterial isolates and the potential of multidrug resistance (MDR). A total of 685 urine samples were submitted from the Small Animal Clinic, St. George's University and the Society for the Prevention of Cruelty to Animals for examination at SGU, School of Veterinary Medicine, Microbiology Laboratory. Bacteria were isolated in 33.6% (230/685) of the urine sampled. Among the Gram-negative isolates, the most common was *Escherichia coli,* followed by *Proteus Mirabilis, Klebsiella spp., Pseudomonas spp., Proteus vulgaris,* and *Enterobacter spp.* The most common Gram-positive isolate was *Staphylococcus spp.*, followed by *Streptococcus spp.,* and *Enterococcus spp.* Results indicated that *E. coli* was most sensitive to ciprofloxacin and highly resistant to cephalothin. *Proteus mirabilis* and *Staphylococcus spp.* were susceptible to gentamicin and most resistant to tetracycline. Variable resistance was observed in beta-lactam antibiotics, tetracycline and extended spectrum cephalosporins. Emerging imipenem resistance is evidenced with *Proteus mirabilis* and *Staphylococcus spp*. A good susceptibility for recommended antimicrobials was evident in *Enterobacter spp.* The emergence of MDR documented in this study reinforces the need for judicious regulation of antimicrobial drugs.

Research Grant: Boehringer Ingelheim Island Veterinary Scholars Program
Student Support: School of Veterinary Medicine, St. George's University

Confirmation and enhancement of a PH-HFpEF murine model

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Pulmonary hypertension-associated heart failure with preserved ejection fraction (PH-HFpEF) is estimated to affect two million Americans. It presents as left ventricular diastolic dysfunction that leads to increased pulmonary artery pressure. Even though PH-HFpEF is the most common type of pulmonary hypertension and is associated with high mortality, there are still no effective therapies. One challenge faced by PH-HFpEF is the lack of animal models that accurately reproduce all aspects of the disease since PH-HFpEF arises from several comorbidities. While HFpEF mouse models have been established, stemming from age, hypertension, or metabolic syndrome, only recently was an all-encompassing PH-HFpEF mouse model published. However, this model uses AKR/J mice, which develop thymic lymphoma as early as six months old, with 100% of the mice developing thymic lymphoma by twelve months old. Therefore, confounding variables are introduced and preventative measures for PH-HFpEF can be studied with this model but evaluating potential therapies for the disease becomes difficult and costly. We tracked PH-HFpEF disease progression by measuring body weight and with echocardiography for the proposed twenty weeks after beginning the AKR/J mice on a 60% lipids/kcal high-fat diet (HFD). We found that AKR/J mice develop PH-HFpEF as early as twelve weeks after beginning HFD, maintaining left ventricular ejection fraction but with significantly increased body weight, left ventricular hypertrophy, and tricuspid regurgitation velocity. Shortening the PH-HFpEF murine model to twelve weeks instead of twenty weeks removes the confounding variable of thymic lymphoma and enables researchers to assess efficacy in therapeutic studies accurately.

Research Grant: None
Student Support: NIH/T35 Interdisciplinary Biomedical Research Program and NCSU Provost's Doctoral Fellowship

Prevalence of feline infectious peritonitis in littermates compared to housemates of diagnosed cats

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Feline infectious peritonitis, or FIP, is a deadly disease in cats derived from a mutated form of the feline coronavirus. While the mutation from feline coronavirus into FIP in a cat is seemingly idiosyncratic, a number of studies have investigated possible factors that may contribute to disease development. However, no study has been done on the relatives of FIP-diagnosed cats in household settings and including mixed domestic breeds. The purpose of this project is to determine the prevalence of FIP in the littermates of cats who have been previously diagnosed with the disease. We hypothesize that these littermates will have a higher risk of developing FIP than the unrelated housemates of FIP-infected cats. Cats with confirmed FIP diagnoses will be recruited from an ongoing study investigating the pathogenesis of FIP (Lashnits/Kirchdoerfer, "From benign feline coronavirus to FIP: a study of viral evolution and host antibody response"). Following the identification of a FIP-infected cat, the source of that cat (e.g. breeder, rescue) will be contacted to identify any littermates. A survey will be designed, validated, and then provided to the owners of the littermates to discreetly determine if any of those cats have been diagnosed with FIP by a veterinarian. This study design will be used for further recruitment of cats with FIP, housemates, and littermates outside of the Lashnits/Kirchdoerfer study. This project will contribute to the current understanding of FIP risk, pathogenesis, and role of genetics in the disease which ultimately has the potential to aid in the diagnostic process as well as breeding strategies to potentially avoid further increasing the risk of FIP susceptibility.

Research Grant: Kirchdoerfer, Robert. Structural Studies of the Coronavirus Life Cycle (DHHS, PHS, NIH, Reference number 4R00AI123498-03)
Student Support: Boehringer Ingelheim Veterinary Scholars Program BIVSP-UW-Madison

Evaluation of canine heartworm prevalence among dogs present in Cumberland Gap Region animal shelters

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Cases of canine heartworm (CHW) disease continue to increase in incidence annually despite efforts to increase awareness of its adverse impact and availability of effective and affordable prophylactic medications. Research in the Cumberland Gap region (CGR) has focused on the occurrence of CHW in the local pet dog and mosquito vector populations. Wild Canidae, primarily coyotes (Canis latrans), have also been surveyed opportunistically to assess their role as a source of microfilariae for transmission to suitable mosquito vectors. This study was undertaken to better estimate the contribution to CHW transmission of stray and surrendered dogs resident in CGR shelters. Shelters are visited weekly and whole blood samples collected and tested for CHW antigen without immune complex disassociation by heat treatment. Blood from CHW antigen positive dogs are examined microscopically for microfilariae. To date, 133 dogs have been tested and 11 (8.2%) positive for CHW antigen. Circulating microfilaria (> 300 um) of Dirofilaria immitis were observed in 8 (72.7%) CHW antigen positive dogs supporting their role as transmission sources for infection of the regional mosquito vector population. Although 70% of CHW antigen positive dogs were surrendered by their owners compared to stray dogs, the difference in prevalence was not significant ($P = 0.69$).

Research Grant: College of Veterinary Medicine, Lincoln Memorial University, Harrogate, TN
Student Support: Boehringer Ingelheim

Disease incidence of backyard poultry in Michigan

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Raising backyard poultry has become increasingly popular as people are becoming more aware of their food sources. However, many of these individuals do not have the experience or resources to identify common diseases and obtain timely care. The goal of this retrospective study is to investigate the primary reasons for backyard poultry producers to submit bird(s) to the Michigan State University Veterinary Diagnostic Laboratory (MSU VDL) for necropsy and to determine the most commonly diagnosed diseases affecting these birds. Based on similar data collected in other states across the US, we hypothesized that a large proportion of backyard poultry in Michigan would present with diseases that can easily be prevented by vaccination or preventative care. A total of 436 necropsy cases, based on the USDA definition of backyard chicken flocks, were collected from the archives at the MSU VDL between January 2010-June 2022 and information was collected, including demographics, the presenting complaint, and the most significant disease finding(s). Infectious diseases were identified in 69.9% (305/436) of cases. Of these, 53.1% (162/305) were viral diseases, followed by bacterial infections in 33.1% (101/305). The most common diagnosis was Marek's disease in 23.1% (101/436) of total cases, followed by *Mycoplasma* spp. in 17.8% (78/436). Nutritional deficiencies accounted for only 9.8% (43/439) of the total cases but represented the largest proportion of noninfectious cases 31.1% (43/138). Results from this study provide strong evidence that efforts and resources in Michigan should focus on educating individuals and directing veterinary care toward more preventative measures, such as vaccination and nutrition.

Research Grant: None
Student Support: NIH Grant 5T35OD016477-20

Epigenetic modifications of HSV-1 genomes during lytic infections

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Herpes simplex virus 1, a double-stranded DNA virus that replicates in the nucleus, lytically infects a wide range of cells but establishes latency only in neurons. HSV-1 produces a wide variety of illnesses in humans, including herpes simplex encephalitis, genital lesions, and stromal keratitis. Other alphaherpesviruses infect almost any animal species, including all veterinary animals. Understanding the mechanisms regulating HSV-1 lytic and latent infections would develop a model to study all other members of the alphaherpesviruses subfamily. In lytic infections, the HSV-1 genome is transcribed and assembled into unstable nucleosomes. Despite intensive efforts, the precise mechanisms of HSV-1 transcription regulation by chromatin and epigenetic modifications remain incompletely understood. The aim of this study is to analyze selected epigenetic changes, namely histone post-translational modifications (PTMs), in HSV-1 chromatin in lytically infected cells. We optimized multiple antibodies that recognize selected epigenetic marks, such as di-methylation of histone H3 lysine 4 (H3K4me2) and mono-methylation of histone H3 lysine 36 (H3K36me1), in western blots. Future experiments will analyze the changes in these and other epigenetic modifications during infection. Combined with immunofluorescence, which determines localization of PTMs of lytically infected cells, and ChIP-qPCR, which tests the interactions of histones bearing specific PTMs with viral genomes, the project analyzes the mechanisms whereby epigenetic modulation directly or indirectly modulate HSV-1 gene expression.

Research Grant: NIH NIAID R01 AI153396
Student Support: Boehringer Ingelheim and Cornell University College of Veterinary Medicine

Single cell RNA sequencing of the canine recurrent laryngeal nerve

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Recurrent laryngeal nerve (RLn) injury most commonly occurs due to iatrogenic injury during surgery. This produces vocal cord paralysis, breathy voice and possibly bilateral airway obstruction requiring a tracheostomy. We are investigating the potential of immunomodulation to change events early in the repair process that will alter downstream remodeling to increase regeneration and recovery. We describe a pipeline to isolate critical cell types from the site of nerve graft and repair with and without immunomodulation using an exogenous anti-inflammatory cytokine interleukin-10. We performed sciatic nerve transection and repair in young mice with harvest for scRNA-seq at 5 days post graft. We also performed 5th cervical nerve to RLn graft in two dogs - one with and one without immunomodulation. Regenerative bridges were harvested, digested to a single cell suspension and sequenced. Cluster analysis identified distinct subpopulations of all critical cell types - fibroblasts, macrophages, Schwann cells and endothelial cells. We were able to distinguish myelinating, pro-myelinating, non-myelinating and proliferating Schwann cell subsets in murine samples. Analysis of canine samples will allow us to test our hypothesis that the effects of immunomodulation persist beyond the presence of the exogenous ligand. Immunomodulation could play a vital role in improving repair and regeneration, leading to a better recovery for patients of all species.

Research Grant: RO1DC017171 (NIDCD)
Student Support: NIH T35 OD010941, Cornell University College of Veterinary Medicine

Antibody response to a novel adenovirus-vector vaccine to prevent cytauxzoonosis in domestic cats

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Cytauxzoon felis is an apicomplexan parasite transmitted by *Amblyomma americanum* ticks that causes severe disease in domestic cats. Even with current treatment protocol (atovaquone + azithromycin) this disease is highly fatal, highlighting the need for an effective prevention strategy. Previous studies have identified transmembrane proteins highly expressed in the acute phase of infection. This study aimed to use a replication-deficient human adenovirus (huAd5) vector with a gene encoding a *C. felis*-specific transmembrane protein (c88) as an alternative to stimulate a cellular immunity response to produce neutralizing antibodies. Twelve (n = 12) specific-pathogen free cats were divided into 2 groups: (1) the experimental group (n = 6) received 2 doses the huAd5 vaccine 4 weeks apart while the (2) control group (n = 6) received a sham vaccine (PBS only). Blood samples were collected regularly over the course of three months and were used to measure *C. felis*-specific antibodies using a multiplex immunoassay (MIA) at each collection time point. This was achieved by coupling c88 recombinant protein to magnetic microspheres, incubating the conjugated beads with plasma samples, and then using a detection antibody to quantify the level of *C. felis*-antibodies present. Preliminary results using samples collected seven weeks after initial vaccination showed a significant antibody response in the vaccinated group compared to the sham-inoculated. Future directions include challenge by infection with *C. felis* to compare severity of disease in the vaccinated group against the control group. Results of this study have potential to produce a vaccine sufficient to provide protective immunity against development of cytauxzoonosis.

Research Grant: Funding for this study was provided by Oklahoma State University College of Veterinary Medicine
Student Support: BI Funds

Efficacy of the topical antihistamine olopatadine in dogs with experimentally induced allergic conjunctivitis

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Allergic conjunctivitis is a common condition in dogs and can be associated with environmental allergens. Topical corticosteroids form the basis of treatment, although there are contraindications that may limit their use. This creates a need for evaluation of alternative treatment options. There is currently little research assessing the use of topical antihistamines in dogs for treatment of allergic conjunctivitis. The purpose of this study was to evaluate the efficacy of a once a day, over the counter topical antihistamine 0.7% olopatadine hydrochloride in treating or preventing experimentally induced clinical signs of allergic conjunctivitis in dogs. Twelve healthy student/staff-owned dogs with no history of allergies or atopic dermatitis were randomly assigned to two groups: “Treatment” (n = 9) which received the topical antihistamine and “Control” (n = 3) which received artificial tears. The study involved two phases with dogs receiving the antihistamine eye drops before (Phase 1) or after (Phase 2) receiving the histamine eye drops. Conjunctival hyperemia, chemosis, follicles, ocular discharge and ocular pruritus were graded, and photographs were used to document the response to the antihistamine. Preliminary results suggest dogs receiving the antihistamine eye drops, 0.7% olopatadine hydrochloride, had lower conjunctivitis scores when compared to the control group ($P < 0.05$). When comparing treatment groups from Phase 1 and 2, dogs receiving the antihistamine eye drops prior to development of conjunctivitis (Phase 1) showed less severe clinical signs ($P < 0.05$). Early indications show that antihistamines may not only be beneficial in the treatment of allergic conjunctivitis but also in prophylaxis.

Research Grant: The Ohio State University Internal Award
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Characterizing the cardiac phenotype of Quarter horses with equine neuroaxonal dystrophy

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Equine neuroaxonal dystrophy (eNAD) is an inherited neurodegenerative disease characterized by early onset ataxia and histologic lesions within the caudal brainstem and spinal cord. The clinical and histologic features of eNAD resemble an inherited neurodegenerative condition in humans known as ataxia with vitamin E deficiency. This disease has neurological deficits indistinguishable from another human condition, Friedreich's Ataxia, which has an established cardiac phenotype. The aim of this study is to further the understanding of the pathogenesis of eNAD through characterizing the cardiac phenotype. Echocardiography, electrocardiography, and cardiac troponin I levels were used to define the cardiac structure and function between Quarter horses with eNAD and breed matched controls. Six standardized equine echocardiographic views in both short and long axis were used to measure chamber size and wall thickness. Blinded electrocardiographic data from 24-hour Holter monitors was used to measure the P-R interval, QRS width, Q-T interval, and R-R interval for each horse. Data was assessed for normality using a Shapiro-Wilk test, and all variables were compared between cases and controls with a student's T-test or Mann Whitney-U test, where appropriate. Horses in the eNAD group had a significantly decreased left ventricular free wall thickness in diastole (P = .02). Relative wall thickness was also significantly decreased in the eNAD group (P = .047). No significant differences between the groups were detected in the ECG measurements nor in cardiac troponin I quantification. Characterizing the cardiac phenotype of horses affected by eNAD will improve our understanding of the systemic effects of this complex heritable condition.

Research Grant: None
Student Support: Students Training in Advanced Research (STAR) Program through NIH T35-OD010956

Assessing the cultural competence of veterinary student caregivers in their community clinic rotation

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The increase in diversity among pet owners, the establishment of Diversity Equity Inclusion programs within the profession, and the presence of stereotypes in our society on pet ownership amongst communities of color has demonstrated the importance of cultural humility in veterinary medicine. To better understand its value in a clinical setting, this study assesses the cultural competence of veterinary student caregivers in their interactions with clients from low-income populations and determines their self-perceived ability to meet their clients' needs with areas where further training may be needed. The students will be assessed using an instrument developed by the University of Denver's Institute for Human-Animal Connection. This instrument was first used by veterinarians and our study will extend its use to veterinary students in their community clinic rotation. A 30-minute survey will be sent out to veterinary students who have completed a rotation between March 2022 and February 2023 at the "Tufts at Tech" clinic, serving low-income populations of the greater Worcester area. This survey will include a series of questions on students' demographics, and areas of cultural competence in their work as caregivers. These responses will be examined with the responses collected from clients for a parallel study on client perspectives to determine any correlations. Through this research, we will be able to validate the measure in use in assessing the cultural competence of veterinary students and utilize the data collected as an educational assessment tool to evaluate the impact of the current veterinary curriculum and identify goals to pursue and guide any future changes to the curriculum.

Research Grant: None
Student Support: Blue Buffalo in conjunction with Cummings School of Veterinary Medicine at Tufts University

Major Histocompatibility Complex Haplotypes in the Nokota Horse Breed

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The equine major histocompatibility complex (MHC) is home to many immune system genes, including the antigen presenting MHC Class I and II genes. The MHC is highly polymorphic and heterozygosity at the MHC increases an individual's adaptive immunity to pathogens. The extent of variation at MHC loci can be considered to reflect overall levels of genomic diversity. Here, we determined the diversity of MHC haplotypes in a sample of 94 Nokota horses, descendants from a feral horse population in North Dakota of largely unknown genetic ancestry, using the Neogen GGP70 SNP array. We phased MHC-anchored SNPs with the software tool SHAPEIT. Using the phased haplotypes as a guide, we selected 45 horses for testing on a validated 12-member panel of intra-MHC microsatellite loci. We confirmed haplotype assignments for 12 SNP haplotypes, 9 of which had not been observed previously in any breed. Three haplotypes were described before in other horse breeds (Thoroughbred, Quarter Horse, and the Arabian horse). SNP analysis demonstrated that two haplotypes were found at a higher abundance than others, including the common Thoroughbred haplotype ELA-A3b (17.5%) and a never described before haplotype (38.3%). Several horses were called homozygous for the MHC region (14/94 horses), all of them for one of the two abundant haplotypes. The high concordance between two methods of assessing MHC haplotypes suggests that the results of this study are robust. This work indicates a high level of MHC homozygosity in the Nokota horses, likely stemming from the high frequency of two abundant haplotypes. The skewed frequency of MHC haplotypes may be the outcome of harem breeding practices and may result in low MHC diversity in the Nokota horse breed.

Research Grant: The Preserve Chester Springs
Student Support: Fellowship from Bostwick Family Foundation / Havemeyer Foundation Summer Fellows Program

Peripheral Blood Markers of Chronic Stress in Production Swine

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Enhancing swine welfare in production environments can have both economic and ethical benefits. Reliably assessing welfare status can be challenging and requires an integration of both behavioral and physiological information. In order to identify specific measures that can be used to assess swine welfare, our team has taken advantage of a natural model of social stress associated with a group housed heard of sows. Sows in this herd develop a relatively strict social hierarchy that can be measured by the order in which they enter an electronic feeding system. Studies in human animals suggest that chronic social stress, which we predict is a feature of sows in the low hierarchy sector, may be reflected in the peripheral blood. Specifically, we predicted that blood cells isolated from sows at the low end of the social hierarchy would have shorter telomeres and would include a higher frequency of inflammatory immune cell susbsets. Our preliminary data support this prediction, at least among older sows in the herd. Interestingly, our data from gilts suggests that those classified in the high hierarchy category may be experiencing more stress.

Research Grant: unknown
Student Support: NIH T35 OD010919, Boehringer-Ingelheim, and the University of Pennsylvania

Size limitations for electroporation of DNA constructs in mouse embryos.

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The use of CRISPR/Cas9 genome editing has led to major advancements in the generation of genetically engineered animal models. CRISPR/Cas9 reagents are commonly delivered by microinjection (MI) directly into embryos, however, MI is technically demanding, expensive and time consuming. Electroporation (EP) offers a cost effective, simple, and efficient alternative to MI. The objective of this study is to evaluate the success of electroporating DNA constructs of varying sizes into embryos. We hypothesize that small DNA constructs ≤ 200 bp will be electroporated efficiently while larger DNA constructs (> 500 bp) will undergo EP less efficiently with increasing size. One-cell embryos will be collected from superovulated, immature C57BL/6 mice, then assigned to 6 experimental groups. Four groups will undergo EP with varying sizes of DNA constructs (200, 500, 700, and 1100 bp) + CRISPR/Cas9 reagents. Control groups will include non-electroporated embryos and a group that will be electroporated without DNA constructs. Embryos will be cultured *in vitro*, monitored for continued survival and development and collected as blastocysts for PCR analysis to assess genomic integration of the DNA constructs. Statistical analysis will be done using a one-way ANOVA with a Tukey post-hoc test. We expect to see a negative correlation between the number of embryos carrying a genomic integration with the increasing size of the electroporated DNA construct. This would suggest that there may be size limitations for EP and provide guidelines for when alternative methods of delivery of larger DNA constructs should be considered.

Research Grant: Bryda Research Incentive Funds.
Student Support: IDEXX-BioAnalytics Endowment Funds.

Targeting the IL-33 Receptor of Mast Cells with an Oligonucleotide to Mediate Inflammatory Responses

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IL-33 is a cytokine produced from endothelial and epithelial cells found in our skin, lungs, and gastrointestinal tract. It plays an important role in type 2 immune responses, and therefore has been implicated in the pathogenesis of different inflammatory diseases such as: asthma, inflammatory bowel disease, anaphylaxis, and atopic dermatitis. It alerts the immune system by activating lymphoid and myeloid cells such as mast cells (MCs) through its membrane-bound receptor ST2. ST2 activation in MCs consequently induces the production of more pro-inflammatory cytokines which creates severe inflammatory effects in the body. In this study, MCs were treated with an oligonucleotide to create a soluble variant of the ST2 receptor (sST2), which can neutralize IL-33 activity by acting as a decoy receptor. We hypothesized that MCs treated with oligonucleotides will be forced to produce more sST2 to capture IL-33 and potentially reduce inflammatory responses. Murine bone marrow mast cells (BMMCs) were treated with a synthesized oligonucleotide that resulted in a change to the template RNA and protein expression of ST2 in these MCs. Preliminary results showed that the treated cells exhibited a reduction of the membrane-bound receptor by nearly 60% as measured by flow cytometry, and an ELISA revealed a mild increase in the sST2 production outside of the cells. These results were consistent across multiple donors, concluding that possible strategies for anti-inflammatory therapies in the future can include targeting the IL-33/ST2 binding with oligonucleotides. This will result in an increase in the sST2 receptor which will modulate the impact of IL-33 in allergic and inflammatory diseases.

Research Grant: Unknown
Student Support: Herbert Benjamin Endowment

Gestational Intermittent Hypoxia increases apneas in neonatal offspring

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Perturbations of the in-utero environment increase susceptibility to disease and dysfunction later in life. One such disturbance in pregnancy is sleep-disordered breathing (SDB) in which the mother stops breathing during sleep (apneas), often hundreds of times each night. SDB is characterized by intermittent hypoxia (IH), a well-known inflammatory stimulus that can disrupt normal fetal brain development. Male offspring exposed to intermittent hypoxia for the last half of gestation (GIH) have increased spontaneous apneas in adulthood compared with controls exposed to intermittent room air (normoxia) during gestation (GNX). However, little is known regarding the impact of GIH on breathing in neonatal offspring. Additionally, information is limited regarding the impact of infection (particularly from Gram-positive bacteria), another inflammatory stimulus, on neonatal breathing. With infection being the second greatest cause of newborn mortality, we sought to determine the impact of inflammation via these two stimuli on neonatal offspring. Thus, we used plethysmography and respirometry in awake, freely moving GIH and GNX neonatal rats with and without i.p. injection of saline or the Gram-positive inflammatory mimetic, peptidoglycan (10 mg/kg; 3h). Preliminary results in non-injected neonatal rats suggest that GIH P3 males (15.1 $\pm$ 0.1 apneas/10 min) and females (15.4 $\pm$ 0.2 apneas/10 min) have significantly increased occurrences of spontaneous apneas compared to GNX males and females (10.5 $\pm$ 1.0 apneas/10 min). Though we currently have no data regarding the impact of Gram-positive inflammation, these data suggest that GIH impacts breathing early in development in both male and female neonates.

Research Grant: National Institutes of Health R01 NS085226 (JJW), R01 HL142752 (JJW, TLB); Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin-Madison
Student Support: AVMA/AVMF 2nd Opportunity Research Scholarship

Zoonotic disease surveillance in the dromedary camel (*Camelus dromedarius*) in Samburu, northern Kenya

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Pastoralism is the main economic activity in Samburu, Kenya with approximately 80% of households keeping mixed herds of livestock, including camels. Camels improve overall herd resilience because of their ability to maintain productivity in the face of a harsh arid climate. Due to the proximity between pastoralists and camels, certain zoonotic diseases such as Q fever (*Coxiella burnetii*), brucellosis (*Brucella abortus* and *Brucella melitensis*), and Middle East respiratory syndrome coronavirus (MERS-CoV) are significant public health concerns. Currently, there is a limited number of published studies reporting the prevalence of these zoonoses in Samburu, highlighting the need for quantifying the risk of zoonotic disease transmission between camels and community members. In 2021, a total of 135 camels were sampled in three areas of Samburu (Ntaparani, Lbaa, and Sirata). Samples collected included blood, feces, ticks, milk, and nasal swabs. Additionally, a questionnaire was distributed to a total of 15 households to collect preliminary data on knowledge, attitudes, and practices of Samburu pastoralists. The project is part of an initiative to establish the first women's camel milk value chain in Samburu. Results of the project will be used to inform One Health policy and practice in Samburu and could be used as a model for other regions where pastoralism is practiced.

Research Grant: None
Student Support: Foundation for Food & Agriculture Research: Veterinary Student Research Fellowship

Characterization of Retroviral Elements in Jamaican Fruit Bats

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Bats are a diverse group of mammals recognized for their role as zoonotic reservoir hosts that transmit numerous pathogenic viruses in the apparent absence of clinical disease. One hypothesis explaining this phenotype is that endogenous retroviruses (ERVs) have conferred viral tolerance. ERVs are retroviral elements integrated into host genomes as proviruses, which are vertically transmitted through the germline. Specifically, ERVs can introduce variation in gene expression, resulting in heritable immunological changes. For this reason, immunity derived from ERVs may play a vital role in bat immune system evolution, relating to virus-tolerant phenotype. While a handful of retroviral infections and ERVs of bats have been defined, retroviral sequences in the Jamaican fruit bat, *Artibeus jamaicensis*, have not been studied. Building on prior studies that identified retroviral transcripts in cells derived from clinically normal colony-reared *A. jamaicensis*, we develop a qPCR assay to measure proviral copies in host DNA samples. Housekeeping gene GAPDH was used as a diploid control. Our results demonstrated that squirrel monkey gamma retrovirus-like ERVs were present in high abundance in normal spleen DNA. Based on GAPDH Ct values, we estimated 6 ERV copies per cell, indicative of the number of ERV genomes per *A. jamaicensis* cell. This is less than the number of enFeLV in domestic cat cells, which contain 10-12 enFeLVs per cell. Further analysis will include quantifying ERV in an array of host tissues, and evaluation of ERV mRNA to assess gene expression in different cell types. These preliminary findings will provide pilot information for future studies evaluating impact of bat ERV on susceptibility to infectious diseases.

Research Grant: This project was funded by CSU intramural funds
Student Support: Supported by NIH Grant Number T35 OD015130

Cooling and vitrification of canine spermatozoa: Interaction between supplements and egg yolk

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Sperm vitrification is an emerging method of cryopreservation and provides certain advantages over conventional freezing. However, post-thaw survival rates after vitrification are sub-optimal. The objective of the present study was to determine the effects of selected supplements in defined medium or in egg-yolk containing extender on the cooling and freeze-thaw cryo-survival and motility of canine spermatozoa. Sperm samples were extracted from cauda epididymis of dogs using phosphate buffered saline (PBS). The following seven treatments were evaluated: 1) Tris-citric acid-sucrose (TCS), 2) TCS + 1 mg/ml myo-inositol (MI), 3) TCS +1 nm vitamin D, 4) TCS + 1 mg/ml beta-alanine (BA), 5) TCS + 1 mg/ml MI + 1 nm vitamin D + 1 mg/ml BA, 6) TCS + 20% egg yolk (TCS-EY), and 7) TCS-EY + 1 mg/ml MI + 1 nm vitamin D + 1 mg/ml BA. After cooling for one hour, samples were vitrified in 33 μl pellets dropped in liquid nitrogen from 11 cm. Pre-freeze and post-thaw total motility (TM), total progressive motility (TPM), percent viability (PV), average path velocity (VAP), and straightness were analyzed by computer-assisted sperm analysis (CASA). Post thaw TM, TPM, PV, VAP, and straightness were 0-14.6%, 0-4.5%, 1.4 98.6%, 0-139.5 μm/s, and 0-99%, respectively. Treatments did not affect ($P \geq 0.05$) post-thaw TM, TPM, PV, VAP, and straightness. However, supplementation of media with myo-inositol seemed most beneficial on sperm cryosurvival. Further investigation of different concentrations of myo-inositol may be valuable. These findings may be beneficial to food animal production and conservation of endangered species.

Research Grant: Boehringer Ingelheim Scholars Program
Student Support: Tuskegee University Veterinary Scholars Program

Comparison of protein hallmarks of ferroptosis in mice with prion disease

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Transmissible spongiform encephalopathies are fatal neurodegenerative diseases associated with an accumulation of misfolded prion protein (PrP^{Sc}). Normally folded cellular prion proteins (PrP^{C}) are expressed in mammalian cells and are involved in the maintenance of cellular iron homeostasis. Prion disorders are associated with iron dyshomeostasis, leading to an imbalance in the brain's metal metabolism. The underlying mechanism of iron dyshomeostasis in animals with prion disease-and its exact role in disease progression-is unclear. In other neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, a new form of iron-related dyshomeostasis has been implicated in disease mechanism. Ferroptosis is a non-apoptotic form of regulated cell death. This specific form of iron dyshomeostasis relies on the accumulation of intracellular iron to trigger lipid peroxidation and rupture the cell membrane. Established protein hallmarks include acyl-CoA synthetase long-chain family member 4 (ACSL4), cyclooxygenase-2, and transferrin receptor. We hypothesized that the expression of these protein hallmarks would differ in the brain tissues of mice with prion disease compared to control mice. Western blots were analyzed comparing the abundance of targeted proteins in PrP^{Sc}-inoculated mice and non-inoculated mice. Initial results demonstrate a difference in the abundance of ACSL4 between these samples. Based on these findings, it is suggested that the expression of protein hallmarks of ferroptosis differs between mice with prion disease and negative controls, and supports further investigation of the remaining protein hallmarks.

Research Grant: United States Department of Agriculture, Agricultural Research Service
Student Support: National Institutes of Health

Correlation of plasma glucagon-like peptide 2 with fecal scores in dogs with chronic enteropathies

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The hormone glucagon-like peptide-2 (GLP-2) is secreted by the small intestine in dogs in response to food intake and by-products of gastrointestinal (GI) microbiota metabolism. GI microbiome dysbiosis is well-documented in dogs with chronic enteropathies (CE). As a worse fecal score (FS) may correlate with dysbiosis, it was hypothesized that GLP-2 plasma concentrations would negatively correlate with FS. Dogs with CE (n = 20) had food withheld (≥ 12 hours) before being fed a standardized diet. Blood samples were collected at baseline and 60 minutes and 180 minutes post-meal. Each blood sample was collected into chilled EDTA tubes on ice. Half of the sample was mixed with 3% volume each of proteinase inhibitors Diprotin A and Aprotinin. A commercial canine ELISA was used to measure plasma GLP-2. A previously established, standardized FS was used to grade same-day stool samples by a single, blinded observer. A mixed model with repeated measures was used to compare GLP-2 concentrations over time. Pearson's correlation was used to compare GLP-2 with FS. GLP-2 concentration did not differ over time (baseline, 430 ± 168 pg/mL; 60 minutes, 447 ± 161 pg/mL; 180 minutes, 485 ± 154 pg/mL; $P = 0.14$). FS ranged from 1.5-5.0 out of 5.0 (median 3.5). There was no correlation between GLP-2 and FS ($r^2 = 0.11$; $P = 0.16$). In dogs with CE, plasma GLP-2 concentrations were not related to FS. Comparison of GLP-2 and FS relation between healthy and CE dogs, as well as targeted microbiome analysis is warranted.

Research Grant: Mark Derrick Canine Research Fund at Kansas State University College of Veterinary Medicine
Student Support: National Institutes of Health T35 Training Grant

Developmental regulation of CD8+ T cell exhaustion

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While advances in CD8+ T cell biology have facilitated revolutionary immunotherapies for cancer and chronic viruses, T cell exhaustion remains a barrier to patient health. CD8+ T cell exhaustion is a hypofunctional state characterized by loss in proliferation, cytokine production, and effector function of CD8+ T cells after chronic stimulation. An important and outstanding question is why some CD8+ T cells become more exhausted than others. Our lab showed that a previously-overlooked source of CD8+ T cell heterogeneity-developmental origin-determines T cell fate after acute infection. Adult T cells (derived from bone marrow stem cells) become memory precursors, while neonatal T cells (derived from fetal liver stem cells) become short-lived effectors. I thus hypothesized that developmental origin also regulates CD8+ T cell fate in chronic infection. To test this, I transferred neonatal and adult CD8+ T cells into recipient mice and compared their responses to a chronic virus (LCMV clone 13) via high-parameter flow cytometry. Neonatal cells became highly functional effectors, whereas adult cells became exhausted. Interestingly, neonatal cells' effector skew corresponded with enhanced proliferation and migration into tissues early in infection. Late in infection, neonatal cells produced more IFNg and may better respond to PD-1 blockade than adult cells. To understand the functional consequences of these differences, we are currently comparing their transcriptomes and behavior in tumors. Collectively, our data suggest that developmental origin plays a deterministic role in CD8+ T cell fate during chronic infection. These findings are clinically relevant to chronic viruses and cancer in human and veterinary medicine alike.

Research Grant: R01 AI110613; Cornell Center for Vertebrate Genomics Scholarship; NIH F30 OD032097
Student Support: None

Characterizing cardiac collagen content in a pre-translational mouse model of osteogenesis imperfecta (*oim*)

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Osteogenesis imperfecta (OI), a heritable connective tissue disorder, results in reduced bone mineral density, bone deformity, increased bone fragility, and intrinsic muscle weakness. OI has an incidence of 1:10,000-20,000 births, with roughly 85% of OI cases resulting from defects in the type I collagen genes, *COL1A1* and *COL1A2*. The second leading cause of death in OI patients is cardiopulmonary complications. Preliminary studies in the osteogenesis imperfecta murine (*oim*) model, which models severe OI due to a mutation in *COL1A2*, demonstrated increased wet heart weights relative to body weights, as well as increased left ventricular blood volumes by 7T-MRI as compared to age-matched wildtype (Wt) littermates, with greater differences in male mice as compared to females. The objective of the present study is to quantify the amount of collagen in *oim* and Wt age- and sex-matched mouse hearts. We hypothesize that *oim* will have reduced total collagen content in their hearts as compared to Wt littermates, with male mice more significantly affected. Cross-sections of 4-month-old male and female Wt and *oim* hearts will be fixed and stained with picrosirius red and semi-quantitatively analyzed using Image-J software. Hydroxyproline, an indirect measure of fibrillar collagen, and total protein content will be determined using hydrolyzed heart tissue to quantify total collagen content. These findings would support the hypothesis that alterations in type I collagen in the extracellular matrix of the *oim* hearts as compared to Wt mouse hearts contributes to cardiac dysfunction of the *oim* mouse heart and may be a major contributor to the development of cardiac disease resulting in the early death of OI patients.

Research Grant: NIH R21 AR778132, 7T MRI facility supported by Truman VA and University of Missouri, Kansas City Consortium on Musculoskeletal Disease
Student Support: Stipend for Ali McAllister-Day is supported by a grant from Boehringer Ingelheim

Chikungunya outbreak epidemiology for informing vaccine trial design

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The increased prevalence and expanded range of chikungunya virus outbreaks within the past decades illustrate an important public health threat and need for a vaccine. However, the characteristics of transmission of mosquito-borne arboviruses complicate the implementation of gold standard randomized clinical trials for vaccine efficacy assessment. We review and synthesize available data on chikungunya outbreaks, describe logistical roadblocks to implementation of randomized clinical trial design, and identify characteristics of chikungunya virus outbreaks that will necessitate modifications to vaccine trial protocols for effective implementation. Implementing a randomized clinical trial during a chikungunya outbreak would be expensive due to the fast action required and would struggle to obtain a significant sample size due to the small nature of chikungunya outbreaks. Randomization efforts would also be frustrated due to the sporadic nature of transmission. We suggest alternative trial design types that may retain statistical significance while limiting risk to the population.

Research Grant: Louisiana State University School of Veterinary Medicine
Student Support: National Institute of Health T35 (LSU # 47349, AWD-002018)

Effect of EXCEDE administration on acute phase proteins in healthy adult horses

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Serum amyloid A (SAA) and fibrinogen (FIB) are positive acute phase proteins (APPs) that increase in response to inflammation. Serial measurements of APPs have been used to monitor disease progression and response to therapy. EXCEDE is a third-generation cephalosporin reported to cause injection site swelling in horses. The study objective was to evaluate if EXCEDE administration results in muscle injury and elicits a measurable increase in APPs in healthy adult horses. Eight horses received two intramuscular injections of EXCEDE on days 5 and 9. Blood samples were taken daily on days 1-15, and every other day on days 16-25. Total white blood cell (WBC) count, WBC differential, FIB, SAA, creatinine kinase (CK), and aspartate aminotransferase (AST) were also analyzed. If present, injection site swelling was measured. Ultrasounds were performed prior to EXCEDE administration, and on the first and third day following both injections to evaluate the musculature for edema. All horses had minor injection site swelling after both injections, which resolved by 6 and 7 days after the first and second injections, respectively. CK and AST both remained within the reference interval for the entire study period. Three of 8 horses had increased SAA 2-3 days following the second injection. Two of the 3 horses with increased SAA had increased FIB after the second injection. One of the 3 horses with an increased SAA had an increased total WBC and neutrophil count on day 10. These findings suggest that a clinically relevant increase in APP occurs in some horses following EXCEDE administration. Further studies are needed in sick horses to assess if EXCEDE administration interferes with APP monitoring of inflammation.

Research Grant: Midwestern University Start-Up Funds
Student Support: Boehringer Ingelheim Veterinary Scholars Program and Federal Work Study

Energy deficient osteoblasts inefficiently mineralize developing bone in sheep with Hypophosphatasia

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Hypophosphatasia (HPP) is a rare genetic disorder characterized by low enzymatic activity of tissue nonspecific alkaline phosphatase (TNSALP), resulting in decreased bone mineralization, muscle weakness, and premature loss of deciduous teeth, all present in gene-edited HPP sheep. Our previous studies identified small, abnormal mitochondria, leading to the hypothesis that reduced bone mineralization may result from decreased osteoblast (OB) activity and cellular energetics. Iliac crest bone biopsies and sternal bone marrow mesenchymal stem cells (MSCs) were harvested from 1 yr old WT and HPP sheep (n = 3/genotype). Histomorphometric analysis of bone biopsies from HPP sheep showed increased unmineralized osteoid matrix compared to WT in vivo, indicative of decreased OB activity. To determine if MSCs are induced to differentiate toward the adipocyte (AD) or OB lineage, MSCs were cultured for 21 days. HPP MSCs had significantly increased AD differentiation but similar OB differentiation when compared to WT. The measurement of oxidative respiration and glycolysis using Seahorse XF analysis on days 0, 7, 14 and 21 of both OB and AD differentiation demonstrated that both undifferentiated and differentiating HPP cells had markedly decreased oxygen consumption rates (OCR) and extracellular acidification rates (ECAR), reflective of diminished oxidative respiration and glycolysis. Reduced TNSALP activity was correlated with increased intracellular ATP, suggesting inefficient ATP utilization. These data suggest that decreased bone mineralization in HPP sheep results from decreased MSC and OB energetics through both oxidative respiration and glycolysis, and that reduced TNSALP activity leads to enhanced bone marrow adipogenesis.

Research Grant: NIH R21-DE028076-01; Soft Bones Foundation
Student Support: NIH T35 OD010991-17, Texas A&M University School of Veterinary Medicine and Biomedical Sciences

Characterizing phenotypic antimicrobial resistance of *E. coli* across two land use gradients in Oregon

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Antimicrobials have been essential for the treatment of serious infections and disease. However, the indiscriminate use of antimicrobials in human and veterinary medicine and in animal agriculture, is a significant global problem as bacterial isolates develop resistance to commonly used antimicrobials. Animal production is considered one of the main sources of antimicrobial resistance (AMR). Antimicrobials are often used to enhance growth and production of livestock, which allows resistance genes to be released into the environment via animal waste. This study aims to identify the presence of *Escherichia coli* AMR in water samples collected from natural water sources along a gradient of human land use from minimally disturbed through agricultural and urbanized areas. Antibiotic susceptibility of *E. coli* isolates against Ampicillin, Tetracycline, Kanamycin, Cephalothin, Sulfamethoxazole-Trimethoprim, Imipenem, Ciprofloxacin, and Chloramphenicol are assessed using Kirby-Bauer disk diffusion. Antibiotic resistance profiles in water samples collected along the Mary's River in the Willamette Valley and the White River, Tygh Creek, and Three Mile Creek in Tygh Valley, Oregon will be compared across sampling locations to determine distribution across the land use types. Based on related studies of *E. coli* resistance in environmental water sources, we expect to see similar differences among land use gradients in samples collected near areas of anthropogenic activity and livestock farmland compared to samples collected near natural habitats. The results of this study will enhance our understanding of the environmental distribution of phenotypic AMR along land use gradients and the influence of human activity on these patterns.

Research Grant: Western University of Health Sciences Intramural Team Grant to Hanselmann and Steinauer; Oregon State University Department of Biomedical Sciences intramural support to Beechler
Student Support: WesternU Research Fellowship and CVM Summer Scholar Program; WesternU Intramural Team Grant

Development of a corneal model as a tool for assessing ocular surface health

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Dry Eye Disease (DED) is a multifactorial disease of the ocular surface characterized by a loss of tear film homeostasis resulting in corneal inflammation and irritation. DED affects 10-30% of the population worldwide. However, early diagnosis of DED remains a challenge whereby patients have symptoms of DED in the absence of diagnostic findings. It has been shown that changes in the quality and quantity of the mucins of the inner most layer of the tear film occur in DED, but there is no agreement on the degree and direction of these changes. The aim of this study is to develop both an *in vitro* and *ex vivo* models for imaging the membrane-associated mucins (MAMs) on the ocular surface with fluorescent confocal microscopy to detect differences in the distribution of mucins in modeled health and disease states. Previous studies have shown that the MAMs of *in vitro* human corneal epithelial cells (hTCEpi) can be fluorescently labeled using an O-glycan binding lectin, called jacalin, bound to biotin and detected with a fluorescent quantum dot (Qdot) bound to streptavidin. It was hypothesized that this labeling procedure can be used on other corneal models expressing O-glycan glycosylated MAMs. Currently, development of *ex vivo* health and disease models, using rabbit and mouse eyes, and an *in vitro* model, using hTCEpi cells, for confocal imaging of MAMs are being investigated. Differences identified in the distribution of mucins in health and disease state models will support the hypothesis that imaging of MAMs on the ocular surface can be used to distinguish health from disease and serve as proof of concept in the development of mucin imaging modalities for the assessment of ocular surface health in patients.

Research Grant: Funds from the University of California, Davis, School of Medicine, Department of Ophthalmology & Vision Science
Student Support: UC Davis School of Veterinary Medicine Endowment Fund (STAR program)

Impacts of jellyfish nematocyst toxins on Atlantic salmon (*Salmo salar*) tissues

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Complex gill disease (CGD) is an important cause of Atlantic salmon (*Salmo salar*) mortality in the marine stage of commercial aquaculture. Depending on the season, jellyfish blooms will form and pass through the mesh of the sea cages. They then come into contact with the skin and gills, releasing nematocysts into the water column, then entering the oral cavity of salmon to cause direct damage to the gills. The purpose of this study was to simulate gill damage resulting from jellyfish nematocyst toxins and assess the pathological changes and expression of specific genetic markers in salmon tissues. Field collections of lion's mane (*Capillata cyprinid*) and moon (*Aurelia aurita*) jellyfish were performed on Prince Edward Island followed by the maceration of their tentacles in a 1:1 solution of PBS to stimulate nematocyst release creating a jellyfish slurry. The resulting slurry was then filter-sterilized and used for exposures on salmon at different concentrations to analyze histological changes and genetic markers such as immune, redox and mucus biomarkers. The results of this study are still pending, but based on a preliminary study, exposure of salmon tissue to homogenized jellyfish nematocysts results in increased cytotoxic effects and will likely result in increased damage to the salmon tissues. This damage is likely to be a contributing factor to the development of complex gill disease in farmed salmon.

Research Grant: Genome Canada - GAPP project
Student Support: VetSRA funding

Evaluating food diagnostic techniques and euthanasia methods in the detection of *Aeromonas* in Siluriformes

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Aquaculture is the fastest-growing food industry globally. Siluriformes production is the major fish species in the USA, with the Southeastern States accounting for over $350 million in total sales. Unfortunately, the Siluriformes industry has been plagued with infectious diseases, primarily but not limited to bacterial pathogens. One of such infectious diseases, Motile *Aeromonas* Septicemia (MAS), is caused by *Aeromonas hydrophila*. Since 2009, this infectious disease has caused an annual loss of over $12 million within the sector. Other *Aeromonas* spp. infections in fish include Red Sore Disease and fin and tail rot. Due to the adverse effect this infectious disease causes within the industry, it is essential that proper screening and diagnostic methods and choices are made before an outbreak occurs. Proper screening and diagnostic approaches will ensure that mitigation strategies are effective. Therefore, this study aimed to (1) evaluate enrichment procedures for detecting *Aeromonas* spp. using food diagnostic approaches and (2) investigating the effect of MS-222 on the detection of *Aeromonas* spp. compared with a nonchemical method of euthanizing fish. Our results showed that it was possible to detect *Aeromonas* spp. using the food diagnostic approach with higher odds of detection using the maceration technique than the rinsing technique. Additionally, MS-222 samples showed a lower prevalence of *Aeromonas* spp. compared with the nonchemical euthanasia option used, suggesting MS-222 may affect the isolation of *Aeromonas* spp.

Research Grant: DHHS/HRSA D34HP00001-35-00, NIH/NIMHD RCMI grant # U54MD007585, and USDA/NIFA NI21AHDRXXXXG022
Student Support: Tuskegee Veterinary Scholars Program

How variable is the urinary microbiota of healthy dogs over time?

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Recent advances in sequencing and culturing techniques challenge the long thought concept that urine is sterile. A few recent cross-sectional studies have characterized the urobiome in healthy and diseased dogs. However, the stability of the canine urobiome over time is unknown. Urine is a highly variable matrix; it can change in pH, concentration, and composition within hours based on a dog's health, diet, and hydration status. Understanding if microbial profiles fluctuate within urine will help drive more informed clinical decisions. Based on preliminary culture results, we hypothesized that the urobiome would be relatively stable over time within dogs but would differ between dogs. To test this, mid-stream free catch urine was collected from 14 healthy dogs (7 female, 7 male) over 12 time points that were hours, days, and months apart. We also collected genital (vulvar/preputial) and perineal swabs and fecal samples from the same dogs at the same time points. DNA was extracted using Qiagen DNA Isolation Kits. The V4 region of the 16S rRNA gene was amplified and sequenced, and the sequence data was processed and analyzed using DADA2 and QIIME 2 . We used Jaccaard, Bray Curtis, and UniFrac distances to assess microbial community similarity and stability within and between dogs. Preliminary results reveal distinct fecal and urine microbial communities that overlap with perineal and genital swab samples. We also observed stability in urinary tract microbiota over three months within dogs, and significant variation in microbial communities between dogs (PERMANOVA, $P < 0.05$). These findings help define normal variation in the urobiome, which can ultimately help better define abnormal changes in the urobiome during disease.

Research Grant: Ohio State University College of Veterinary Medicine Canine Funds
Student Support: NIH T35 OD010977

The morphological difference between Oocytes during Pre and Post LH surge

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To date there is not an effective protocol to mature canine oocytes in vitro due to a paucity of research in the area. To mitigate this issue we conducted a study in which we collected ovaries from bitches in heat, during the pre and post (luteinizing hormone) LH surge. Blood and vaginal cytologies were used to test progesterone levels and percent cornification respectively. In most animals the LH surge initiates the final step of oocyte maturation before it's released from the follicle during ovulation. The canine oocytes don't reach maturity until day 2-3 in the oviducts after being released from the follicle in an immature state. Furthermore, the effect of the LH surge on canine oocyte maturity is unknown. The purpose of this study is to assess the morphologic characteristics of the cumulus cells that surround oocytes collected Pre or Post LH surge and the effect the cumulus cells morphology has on maturation rate in vitro. The information will be used to develop a protocol to mature canine oocytes in vitro and provide valuable input to this area of research.

Research Grant: None
Student Support: NIH T35OD011070 Interdisciplinary Biomedical Research Training Program

Transcutaneous identification of implanted microchips as a method of tracking equine large colon migration

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Colic due to large colon displacement is one of the leading causes for equine hospitalization and surgery, yet there is not an adequate model to study the pathophysiology of this condition. The objective of this proof-of-concept study was to determine if subserosal implantation of bioinert microchips in the large intestine would be detectable by a RFID (radio-frequency identification) receiver when the implanted microchips were adjacent to the body wall, thus identifying the location of the colon within the abdomen. The goal is to develop a model that can be used in future studies to monitor colonic movement in relation to various stimuli. A horse with no history of gastrointestinal disease underwent a ventral midline celiotomy to implant twelve bioinert microchips into the subserosa at predetermined locations within the large colon and cecum. A RFID scanner was used to monitor the location of the colon via transcutaneous identification 1-5 times daily for a one-month period. Following humane euthanasia, postmortem examination of the horse was performed to assess microchip implantation sites for migration and histologic assessment. Eleven out of the twelve implanted microchips were successfully identified transcutaneously at occurrences as high as 100%. Two microchips were lost into the intestinal lumen but the remaining microchips were found intact at their appropriate locations on necropsy. Microchips implanted into the subserosa of the equine large colon can be used as a means of identifying the approximate location of the equine large colon via transcutaneous identification by a RFID scanner.

Research Grant: Department of Clinical Sciences, Colorado State University
Student Support: USDA and Merial Veterinary Research Scholars Program

High-dose carprofen in mice provides poor levels of analgesia post-surgery despite high blood plasma levels

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Carprofen is a commonly used NSAID for pain management in laboratory mice. However, based on recent literature, the standard dosage of 5mg/kg every 12 hours (BID) may not provide sufficient analgesia. This study aimed to evaluate whether a higher dose of carprofen would improve analgesia. A pharmacokinetic study was performed in mice given 10 or 20mg/kg. Mice were dosed subcutaneously (SC) once, and blood was collected for plasma concentrations prior to dosing, and at 0.25, 0.5, 1, 2, 4, 8, 12, 14, an 48h after dosing. An efficacy study was then completed by performing an ovariectomy via laparotomy and evaluating pain responses by activity levels and nesting behavior measured by radio frequency identification (RFID) motion tracking, and von Frey assessment of mechanical pain at the incision site at -24, 4, 12, 24, and 48h postoperatively. Mice were split into surgery and anesthesia groups, with each group stratified into no (saline), low (5mg/kg), or high (10mg/kg) carprofen doses administered SC BID. For the PK study, both dosages had a peak plasma level at 2h and achieved the purported therapeutic plasma level up to 12h. Based on RFID motion data, there were no differences between treatment groups in the amount of time spent in and out of the nest or in general activity levels over time. There were no significant differences seen in the von Frey assessment, though it showed a decrease response to the stimulus over time. These findings suggest that despite high plasma concentrations of carprofen, the higher dosing used may not provide adequate levels of analgesia. Therefore, SC carprofen as a sole analgesic at these dosages may not be effective at alleviating post-ovariectomy pain in female mice.

Research Grant: Office of the Vice President for Research, Colorado State University
Student Support: American Society of Laboratory Animal Practitioners Foundation

Advancing tools for studying drug effects on *Toxocara canis* third stage larvae

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Toxocara canis is a zoonotic intestinal parasite of canines that causes of visceral and ocular larval migrans in humans (toxocariasis). Dogs are the reservoir for *T. canis* due to tissue-dwelling third stage larvae that are transmitted to puppies in utero. The problem is that tissue-dwelling larvae are not killed by anthelmintics and the mechanism for this tolerance is not resolved. In the past, studies of tissue-dwelling larvae were difficult due to their small size and distribution throughout the animal tissues. In this study, we hypothesized that 1) the L3 stage of *T. canis* are susceptible to macrocyclic lactones (MLs) and 2) a device containing L3 larvae can be implanted subcutaneously and recovered for assessment of parasite killing. Computer aided motility studies using wMicrotracker revealed that larvae had decreased but not ceased motility when exposed to (MLs) in vitro. In addition, these assays demonstrated that P-glycoprotein inhibitors worked synergistically with MLs to paralyze larvae. Polymeric implant devices were loaded with 500 larvae and implanted subcutaneously in mice which were subsequently treated with a subset of MLs and P-glycoprotein inhibitors. Implants were removed and parasites recovered were suitable for transcriptomic analysis. Our results demonstrate that 1) P-glycoprotein inhibition potentiates the effects of MLs on *T. canis* larvae. Importantly, the implant device will enable the study migrating parasite stages that was not possible previously in vertebrate hosts.

Research Grant: Research Grant: NIH
Student Support: Student Support: Boehringer Ingelheim and Ramsey Professorship

The association of pet ownership and sleep quality

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This cross-sectional study aimed to determine if there is an association between dog and cat ownership and sleep quality using data from the National Health and Nutrition Examination Survey (NHANES) conducted in 2005-2006. Owning a cat or dog may be beneficial for an owner's quality of sleep due to the social support that pets provide their owners as the pets offer a sense of security and companionship; possibly resulting in improvements in levels of anxiety, stress, and depression. Alternatively, pets may disrupt their owners' sleep. Multiple multivariable logistic regression models were built to assess the association of pet ownership and the sleep quality outcomes which included the following: snoring, snorting, sleep disorder diagnoses, trouble sleeping, trouble falling asleep, waking up during the night, waking up too early, feeling unrested, feeling sleepy, not getting enough sleep, needing pills to sleep, leg jerks and cramps, taking longer than 15 minutes to fall asleep, and having less than 6 hours of sleep. Demographics and body mass index (BMI) were adjusted for in hierarchical regression analyses. Our results indicated that having a dog was associated with greater odds of having a sleep disorder and having trouble sleeping. Having a cat was associated with greater odds of having leg jerks. While the casual nature of co-sleeping and sleep disorders was unable to be established, results of this study provide a possible explanation for pet owners that experience certain sleep issues, but future studies should confirm our findings.

Research Grant: None
Student Support: Lincoln Memorial University CVM research student funds

Effect of the industrial chemical Bisphenol A on endocrine function of the male gonad

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Bisphenol A (BPA) is a plasticizing agent used in the manufacture of common household items such as water bottles, food packaging, and baby toys. Although BPA is a known endocrine-disruptor, little is known about its mechanism of action in the male gonad. Therefore, the present study aims to elucidate the effects of BPA on steroidogenesis in the male rate gonad. First, male Long-Evans rats were provided drinking water without (control) and with 1, 5, 10, 15, and 20 μg/L BPA for 56 days, from 21-77 days of age. Following sacrifice, serum and pituitary were collected, then testicular tissue and Leydig cells were incubated in DMEM/Ham's F-12 culture medium without (basal) or with 100 ng/mL ovine LH for 3 hours. The concentrations of testosterone (T) and estradiol (E2) in serum and spent media were determined by RIA. Additionally, gene expression in testicular tissue and pituitary gland tissue were measured by Western blot analysis. Results showed BPA increased LH-stimulated testicular T production compared to control, increased basal Leydig cell T production dose-dependently, and decreased LH-stimulated T production, except at the greatest BPA dose which was greater than control. Additionally, BPA exposure decreased basal testicular E2 production dose-dependently and increased LH-stimulated Leydig cell E2 production in the 20 μg/L exposure group. Analysis demonstrated that BPA exposure increased StAR protein expression in the 1 μg/L exposure group and LHβ subunit protein in the 15 μg/L exposure group. These data suggest that BPA exposure exerts dose-dependent effects on steroidogenesis in the male gonad. Identification of the mechanisms for chemical exposure effects facilitate risk assessment of the population.

Research Grant: Animal Health and Disease Research Grant, AUCVM
Student Support: Boehringer Ingelheim Vetmedica, Inc. Veterinary Scholars Program and the AUCVM

Evaluation of the nutritional content in senior vs adult dog food diets

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There is limited information regarding how the nutritional needs of senior pets differ from those of adult pets. Currently the Association of American Feed Control Officials (AAFCO) does not have any specific requirements for diets labeled as senior dog food. As a result, diets formulated for senior dogs can vary depending on the philosophy of the pet food manufacturer. This prompted our study to evaluate nutritional content differences between senior dog food and adult dog food. The caloric density, protein, fat, fiber, moisture, ash and mineral content of diets labeled for senior dogs were compared to diets intended for adult dogs. In addition, the nutrient profile of diets labeled for all life stages were compared to both diets. A list of dog food sold in northern Colorado pet food supply stores was created; 1188 diets were randomized and 71 canned and dry canine diets were selected. Thirty diets labeled for senior dogs, 30 diets labeled for adult dogs and 11 diets labeled for all life stages. Two hundred and fifty grams of each diet were sent to Midwest Laboratories for complete proximate analysis with minerals. All data from each life stage will be averaged and nutrition contents between groups will be compared. Without proposed AAFCO guidelines regarding the specific nutrient profiles for senior dogs, manufacturers formulate senior diets to meet the minimum requirements for adult dog maintenance resulting in the potential for wide variability between senior diets. Based on the wide variation of senior diets for dogs, our hypothesis is that the nutrient profile of diets marketed for senior dogs will not be statistically different than those marketed for adult dogs.

Research Grant: Center for Companion Animal Studies
Student Support: Center for Companion Animal Studies

The impact of feeding enrichment on shelter cat approachability and stress

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Nearly 3.2 million cats enter United States shelters annually. Rapid reduction of stress after admission is crucial to prevent negative health and behavioral consequences that impact adoptability. Previous studies have shown that feeding enrichment encourages species-specific behaviors, provides cognitive stimulation, and decreases fear in unowned group-housed cats. However, this needs further exploration in single-housed cats that are vulnerable to confinement stress in shelters. Our objective was to determine the impact of a puzzle feeder on the stress levels of newly arrived shelter cats over a five-day acclimation period. Thirty-two adult cats were randomly allocated to a treatment (TREAT) or control (CON) group. TREAT cats were provided a puzzle feeder consisting of a teaspoon of wet cat food wrapped in a newspaper tube once daily on d1, 3, and 5 post-intake followed by 30 seconds of researcher visibility. All cats were assessed using a human approach test (HAT) on d1 and 5 and the Cat Stress Score (CSS) on d1, 3, and 5. Pearson's chi-square and McNemar's test evaluated between and within group changes in HAT, while the Wilcoxon rank-sum and Friedman test assessed similar for the CSS. The HAT did not differ between TREAT and CON cats on d1 ($P = 0.144$), but TREAT cats became more approachable over the trial period ($P = 0.0455$) and differed from CON cats on d5 ($P = 0.013$). CSS between groups did not significantly differ on d1 ($P = 0.1465$), d3 ($P = 0.0743$) or d5 ($P = 0.0543$), but both groups (CON $P = 0.0008$; TREAT $P = 0.02$) had decreased stress scores over time. This form of feeding enrichment may be a viable low-cost, low-time commitment intervention that can improve the quality of human-cat interactions in the shelter environment.

Research Grant: None
Student Support: Boehringer Ingelheim VSP, Texas A&M School of Veterinary Medicine & Biomedical Sciences

Bovine babesiosis risk of reintroduction into the U.S.

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Rhipicephalus (Boophilus) annulatus and *Rhipicephalus (Boophilus) microplus* serve as vectors for the transmission of the apicomplexan parasites *Babesia bovis* and *Babesia bigemina*, the causative agents of cattle fever or bovine babesiosis. *R. annulatus* and *R. microplus* are commonly found throughout Mexico and intermittently found in Texas in counties bordering the Rio Grande. White-tailed deer serve as secondary hosts for these ectoparasites and frequently travel unregulated between these regions. Transovarial transmission of *B. bovis* and *B. bigemina* establishes concern for the potential reintroduction of these protozoa via movement of infected adult *Rhipicephalus* ticks aboard white-tailed deer near the border and subsequent laying of infected eggs in the U.S. The aim of this study was to determine the number of *Rhipicephalus* ticks infected with *B. bovis* and *B. bigemina* crossing the border via white-tailed deer. A minimum of 50 *Rhipicephalus* ticks per deer were removed from 37 deer crossing the border into Zapata County, Texas. From each deer's assortment of ticks, 16 ticks at various life stages were selected and processed using a grind over liquid nitrogen and Qiagen DNA extraction. Isolated genomic DNA samples were tested for the presence of *B. bovis* and *B. bigemina* using PCR assays to detect kinete-specific protein (ksp). Of the total 598 ticks tested for the presence of *B. bovis* and *B. bigemina*, 2 ticks tested positive for *B. bigemina* and zero tested positive for *B. bovis*. The results indicate that while efforts have been successful to eradicate cattle fever from the U.S., sustained vigilance is necessary in terms of wildlife movement to prevent the reintroduction of these protozoa into the US mainland.

Research Grant: USDA ARS NBAF AGREEMENT NO: ARS 59-3022-1-003 and Mississippi State University College of Veterinary Medicine
Student Support: USDA Agricultural Research Service Scholars, Boehringer Ingelheim Veterinary Scholars Program

***Peromyscus leucopus* population structure and infestation rate with *Ixodes sp.*, a Lyme disease vector**

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Lyme disease is a debilitating flulike illness caused by *Borrelia* bacteria and is transmitted through tick bites. The most common vectors in North America are *Ixodes scapularis* and *Ixodes pacificus*. Over the last two decades, Lyme disease cases have tripled in the United States, with now 300,000 Americans affected each year, especially in the Midwest and Northeast. One crucial determinant in Lyme disease ecology is *Peromyscus leucopus*, the white-footed mouse, which has been widely recognized as the main reservoir host infecting ticks with *Borrelia*. Tick distribution on mice is highly heterogeneous, and some mice have many ticks whereas others have very few. Therefore, understanding the factors that influence tick burden in *P. leucopus* populations could lead to a better understanding of Lyme disease risk factors in humans and their pets. Here, we study datasets provided by NEON (National Ecology Observatory Network), a network of 81 field research sites across the US. We use Mammal Trapping Data from 2015 to 2020, and focus on three sites located in Michigan and Wisconsin, where Lyme disease is highly prevalent. We identify the effects of *P. leucopus* weight, life stage and sex on tick burden, as well as the impact of mice density on tick infestation rate. We also study how the density of rodents influences their home range, and thus their probability of encountering a tick in the environment. Results from this work can inform on potential characteristics of rodents most responsible for *Borrelia* transmission, and therefore could prove useful for disease vector control.

Research Grant: None
Student Support: Boehringer Ingelheim

Biomechanics of the LDE and ACL in ovine stifle stability for a novel model of posttraumatic osteoarthritis

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Posttraumatic osteoarthritis (PTOA) is a degenerative disease that affects 630 million people worldwide. Surgical ovine models via anterior cruciate ligament (ACL) transection require 40 weeks to develop clinical signs associated with PTOA. This may be due to the long digital extensor (LDE) tendon on the craniolateral aspect of the femoral condyle. We hypothesized that the LDE tendon provides sufficient anterior support for the stifle following injury to the ACL, thus slowing the progression of PTOA in ovine models. The study aim was to identify the biomechanical influence of the LDE tendon on joint stability. For this, ovine cadaver stifles were assessed for joint mobility using a load frame to simulate a cranial drawer test. To delineate the role of the LDE tendon and/or ACL, specimens were divided into two groups which differed only in order of ACL/LDE transection. For each testing sequence, specimens were pulled at 0.1mm/sec until a standard load of 50N was achieved, with 5 minutes of rest between testing. Interestingly, transecting the ACL first resulted in a 6.1x increase in maximum displacement and a 4.2x reduction in stifle stiffness relative to an intact limb. Sequentially cutting the LDE resulted in an additional 1.3x increase in maximum displacement with no further decrease in stifle stiffness. Notably, when transecting the LDE first, a change in these parameters was not detected until the ACL was sequentially transected. These data suggest that the ACL provides the major stability in the ovine stifle, and that transecting the LDE in conjunction with the ACL increases joint instability. Future studies will elucidate the effects of PTOA development in the ovine stifle through a dual ACL/LDE transection model.

Research Grant: Young Investigator Award Program (Center for Companion Animals, Colorado State University)
Student Support: Boehringer Ingelheim

Spatial and social structure of rewilded laboratory mice

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As an essential biomedical model organism, house mice have been studied intensely under laboratory conditions, yet they evolved to survive and reproduce in complex and dynamic environments. There has been recent interest in the study of 'rewilded' mice reared in complex outdoor environments, particularly for understanding the brain and behavior. Yet little work has examined lab mouse behavior under free-living conditions. Here, we characterize the emergent spatial and social structure of replicated populations of C57BL/6J (C57) mice over 10 days in large outdoor field enclosures and compare them to populations of recently wild-derived outbred house mice under the same conditions. We observed shared aspects of space use and social structure focusing on agressive male-male interactions across all trials. Female C57 mice spent more time with other individuals and explored more space relative to all other groups, which resulted in C57 mice rapidly forming less stable, but more densely connected, social networks than outbred wild-derived mice. Male C57 mice seemed to wait longer before establihing hierarchies and kept displaying agressive behavior towards males for a longer time than outbred mice. Importantly, this work demonstrates that C57 mice recapitulate many, but not all, aspects of social structures generated by wild mice in outdoor conditions. Rewilding allows for tractable, replicable, and ecologically realistic approaches to studying mouse behavior and can facilitate the study of the biological basis of higher order social organization.

Research Grant: Cornell University intramural funds, USDA Hatch Grant NYC-341191428, NIH R35 GM138284
Student Support: Fellowship from the Bostwick Family Foundation, Cornell Leadership Program

Consequences of parasitism in a threatened population of marbled murrelets on the Oregon Coast

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Protozoans in the *Leucocytozoon* genus are malaria-like parasites that develop in the erythrocytes and leukocytes of avian hosts. Past studies have shown that each species of bird tends to be infected with its own unique species of *Leucocytozoon*. The aim of this research was to identify and describe this parasite in the marbled murrelet population on the Oregon Coast. Marbled murrelets are a threatened seabird species found along the Pacific Coast from Alaska to Northern California. Murrelets differ from other seabirds in that they utilize late successional and old-growth forests, up to 80 kilometers inland, for nesting. Their distinctive lifestyle means they contend with both changing ocean conditions as well as factors that reduce the quality of nesting habitat. *Leucocytozoon* parasites have never been described in murrelets prior to this study, and it is unknown what affects this parasite has on the declining Oregon murrelet populations. Of 295 birds sampled between 2017 and 2022, blood smears showed 71% having *Leucocytozoon* parasites. We obtained primers specific to the mitochondrial cytochrome b gene of the apicomplexan parasite genome. Using these primers, we performed a nested PCR reaction and submitted the amplified DNA to be sequenced to identify the *Leucocytozoon* species. We have calculated visual abundance on the blood smear for a subset of birds, and those that were positive had an average of 16 parasites seen in 100 WBCs (range 78 to 1). The murrelets we drew blood samples on were tagged so that we could collect data on their survival, movement patterns, and breeding success up to four months post-capture. We plan to obtain parasite abundance data with qPCR to analyze how this impacts these parameters.

Research Grant: College of Forestry at Oregon State University and the USDA National Institute of Food and Agriculture, McIntire Stennis project 1014995; Carlson College of Vet Med Biomedical Sciences Internal Grant
Student Support: Funded by Boehringer Ingelheim and Oregon State University

The effects of m^{6}A RNA modifications on the oncogenic retrovirus HTLV-1

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Human T-cell leukemia virus type 1 (HTLV-1) is an oncogenic retrovirus that infects 5-10 million people worldwide and ~5-10% of those infected develop disease (adult T-cell leukemia or neurodegenerative disease) after a clinical latency period of several decades. Patient prognosis is poor and there are few effective therapeutic options available for patients with HTLV-1-mediated diseases. Two viral genes, *Tax* and *Hbz*, have been previously identified as critical to viral persistence and pathogenesis. Additionally, posttranscriptional modifications to RNA are common in eukaryotes and methylation of the N6 position of adenine (m^{6}A) is the most common modification. Reader proteins (YTHDF1-3, YTHDC1-2) have the ability to recognize these m^{6}A modifications and regulate target gene expression. We hypothesize viral transcripts *tax* and *hbz* contain m^{6}A modifications and that these modifications affect viral gene expression and immortalization. Using immunoprecipitation (IP) we found both *tax* and *hbz* mRNAs contain m^{6}A modifications and through crosslinking IP (CLIP) we determined several reader proteins interact with the viral transcripts. qPCR analysis in HTLV-1 transformed cell lines revealed that shRNA-mediated m^{6}A reader protein knockdown of YTHDF1-3 or YTHDC1 results in increased viral gene expression. Interestingly, YTHDF1 shRNA-mediated knockdown in virus producing cells resulted in a significant decrease in viral p19 in the supernatant, a measure of virus release. Taken together, our results suggest several reader proteins play a role in regulating HTLV-1 gene expression and regulating the viral lifecycle. This work helps further define the mechanisms of HTLV-1 infection and is critical for understanding viral pathobiology.

Research Grant: Ohio Cancer Research Seed Award (PI: Panfil)
Student Support: AVMA/AVMF Second Opportunity Research Scholarship

Examining veterinarians' decision-making process surrounding antimicrobial use and resistance

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Antimicrobial resistance (AMR) is a growing problem that affects both human and veterinary healthcare professionals and their patients. AMR is spread among humans, animals, and the environment. According to the CDC, approximately 2 million people in the U.S. are affected by antimicrobial-resistant bacteria annually. Therefore, combatting AMR requires a complex approach that considers the prescribing practices in both veterinary and human medicine. Our goal is to examine the decision-making process and knowledge of veterinarians regarding antimicrobial medications. Veterinary clinics throughout Central Illinois were identified using an online search. Veterinarians were recruited by cold-calling and emailing veterinary clinics, as well as announcements on the University of Illinois listserv for referring veterinarians. Participating veterinarians were then interviewed over video call about their experiences with AMR, prescribing antimicrobials, education on antimicrobials, and the scope of their practice. At the conclusion of our study we will identify the recurring themes in the decision-making process of veterinarians when prescribing antimicrobials. This effort is part of a larger One Health study that is conducting similar interviews with healthcare providers for humans, pet owners, and community members in Central Illinois. This information will be used to inform future studies and educational materials to understand and combat the growing issue of AMR.

Research Grant: Jump ARCHES P3331
Student Support: Office of the Director, NIH, T35 OD011145

Proteoglycans and innate immunity in xylosyltransferase II (Xylt2) deficient mice

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Proteoglycans (PGs) are highly heterogenous glycoproteins found in connective tissue and at the surface of cells throughout the body, and these proteins are important co-receptors for growth factors and cytokines, extracellular matrix repositories for enzymes, and are structurally integral to tissues. A PG is a core protein with assembled glycosaminoglycan (GAG) chains. These GAG chains are initiated by a xylosyltransferase that transfers a single xylose molecule to specified serine(s) in the core protein. Loss of this reaction in mice due to Xylt2 deficiency results in loss of GAG assembly and leads to changes in core protein GAG levels in tissues. Xylt2 deficiency in mice results in increased lung weight and decreased GAG content. Furthermore, the lungs of these mice have an exaggerated lipopolysaccharide (LPS) reaction demonstrated by significantly higher hemorrhage and inflammatory cell infiltration as compared to controls. Major cellular constituents of the lung include epithelial cells, macrophages, and endothelial cells. Since alveolar macrophages are the first line of defense in the lung, our hypothesis is that the alveolar macrophages are paramount to the exaggerated LPS response we observe in the Xylt2 deficient lungs. A total of 18 mice were used, 9 controls and 9 Xylt2 deficient (Xylt2-/-). Alveolar macrophages were isolated, cultured, and challenged with LPS, and LPS signaling was evaluated by the following: rtPCR, pro-inflammatory cytokine analysis, and Western blot for NF-kb p65 analysis.

Research Grant: None
Student Support: Physiological Sciences, CVM

Dogs are great tick collectors: off-leash dog parks as tick-sinks?

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Ticks serve as vectors for a number of pathogens in North America and are considered a public health risk. In summer 2022 in Saskatchewan, we conducted passive and active surveillance in order to determine the risk of tick-borne illnesses and the potential for establishment of *Ixodes scapularis*, the principal vector for Lyme Disease. To date, this species has not been collected via active sampling, but 32 adult females were detected through eTick, 69% from dogs. *Dermacentor variabilis* and to a lesser extent, *D. andersoni*, are endemic to Saskatchewan and frequently feed on mammals, including humans and dogs. Off-leash dog parks are increasingly popular in cities, but may serve as a reservoir for pathogen transmission, including tick-borne pathogens. In this study, we compared environmental tick burdens using standardized dragging protocols in 2 off-leash dog parks with 3 urban green areas not frequented by dogs in the city of Saskatoon. Off-leash dog parks had lower abundance of ticks compared to both conservancies and agricultural land. *Dermacentor variabilis* was the sole species found at all the surveyed sites except for one conservancy where dogs are not allowed, where *D. andersoni* was also present. In order to assess the potential for interspecies hybridization, the second nuclear internal transcribed spacer was amplified and compared using electrophoresis. Additionally, all ticks collected from the sympatric population were screened to determine and compare the prevalence of *Rickettsia* species. Our findings suggest that off-leash dog parks have a lower abundance and diversity of ticks, which may pose important ecological and public health implications.

Research Grant: Bishops University, Saskatchewan Health, ClyDRN
Student Support: Interprovincial Undergraduate Summer Research, Boehringer Ingelheim Veterinary Scholarship

A retrospective analysis of the completeness of wildlife intake forms at a veterinary teaching hospital

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There has been an extensive level of research evaluating the completeness and efficacy of medical records in human medicine. However, such efforts are not as widely implemented in veterinary medicine and, in particular, in wildlife rehabilitation centers despite the ability of intake related information to have direct implications for both patient care and population health. This retrospective study analyzed intake forms from 1489 wildlife intakes presenting to the AVC Wildlife Service (AVCWS) between 2016 and 2020. Level of completeness was assessed for nine sections, and associated subsections, of the intake sheet: Intake Form Presence, Finder Information, Animal Intake Information, Intake Form Properly Scanned, Signalment, Physical Examination, Indication of Radiographs Taken, End of Care Information, and Sent to Pathology. Based on defined characteristics, each category was assessed regarding its degree of completeness. Results demonstrated that information that is traditionally completed by the finder (Finder Information) is most often complete, with the subsection with the lowest percent of incompletion being 1.07% (date of admission) and the highest being 18.33% (location found). The highest percent of incompletion noted overall for sections was 61.85% (Indication of Radiographs Taken), and 52.72% (hind limb, a subsection of Physical Examination) for subsections. The section of the intake form that was deemed most incomplete was Physical Examination, with subsections ranging from 42.92% to 52.72% incomplete. Factors that likely contributed to level of completeness of intake forms, such as staffing levels, wildlife related training level of staff, and resource (including time) availability, were identified.

Research Grant: None
Student Support: AVC Veterinary Summer Research Award

Cannabinoid vehicle effects on immune function in dog PBMCs

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With more states each year passing laws legalizing the medicinal and recreational use of cannabis, the crucial plight to understand the effects of cannabis becoming increasingly prevalent. Due to the legalization of hemp, unregulated low-dose "nutraceuticals" of CBD such as treats, oils, tinctures, or topicals are being marketed for humans, canines, and felines with buyers wanting relief for their or their pet's anxiety, inflammation, or pain. However, it is currently unclear as to what immunological effects of cannabis treatment or consumption has in canines. This study aims to examine the effects of two plant-derived cannabinoids, cannabidiol (CBD) and Δ9-tetrahydrocannabinol (THC), on immune function in dog peripheral blood mononuclear cells (PBMCs). The studies will first focus on cannabinoid effects on cytokine proteins; specifically levels of IL-2 and IFN-γ generated from activated canine PBMCs treated with CBD or THC and stimulated with phorbol ester plus calcium ionophore (P/I). Second, these studies will determine if delivery vehicle alters cannabinoid effects by comparing immune function effects of CBD and THC delivered in ethanol or dimethyl sulfoxide (DMSO). The results showed that there was a modest decrease in production, expression, and secretion of IL-2 with cannabinoids delivered in DMSO as compared to ethanol. There was also a modest decrease in the population of cells producing IFN-γ both with cannabinoids delivered in DMSO and with increasing concentrations of cannabinoids. Overall, there appears to be a difference in the efficacy of the cannabinoid effects on canine PBMCs depending on the vehicle used to deliver the compounds, suggesting that nutraceutical potency might vary depending on vehicle.

Research Grant: National Institute of Health P20GM103646 core C
Student Support: National Institute of Health T35OD010432

Effects of TGFβ stimulation on mitral valvular interstitial cell phenotype and miRNA expression

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Although myxomatous mitral valve disease is a common heart condition in dogs, its etiology is still unknown. It is a heart disease that currently has no medical treatment available, and patients can advance to congestive heart failure if significant valvular insufficiency develops as a result of the valve pathology. Valvular interstitial cells (VICs) in healthy mitral valves express low levels of alpha- smooth muscle actin (α-SMA), which is consistent with a fibroblastic phenotype. On the other hand, VICs in affected valve leaflets undergo a phenotypic transition to myofibroblastic VICs with increased expression of α-SMA, eventually leading to the formation of myxomatous nodules. Past research has suggested that transforming growth factor-β (TGFβ) may mediate these phenotypical changes. Another possible factor that could promote the fibroblast-to-myofibroblastic cell transition is micro-RNA (miRNA) post transcriptional gene modification. Based upon our preliminary data, miRNA-145 expression is increased in myofibroblastic VICs, and overexpression of miRNA-145 can induce this phenotypical transition. However, it is unknown what caused the overexpression of miRNA-145 in these myofibroblastic VICs. This current research examines if TGFβ plays a role in miRNA expression dysregulation, inducing overexpression of selected miRNAs, including miR-26a, miR-133c, miR-145 and miR-574, with particular interest in miRNA-145. Our results are in progress but will further inform the mechanism by which mitral valve disease develops and enable the design of a therapy for canine myxomatous mitral valve disease.

Research Grant: NIH K01 OD028205
Student Support: Boehringer Ingelheim

Inhibition of cytokine production in dendritic cells by the Nrf2 activator, tBHQ, in female C57BL/6 mice

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Tert-butylhydroquinone (tBHQ) is a food additive and antioxidant used to extend shelf life. It is commonly found in cooking oils, crackers, and frozen fast food. In addition, tBHQ activates the nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2 is a master regulator of oxidative stress and modulates inflammatory genes as a cytoprotection mechanism. tBHQ inhibits T cell, NK cell, and B cell functions in a Nrf2-dependent manner, marked by a decrease in IL-2 and IFN-É£ production, a decrease in cell activation, and an increase in IgM antibody. However, the effects of tBHQ on dendritic cells (DCs) has not been well explored. The reduced inflammatory function of immune cells in the presence of tBHQ presents a major health risk for susceptibility to pathogens. Since Influenza A virus is one of the most common and deadliest viral infection in the world and given the effects of tBHQ on other immune cells, we hypothesize that tBHQ decreases DC cytokine productions such as IL-6, IL-12, and TNF-α in a Nrf2-dependent manner. Splenocytes were collected from wildtype and Nrf2 knockout C57BL/6 mice and treated with 0.005% EtOH, 0.05 μM tBHQ, 1 μM tBHQ, or 5 μM tBHQ for 30 mins prior to activation with influenza A virus for 24 hours. Supernatants were collected and will be used to perform ELISAs for the cytokines IL-12, IL-6, and TNF- α to determine tBHQ-induced changes in DC function. This study will help determine the adverse impact that tBHQ has on function and maturation of DCs.

Research Grant: ROI ES024966
Student Support: NIH R25

Xenobiotic from dogs with gallbladder mucocele formation inhibits secretion by organoids

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Mucocele formation in dogs is characterized by a cystic fibrosis-like accumulation of thick mucus in the gallbladder. Eventual bile duct obstruction or rupture of the gallbladder necessitates surgery and carries a 15% chance of mortality. The cause of mucocele formation is unknown. Our purpose was to investigate a biliary-excreted xenobiotic (XENO) that has been shown to bioaccumulate in dogs with gallbladder mucocele formation. We hypothesize that XENO inhibits gallbladder cystic fibrosis transmembrane conductance regulators (CFTR) thereby preventing the hydration of mucus in dogs with mucocele formation. We developed an organoid model to test the ability of XENO to inhibit CFTR-mediated secretion by canine gallbladder epithelium using a classical "swelling assay". Epithelial cells were mechanically and enzymatically dissociated from gallbladders of healthy euthanized dogs, supplemented with growth factors, seeded into cultrex, and incubated for 2 weeks to form organoids. Organoids were pretreated with XENO (range, 0uM to 15μM for 72hrs prior to stimulation with CFTR agonists (IBMX and forskolin). Images of organoid swelling were captured every 10 minutes for 2 hours and the average number and change in area of swelled organoids were calculated. Preliminary findings suggest a likely dose-response inhibitory effect of XENO on canine gallbladder organoid swelling. Therefore, XENO found in dogs with gallbladder mucocele formation may be sufficient to inhibit CFTR function of canine gallbladder epithelium.

Research Grant: North Carolina State University Faculty Scholar Award (JLG)
Student Support: NIH T35 Interdisciplinary Biomedical Research Training Program

Establishment and characterization of a new canine Langerhans cell histiocytosis cell line

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Langerhans cell histiocytosis (LCH) is a rare, aggressive histiocytic proliferative disorder in canines. Currently, there are no effective treatments for this devastating disease. The purpose of this study was to develop and characterize a canine LCH cell line to better understand canine LCH with the ultimate goal of discovering a new therapeutic target for this lethal disease. We developed the CLCH-A cell line from a lymph node aspirate of a 3-year-old, intact male Scottish Terrier dog. The CLCH-A cell line has been maintained in culture for 10 months over 80 passages. The cells grew in suspension with a doubling time of 20 hours 31 minutes at passage 42. Wright-Giemsa staining revealed that CLCH-A showed a round cell morphology that resembled the primary neoplastic histiocytes. Birbeck bodies, cytoplasmic organelles unique to Langerhans cells, were not observed in the CLCH-A cell line while using transmission electron microscopy. This observation, however, agrees with the previous finding that canine Langerhans cells lack Birbeck bodies. Flow cytometry analysis revealed that the CLCH-A cell line exhibits the same immunophenotype as the neoplastic histiocytes: CD11c-high CD11b-low CD45+CD18+MHCII+CD4-, consistent with the Langerhans cell origin. Overall, CLCH-A was shown to retain morphological and immunophenotypical characteristics of the primary neoplastic Langerhans cells. Experiments are underway such as immunofluorescence, whole genome sequencing to discover somatic mutations responsible for tumorigenesis, and functional analysis as antigen-presenting cells to further characterize the cell line. CLCH-A may represent a useful *in vitro* model to study canine Langerhans cells and LCH.

Research Grant: NC State Faculty Startup Fund
Student Support: NC State University Fluoroscience Endowment

Exploring canine polymorphonuclear myeloid-derived suppressor cells in health and disease

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Myeloid-derived suppressor cells (MDSC) are a heterogeneous group of leukocytes defined functionally by their ability to suppress T-cell responses. Polymorphonuclear-MDSC (PMN-MDSC) activity contributes to immune dysregulation in cancer and autoimmune disease in humans. The bioactive vitamin D metabolite calcitriol is thought to play a role in the differentiation and function of MDSCs, and low serum calcitriol occurs in several canine immune-mediated diseases. Given the potential role of PMN-MDSC in canine health and disease, we sought to begin exploring functional differences between PMN-MDSC and conventional neutrophils by testing the effects of calcitriol on cytokine secretion profiles and by monitoring fluctuations in peripheral blood levels of these cells over time. Conventional neutrophils and PMN-MDSCs were isolated from the whole blood of Alaskan sled dogs via density gradient fractionation followed by flow-assisted cell sorting of the hypodense layer (PMN-MDSC) and hyperdense layer (conventional neutrophil) based on the unidentified canine neutrophil antigen CADO48A. A thirteen cytokine panel run on two dogs identified KC-Like and IL-8 secretion as dependent on cell type and calcitriol treatment and IFN-g and GMCSF as secreted at baseline levels independent of cell type or treatment group. Secretion of these four cytokines was measured in an additional three dogs. Additionally, PMN-MDSC levels in the peripheral blood of ten individuals were monitored over two weeks. These results begin to further our understanding of the functional properties and fundamental characteristics of MDSCs.

Research Grant: Cornell University Internal funding
Student Support: NIH T35 OD010941 and the Cornell University College of Veterinary Medicine

Sheep immune response of *Culicoides* salivary proteins incorporated into inactivated BTV-17 vaccine

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Bluetongue virus (BTV) is an increasing concern with climate change, urbanization, and global trade. Unfortunately, implementing preemptive control measures proves challenging due to lack of vaccine cross-protection between serotypes and vector control. Research has shown that viral capsid proteins alter in the presence of proteases found in the arthropod saliva, thus creating more infectious sub-viral particles. Given that the primary vector for BTV is the *Culicoides* spp., this study compared the immune effects using an inactivated BTV-17 vaccine, with and without recombinant salivary proteins from *C. sonorensis* in Dorper sheep. Previously, researchers classified important members of *C. sonorensis's* secretome: D7 odorant binding protein family, Kunitz-like serine protease inhibitor, and maltase. Blood collection occurred prior to treatment, 1 week post-initial dose, and 1 week post-booster dose. qRT-PCR was utilized to amplify cytokinesgenes depicting TH1 (IL-2, IFN-gamma, TNF-alpha) and TH2 (IL-6, IL-10) responses. The 3 TH1 cytokines analyzed were upregulated for both vaccinations; however, the addition of salivary protein had a 5 and 4 fold increase over the inactivated virus alone when analyzing the IL-2 and TNF-alpha responses, respectively. In comparison, the IFN-gamma results revealed the salivary proteins were upregulated only half that of the inactivated virus alone. For the TH2 response, IL-6 was upregulated a week after both vaccines with salivary proteins, while IL-10 was downregulated for both vaccination groups overall. In summary, the addition of salivary proteins to the inactivated BTV-17 vaccine depicted a proinflammatory response via the measured cytokines.

Research Grant: NBAF Workforce Development (USDA-ARS), NIH T35
Student Support: NIH T35

Effect of differing concentrations of netarsudil ophthalmic solution on intraocular pressure in cats

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Glaucoma is a common cause of irreversible blindness in humans and veterinary species. The elevated intraocular pressure (IOP) associated with glaucoma is caused by impaired aqueous humor outflow. Netarsudil (a Rho kinase inhibitor) is a new human glaucoma therapy that improves aqueous outflow by reducing the rigidity of the trabecular meshwork, a vital tissue in this pathway. While effective in humans at the commercially available (0.02%) concentration, it does not reduce IOP to a clinically significant degree in dogs at this concentration and has not yet been tested in cats. Due to substantially greater anterior chamber volume in dogs and cats compared to humans, we hypothesized that a higher concentration of drug would be necessary to achieve a clinically relevant IOP reduction in these species. We tested this hypothesis in normal cats and cats with primary glaucoma due to *LTBP2* mutation. We compared the effects of 0.02% netarsudil (Rhopressa, Aerie Pharmaceuticals Inc., Durham, NC) and 0.04% netarsudil in a prospective randomized crossover study design, predicting that a clinically relevant IOP reduction would only be observed in response to 0.04% netarsudil. Cats were acclimated to IOP measurements, and assignment of treatment by eye was randomized for each cat, with one eye netarsudil-treated and the opposite assigned as a vehicle-treated control. Treatment was administered every 12 hours for 7 days (7am, 7pm). An observer masked to treatment measured IOPs daily using rebound tonometry at 8am, 11am, and 3pm. Each of the two phases were followed by a 7-day washout period. Preliminary data support a lack of IOP-lowering efficacy of 0.02% netarsudil in cats. Study of the effects of 0.04% netarsudil is ongoing.

Research Grant: Supported by an Unrestricted Award to the Department of Ophthalmology and Visual Sciences from Research to Prevent Blindness and gift of 0.04% netarsudil from Aerie Pharmaceuticals Inc.
Student Support: NIH T35 Training Grant OD011078-12

The Potential Role of Cognitive Testing in the Diagnosis of Canine Cognitive Dysfunction

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Canine Cognitive Dysfunction (CCD) is a disease akin to Alzheimer's disease in humans that strikes the aging population. The prevalence of CCD ranges from 14-35% of senior dogs. It presents with reduction in activity, decreased social interactions, disruption in sleep-wake cycles, increased wandering and disorientation, and house-soiling. Currently, there is unfortunately no definitive antemortem test to diagnose CCD. Owner completed surveys, the Canine Dementia Scale (CADES) and Canine Cognitive Dysfunction Rating (CCDR) scales, are commonly used to identify dogs with apparent cognitive impairment, but there are no objective behavioral tests that have been validated to aid in diagnosis. Interestingly, recent studies utilizing cognitive testing have demonstrated feasibility in older dogs. Therefore by using a battery of behavioral tests to evaluate memory and executive function in dogs with and without signs of cognitive dysfunction, we aim to assess their ability to accurately diagnose CCD and to correlate the findings with biochemical markers in blood and CSF. Dogs in multiple age groups with and without signs of CCD will undergo serial neurological exams, blood and CSF collection, surveys, and behavioral testing every three to six months for two years. To assess for potential biomarkers, levels of glial fibrillary acidic protein (GFAP), amyloid-β, neurofilament light chain protein and phosphorylated tau protein will be measured in the blood and CSF. In summary, the availability of simple, objective testing may enable earlier diagnosis and allow for monitoring of response to treatment and progression of disease. Further, advances in the diagnosis of CCD may catalyze advances in Alzheimer's disease research.

Research Grant: Unknown
Student Support: Supported by NIH Grant Number T35 OD015130

Effects of Genetic Manipulation of FASTKD1 and FASTKD4 on Mitochondrial Protein Expression and Function

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Mitochondria play an important role in cell homeostasis and dysfunction of mitochondria can cause several diseases in multiple organs. Mitochondria have their own unique genome (mtDNA), which encodes for 13 mtRNAs and the respective proteins, all involved in the electron transport chain (ETC) responsible for ATP production. Consequently, regulation of this genome can play a huge part in how the organelle functions. However, attention has primarily focused on mtDNA and less is known about mtRNA and its regulation. A family of proteins called FASTKDs has been proposed to play a key role in regulating mtRNA and therefore ETC protein expression. However, their specific function remains mostly unknown. The objective of this study is to examine how manipulation of two FASTKD isoforms, FASTKD1 and FASTKD4, will affect mitochondrial health and function. We hypothesize that FASTKD1 and FASTKD4 regulate the expression of mtRNA-encoded ETC proteins, ATP levels and mitochondrial potential. Using cultured mouse embryonic fibroblasts, FASTKD1 and FASTKD4 will be either overexpressed by adenoviruses or depleted by siRNAs. We will utilize Western blotting, luciferase-based assay, and TMRE staining to measure ETC protein expression, ATP levels and mitochondrial potential, respectively. We expect that fibroblasts with depleted FASTKD1 and FASTKD4 will have reduced ETC protein levels, ATP levels and decreased mitochondrial potential whereas overexpression will have the opposite effects. These results would indicate that both FASTKD isoforms are vital to regulation of the mitochondrial genome and function. This will provide new insight into how the organelle works and this knowledge will be critical for the study of mitochondrial diseases.

Research Grant: NIH R21 AG067702
Student Support: An endowment established by IDEXX-BioAnalytics

Evaluating Adjuvant-Based Vaccines for Induction of Humoral and T-Cell Immunity to Respiratory Syncytial Virus

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Respiratory Syncytial Virus (RSV) is one of the leading causes of pneumonia in infants worldwide. This virus is unique in that recovered individuals develop poor immunity and are susceptible to reinfections. In a clinical trial for a RSV vaccine, development of an undesirable Type 2 immune response resulted in deaths of vaccinated toddlers upon exposure to RSV and the termination of further trials. The Suresh Lab has identified that a subunit vaccine consisting of a nanoemulsion adjuvant Adjuplex (ADJ) in combination with Toll-like receptor agonists glucopyranosyl lipid A (GLA) or CpG elicited broad and potent immunity to SARS-CoV-2 and influenza viruses. We investigated whether a subunit vaccine containing RSV proteins (NS1, M, and Pre-fusion), ADJ, and GLA/CpG elicited T-Cell and antibody immunity against RSV in the respiratory tract of C57BL/6 X BALB/c F1 mice. We used MHC I tetramer/peptide complexes to visualize RSV antigen-specific CD8 T cells by flow cytometry. We find that both vaccine formulations elicited high numbers of RSV-specific CD8 T cells in lungs and airways at day 8 after booster vaccination. Other analyses to characterize the differentiation status, mucosal imprinting and development of tissue-resident memory T cells, and the functional polarization (T1, T2 and T17) of RSV-specific T cells are in progress. Additionally, we will quantify RSV-neutralizing antibodies in serum and airways using a Vero cells-based plaque assay. Follow-up studies will test whether these vaccines accelerate viral control and protect against a challenge with virulent RSV. These studies are expected to provide new insights into mechanisms of vaccine immunity to RSV and pave the way for testing the vaccine approach in humans.

Research Grant: PHS grants U01 AI124299, R21 AI149793 and T35OD011078 from National Institutes of Health
Student Support: Short-term Research Training for Veterinary Students in Wisconsin - NIH T35 OD011078-12

Surveillance for novel paramyxoviruses in West African bats

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Viruses within the family *Paramyxoviridae* have the potential for significant human and animal disease. However, our understanding of the diversity of these viruses in nature is still very poor. Describing the pre-emergent diversity of viruses in wildlife can help with pandemic preparedness efforts by targeting behavioral, ecological, and medical interventions towards viruses with the highest risk of emergence. The aim of this study was to screen samples collected from different species of bats in Liberia for the presence of paramyxoviruses to better understand the diversity of viruses circulating in this region. Epidemiological models were used to identify the factors associated with the highest odds of detecting paramyxoviruses to understand more about their biology (e.g., presence in different sample types) and refine future surveillance efforts (e.g., targeting specific species). About 1,200 samples were screened for paramyxoviruses. 18 paramyxovirus sequences and 2 co-infections were found during screening. The paramyxoviruses were phylogenetically analyzed to compare the diversity of viruses between sample types, individuals, and species.

Research Grant: UC Davis Starter Funds for the Anthony Lab
Student Support: NIH T35 Training Grant OD010956-22 grant

Impacts of diet-induced obesity on ion channel and mRNA expression in murine hypoglossal motoneurons

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Obstructive sleep apnea (OSA) is characterized by loss of airway patency during sleep, with obesity being a strong risk factor for OSA. Disease prevalence is approximately double that of normal weight patients, a trend which has been documented in humans, rats, pigs, and brachycephalic dog breeds. Mechanisms of obesity-related contributions to OSA remain to be fully elucidated. We hypothesize that dysregulated leptin signaling may contribute. Leptin is normally transported from peripheral circulation across the blood brain barrier where it binds receptors in the central nervous system (CNS). Upon leptin binding to its receptor, the activated signaling cascade leads to restructuring of actin skeletons to activate ion channels, including K_{ATP} and BK potassium channels, which are expressed in hypoglossal motoneurons. Peripheral hyperleptinemia associated with obesity saturates and downregulates transport proteins at the blood brain barrier, leading to a relative leptin deficiency in the CNS. This relative deficiency may be partially responsible for OSA because, regardless of weight, OSA patients have plasma leptin levels 50% higher than controls, and severity of disease is proportional to the degree of hyperleptinemia. We hypothesize that CNS leptin insensitivity due to diet-induced obesity will decrease potassium channel trafficking in the hypoglossal motor nucleus, as well as affect other ion channels and receptors. We used RNA sequencing and will use immunofluorescence methods to investigate mRNA and protein level changes in hypoglossal motoneurons in response to diet-induced obesity and leptin deficits in the CNS. This pilot study is a first step in elucidating a mechanism between obesity and the onset of OSA.

Research Grant: Midwestern University One Health Grant Student Award, Midwestern University One Health Grant Faculty Award
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Investigating the hind limb musculature of the hinge-back tortoise (*Kinixys erosa*)

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Limb pathologies are common among pet tortoises (Testudinidae). Since they are long-lived terrestrial animals, they are often subject to degenerative joint disease, osteomyelitis, and septic arthritis. Luxation and fractures are also often seen due to mishandling. Additionally, poor husbandry can lead to nutritional secondary hyperparathyroidism, resulting in metabolic bone disease and limb abnormalities, such as broken bones and associated soft tissue swelling. Veterinarians must treat various species of tortoise, which may be challenging due to minimal documentation regarding anatomical differences among species. *Kinixys erosa*, the hinge-back tortoise, is a species of tortoise distributed in the Guinea-Congo rainforest region in West and Central Africa. In this comparative anatomy study, we dissected and reviewed CT scans of the hind limbs of a male *K. erosa* and female *Centrochelys sulcata*. Findings revealed several major differences in our study taxa (tortoises) compared to previous descriptions of other turtles. In *K. erosa*, the flexor cruris complex differs from previously published taxa in that the semimembranosus is positioned deep to the semitendinosus. The triceps femoris muscle group has two additional muscle bellies than previously described. Brief mentions in the literature of the gopher tortoise (*Gopherus polyphemus*) are consistent with our findings. The literature acknowledges that additional investigations of these muscle complexes are warranted. Ultimately, these anatomical differences support the locomotor pattern specific to terrestrial tortoises versus other turtles.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program and Federal Work Study

Substance P innervation to the nucleus accumbens shell and its role in depressive behavior

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The pathophysiology of stress disorders, such as depression and anxiety, requires more robust characterization to improve diagnostics and drive the development of effective treatments. The neuropeptide Substance P (SP) is implicated in processes such as stress, anxiety, and addiction. Furthermore, the Schank lab has found that the SP endogenous receptor, neurokinin-1 receptor (NK1R), has increased expression in the nucleus accumbens shell (NAc shell) following exposure to chronic social defeat stress (SDS). This study aims to demonstrate the functional role of the NK1R in the NAc shell as a mediator of behavioral responses to SDS. To test this, we used chemogenetic interventions to selectively express inhibitory DREADD receptors in SP cells that innervate the NAc shell. Specifically, a Cre-dependent retrograde AAV (pAAV-hSyn-DIO-hM4D(Gi)-mCherry) was bilaterally infused into the NAc shell of Tac1-Cre mice; Tac1 gene produces the propeptide from which SP is derived. For comparison, pAAV-hSyn-DIO-mCherry was bilaterally infused into the NAc shell of control Tac1-Cre mice. Next, mice will be exposed to SDS for 11 consecutive days. Prior to each exposure, mice will be injected with Clozapine N-oxide, a synthetic compound that selectively targets DREADD receptors, thus inhibiting SP innervation. The mice will then undergo behavior tests to measure depressive and anxiety-like behaviors, such as social interaction test (SI) and elevated plus maze. We hypothesize mice exposed to SDS will exhibit a decreased SI ratio and higher avoidance behavior relative to unstressed controls. Moreover, inhibition of the SP innervation to the NAc shell will attenuate sensitivity to SDS. The full data set and results are pending.

Research Grant: NIH RO1 AA026362
Student Support: NIH T35 OD 010433 Georgia Veterinary Scholars Summer Research Program

Effect of isoprostane type (F2 vs. F3) on bovine monocyte-derived macrophages during endotoxin challenge

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A critical transition period in the life of a dairy cow is between late gestation and early lactation, during which 75% of all health issues affecting dairy cattle occur. A principal reason that cows are so likely to develop disease during this transition period is due to the major metabolic and physiologic changes they need to undergo around the time of calving. These increased demands predispose dairy cows to developing oxidative stress (OS). OS has many negative effects on the function of immune cells, such as reducing phagocyte function and migration to sites of infection, which increases susceptibility to disease. Isoprostanes are compounds formed through reactive oxygen species-induced peroxidation of polyunsaturated fatty acids (PUFAs) and may have an effect on immune cells such as macrophages. The aim of this study is to assess the effects of 8-iso Prostaglandin F2α (F2-isoP, derived from Omega-6 PUFAs) and 8-iso Prostaglandin F3α (F3-isoP, derived from Omega-3 PUFAs) on the function of bovine monocyte-derived macrophages (MDMs) when challenged with lipopolysaccharides (inflammatory challenge). The effects of F2-isoP and F3-isoP on cell viability were assessed using LDH cytotoxicity and apoptosis/necrosis assays, as well as cell function using wound healing and phagocytosis assays. Based on preliminary results, it is anticipated that F2-isoP will enhance the inflammatory response of macrophages whereas F3-isoP will have anti-inflammatory capabilities. If there exists a significant difference in the effect that F2-isoP and F3-isoP have on bovine macrophages, the results will be critical for developing a strategy to manipulate the inflammatory response in early lactation dairy cows.

Research Grant: USDA NIFA 67015-36350
Student Support: Boehringer Ingelheim, Michigan State University Graduate School

Comparison of Blood Leukocytes and Cancer Cells Transcriptomic Responses to Triple Drug Immunotherapy

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Cancer results from multiple acquired genomic changes and an environment of chronic inflammation. Identifying treatment for cancer poses a difficult challenge due to the heterogeneous interactions within the tumor microenvironment, composed of cancer and immune cells that are continually evolving and adapting to escape elimination. However, manipulation of myeloid-derived suppressor cells (MDSCs), a regulatory subset of monocytes and neutrophils, may offer therapeutic potential. By performing comparative transcriptome analysis of responses to a triple drug immunotherapy targeting MDSCs, distinct genes involved in neoplasia growth, angiogenesis, and metastasis were identified in blood leukocytes and cancer cells. The dual drug therapy including Losartan, an angiotensin receptor blocker, in combination with Toceranib, a tyrosine kinase inhibitor, has demonstrated success by suppressing metastasis through blockade of CCL2-CCR2 monocyte recruitment. Ladarixin, a dual chemokine receptor CXCR1/2 inhibitor, attenuates CXCL8 signaling resulting in decreased recruitment of neutrophils. In combination, this triple drug immunotherapy may synergistically inhibit recruitment of MDSCs resulting in decreased immune suppression and clinical benefit.

Research Grant: Unknown
Student Support: Unknown

Metagenome sequencing of RNA polymerase β subunit gene (*rpoB*) sequence of *Ehrlichia ewingii*

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Ehrlichia ewingii are gram-positive, obligate intracellular bacteria that have been implicated as the predominant agent of canine ehrlichial exposure in the southern and central United States and accounts for up to 9.2% of all human ehrlichiosis cases. Existing diagnostic techniques to differentiate between *Ehrlichia spp.* are limited and *E. ewingii* remains unreliably cultured. Polymerase chain reaction (PCR) testing is the current gold standard for species identification; however, the multi-copy 16S rRNA gene target lacks specificity. Our study targets the RNA polymerase β subunit gene (*rpoB*) as it provides increased resolution when identifying bacterial species at the species and subspecies level. The objective of this study was to sequence the *rpoB* gene of *E. ewingii* from four naturally infected dogs using two distinct sequencing methodologies and compare the results. Using next-generation sequencing via Illumina iSeq 100 and traditional Sanger sequencing, we obtained a 4703 base pair sequence that contained the full *rpoB* gene sequence. With these results, the *E. ewingii rpoB* gene may be further developed and used as both a target for future diagnostic PCR tests and metagenomic phylogenetic analyses.

Research Grant: This study was supported in part by a grant from the Office for Research at Western University of Health Sciences, College of Veterinary Medicine
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Characterization of tick-borne pathogen epidemiology in companion animals in Boone County, Missouri

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With a wide pathogen distribution and increasing geographical range of vectors, tick-borne ehrlichiosis is a risk for humans and dogs across the country, but Missouri and Arkansas have shown the largest increasing trends for disease and highest risk of infection. Ehrlichiosis can manifest variably, from an asymptomatic infection to a fatal multi-organ monocytic ehrlichiosis, with the causative agent being *Ehrlichia chaffeensis, E. ewingii,* or *E. canis* (exclusively canine). Dated prior research has found *E. ewingii* to be the predominant species in Missouri; however, with changing climatic and ecological patterns, there is a need for an updated evaluation of local *Ehrlichia* spp. The objective of this study is to characterize an initial epidemiological picture of canine *Ehrlichia* infection and disease risk in Boone County. With our preliminary clinical data from January 2021 to June 2022, we hypothesize that *Ehrlichia* infections will be more common than that of the other tick-borne pathogens analyzed, and that *E. ewingii* will be the dominant species in screened ticks. We will evaluate positive infection rates and diagnoses from SNAP 4Dx Plus tests ordered in the past five years at the University of Missouri Veterinary Health Center, and ticks collected from dog parks and off of shelter dogs will be screened for pathogens. These results would suggest that *Ehrlichia* spp., *E. ewingii* particularly, are responsible for the majority of tick-borne disease in Boone County. Such results would allow better local understanding of infection and disease risk from *Ehrlichia* bacteria, in addition to detecting other pathogens posing health threats to both pets and their owners.

Research Grant: Tier 2 award from the UM System Strategic Investment Program
Student Support: University of Missouri College of Veterinary Medicine Office of Research

Injectable in situ forming implant for sustained release of punicalagin as osteoarthritis therapy

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In the treatment of osteoarthritis (OA), intra-articular injection is advantageous because it maximizes drug activity in the joint while minimizing the risk of unwanted side effects to other organs. In situ forming implants have not yet been researched in connection with intra-articular OA therapy but are worth investigating due to the simple assembly and potential for sustained and tunable drug release. Punicalagin is a polyphenol derived from pomegranate (*Punica granatum L.*) and is an ideal candidate for a disease modifying OA drug used in this delivery system because of its anti-inflammatory and chondroprotective properties that could slow the progression of articular cartilage degeneration. Punicalagin inhibits activation of transcription factors in signaling pathways involved with synovial inflammation, as well as binds type II collagen and inhibits collagenase activity. This study aims to assess 1) the release kinetics of punicalagin in implants made from various combinations of polymer and solvent ratios, 2) punicalagin's ability to inhibit degeneration of cartilage in 0.025 mg/mL collagenase over 12 days, and 3) punicalagin's ability to attenuate LPS-stimulated production of IL-1β from human THP-1 differentiated macrophages. The results showed this drug delivery system was able to sustain release of punicalagin over several weeks and will likely extend to months. Punicalagin [100μM] significantly inhibited degeneration of cartilage compared to the control. These findings confirm the potential benefit of utilizing in situ forming implants for sustained drug release and support further investigation of punicalagin releasing in situ forming implants as an intra-articular therapy for modifying early-stage OA.

Research Grant: NIH T35 OD010432, NIH R25
Student Support: NIH T35 OD010432

Why do bird owners ask for medical advice on social media?

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Veterinarians may be underestimating pet owners' reliance on the internet for medical, husbandry, and general care information for pets, especially non-domestic pets like birds. While training in exotic medicine is not a staple for veterinary school curriculums, according to the AVMA, more than 13 percent of U.S. households owned an exotic or specialty pet at year-end 2016, which is a 25 percent increase from 2011. With birds being the third most popular pet in the US, many bird owners are left with the question of how to provide medical care for their pet, with some turning to Facebook groups for peer advice Factors that may contribute to why clients ask for medical advice on social media instead of consulting a veterinarian include dynamics of social media platforms such as Facebook, geographic location or distance to an avian veterinarian, convenience, perceived availability of veterinarians trained in avian medicine, trust of health care providers, perceived cost of veterinary care, and owner's confidence in their ability to find answers without involving an expert. Data is being obtained via Qualtrics, an online survey platform. Permission from Facebook group moderators is being obtained, and the survey posted by the forum moderators. Voluntary Facebook user responses will be recorded for 30 days. Descriptive statistical analysis will be performed based on answers provided. Results are anticipated to identify and describe factors that play a role in pet owners' decision to ask for medical advice on social media.

Research Grant: None
Student Support: Boehringer Ingelheim and the Graduate School at Michigan State University

Histological changes of the equine placenta in the presence of insulin dysregulation

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Gestational diabetes mellitus (GDM) in humans can lead to significant histological and secondary functional placental changes which can lead to fetal hypoxia and growth abnormalities. It is unknown if this occurs in horses. We hypothesize that the equine placenta from horses with insulin dysregulation (ID; similar to GDM) will show increased weight, immature villi, choriangiosis and ischemic lesions as compared to insulin sensitive (IS) horses. Placentas were collected immediately after expulsion from eleven adult Arabian mares. The weight and gross characteristics of the placenta were recorded, along with mare reproductive history. Histological samples were collected from various areas of the placenta and H & E slides were reviewed for any changes. Endocrine testing (baseline ACTH and Oral Sugar Tests) was performed on all mares, and data was recorded on body condition score and regions of adiposity. Comparisons were made between ID and IS mares using Student's t-tests, Chi Square tests and Spearman's Correlation Coefficients (significant at $P < 0.05$). We had seven IS mares and five ID mares. We predict mares with ID will have more histologic lesions and higher placental weights than IS mares. These findings could support the hypothesis that the equine placenta has distinct histologic characteristics when ID is present. Findings from this study could support an equine translational model of placental dysfunction as well as guide peri-natal mare and foal care.

Research Grant: MSU Spellbound Equine Endocrine Research Fund
Student Support: Michigan State University College of Veterinary Medicine Student, NIH T35

The prognostic potential of microRNAs in canine splenic hemangiosarcoma

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Background: Canine hemangiosarcoma (HSA) commonly manifests as a visceral tumor that constitutes about 5% of cancer cases in dogs. The prognosis for visceral HSA is poor due to the aggressive nature of the tumor and lack of specific clinical signs until significant infiltration has occurred. Hence, most dogs present with metastatic disease that responds poorly to standard surgical and chemotherapeutic intervention. Moreover, grading systems for HSA have poor prognostic significance. As such, improved markers are imperative to guide a patient's course of treatment. Non-coding microRNAs regulate gene expression and may serve as predictive biomarkers for HSA. Objective: To investigate the potential role of microRNAs in the prognostic assessment of canine splenic HSA. Methods: Retrospective analysis of 15 cases of canine splenic HSA divided into three groups based on survival times (< 90 days, 90-180 days, and > 180 days) to assess microRNA expression in FFPE splenic biopsies by quantitative real-time PCR. Results and Conclusions: Total RNA and microRNA was measured via Qubit assays and the relative expression of miR-126, miR-452, miR-150, and miR-214 will be compared to the exogenous control UniSp6 using the $2^{-\Delta\Delta Cq}$ method.

Research Grant: None
Student Support: Boehringer Ingelheim, Purdue College of Veterinary Medicine

Investigating the impact of novel placental extract on equine intestinal epithelial repair

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In severe colic, a leading cause of death in horses, intestinal hypoxia causes epithelial cell injury and neutrophilic influx, resulting in severe intestinal barrier damage. Placenta derived amniotic fluid improves intestinal epithelial maturity and prevents barrier damage in models of epithelial disease. We hypothesize that a novel equine placental extract will improve epithelial healing and suppress neutrophilic inflammation in an ex vivo model of intestinal hypoxia and injury. Placenta was collected from 3 mares and processed into acellular placental extract (PE). Jejunal enteroids were isolated from a horse (n = 1) euthanized for non-gastrointestinal causes. Enteroids were dissociated and plated in two-dimensional monolayers. Once confluent, monolayers were subjected to 4-hour hypoxia and scratched. Placental extract (0.05 mg/mL, 0.075 mg/mL, 0.1 mg/mL) or bovine serum albumin control (BSA, 0.075 mg/mL) were applied and images taken every 6 hours. Monolayers were EdU-pulse labeled and stained for Ki67 and DAPI at 6 and 12 hours. Once healed, trans-epithelial electrical resistance (TEER) was measured every 6 hours. Equine peripheral neutrophils (n = 3) were isolated by density centrifugation, loaded with a fluorescent dye (cacein-AM), and treated with PE (0.075 mg/mL, 0.15 mg/mL, 0.3 mg/mL) or BSA control for 30 minutes. Neutrophil migration across a membrane toward IL-8 (100 μg/mL) or LTB4 (100 nM) was detected using a fluorescence plate reader. Preliminary results show that PE treatment did not reduce neutrophil migration. Treatment with 0.075 mg/mL PE increased TEER recovery in injured intestinal monolayer cultures compared to other doses and BSA control; additional replicates are needed to determine significance.

Research Grant: North Carolina Horse Council
Student Support: NIH T35OD011070 Interdisciplinary Biomedical Research Training Program

Sudden death and cardiomyopathy associated with LMNA in the Nova Scotia Duck Tolling Retriever

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Dilated-cardiomyopathy (DCM) is characterized by decreased systolic function and dilation of one or both ventricles, often leading to heart failure. In humans, DCM has an autosomal dominant mode of inheritance and a non-genetic form. Familial DCM has also been reported in canines and has been associated with genes that encode structural proteins of the cardiac myocyte, although familial DCM has not been reported in Nova Scotia Duck Tolling Retrievers (NSDTR). Five young related NSDTR died acutely with evidence of dilated cardiomyopathy with myocardial fibrosis (DCM-MCF). Association and homozygosity mapping using two cases of DCM-MCF NSDTR and 35 unaffected controls identified three candidate regions homozygous in the two cases on canine chromosome (CFA) 7, 24, and 19. Whole genome sequencing data from an affected NSDTR, two obligate carriers, and 155 unaffected control dogs were then analyzed for protein-coding variants unique to the affected and carrier dogs. A deletion causing a frameshift mutation in the LMNA gene was identified in the affected NSDTR. LMNA encodes for lamin A/C proteins which are type 3 intermediate filaments that provide structural support to the nuclear membrane. In humans, LMNA mutations, known as laminopathies, cause DCM with sudden death as well as diseases of striated muscles, lipodystrophy, neuropathies, and accelerated aging disorders. The frameshift mutation is hypothesized to impair the processing of prelamin A to mature lamin A, disrupting the structural integrity of the nuclear membrane. Genotyping of 300 NSDTR has revealed a carrier frequency of approximately 10% in the breed. Future genetic testing of the LMNA deletion can be used to reduce the occurrence of DCM in NSDTR.

Research Grant: Center for Companion Animal Health Grant 2021-88F
Student Support: STAR Program at UC Davis School of Veterinary Medicine NIH T35 Training Grant T35-OD010956

Ubiquilin-1/PLIC-1 knockdown differentially regulates cardiac and cancer cell growth

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Anthracyclines such as Doxorubicin (DOX) are a commonly used chemotherapeutic in several cancer types. Unfortunately, their efficacy is limited by induced cardiotoxicity as a result of prolonged usage. Ubiquilins are proteins that serve as adaptors to coordinate the degradation of specific substrates via both proteasome and autophagy pathways. Ubiquilin-1, specifically PLIC-1, has been a subject of interest due to its association with the protein CD47. CD47 is an integral membrane protein that controls cell fate, which is highly expressed in several types of cancer. The PLIC-1 and CD47 interaction display pro-tumor effects, including inhibiting apoptosis and promoting cancer proliferation, thus making PLIC-1 a potential target for cancer treatment. Therefore, we targeted PLIC-1 using an antisense morpholino in a syngeneic breast cancer model. Our data showed that PLIC1 blockade in combination with DOX significantly reduced tumor volume and weight by over 40% compared to monotherapy alone. Thus, suggesting that targeting PLIC1 in combination with DOX reduces tumor burden. In vivo ultrasound revealed changes in cardiac function ameliorated by anti-PLIC1 treatment. In vitro studies suggested that PLIC1 reduced cell proliferation in the 4T1 breast cancer cells, but no effect was observed when administering anti-PLIC1 antisense morpholino to cardiac myoblast cells. Therefore, our data suggest that targeting PLIC1 could be a potential strategy to prevent cardiotoxicity of anthracyclines while enhancing the oncologic efficacy of these drugs, thus increasing the potential for increasing curative responses while improving cancer patient quality of life.

Research Grant: T32 Laboratory Animal & Comparative Medicine Training T32OD010957 (SMB) American Cancer Society Research Scholar Grant RSG-19-150-01-LIB (DSP)
Student Support: T35 Wake Forest University Summer Veterinary Student Research Fellowship T35OD010946 (A.O)

Chemical evolution to select for protective epitopes against biologic tick transmission of *Anaplasma marginale*

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Host immunity to ticks is an alternative to chemical pesticides for tick control, and research in this area is usually focused on reduction of tick feeding and fecundity, often overlooking interference with the tick-pathogen interface as an important metric of immune protection. Preliminary work has indicated that host immunity to tick-derived antigens can interfere with biologic transmission of the tick-borne pathogen, *Anaplasma marginale*. The objective of this study is to identify epitopes and mimotopes that are uniquely reactive to antisera from hosts protected against tick transmission of *A. marginale*. The approach to this objective is based on molecular evolution of a M13 phage library displaying random peptides, starting with negative selection to remove M13 reactive to IgG from unprotected hosts, followed by positive selection of clones reactive to IgG collected from protected hosts. Antiserum from an unprotected host was used to remove M13 from the library, showing a consistent reduction in M13 removed after each round of negative selection. Work is underway to confirm clearance of M13 bound by unprotected host IgG, and subsequently to select for M13 reactive to protected host IgG. Future work is expected to identify protected host IgG-specific peptide motifs that can be further tested for the ability to elicit protection against biologic transmission of *A. marginale* with an established tick transmission model system.

Research Grant: USDA NIFA2017-67015-26630; Fulbright Grant #PS00217781 (SS), and the Foundation for Food and Agricultural Research Veterinary Fellows Program (SK)
Student Support: University of Missouri, College of Veterinary Medicine Office of Research (Olowu B.I).

Examination of *In Vitro* Proliferation Properties of Mesenchymal Stem Cells Harvested from Osteoporotic Sheep

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Osteoporosis results in weak and brittle bones due to an increase in bone resorption and decrease in bone density. Mesenchymal stem cells (MSCs) are important key players in the development and remodeling of bone via self-renewal and differentiation into osteoblasts, adipocytes and chondrocytes. Although the majority of MSC research studies utilize cells isolated from rodents to investigate their role in osteoporosis, ovine MSCs could potentially serve as an improved translational model due to shared Haversian bone remodeling characteristics with humans, which rodents lack. The overall objective of this study was to compare MSC viability, proliferation, and cell differentiation properties between osteoporotic and healthy sheep. Bone marrow aspirates (BMA) were collected from the iliac crests of four (n = 4) osteoporotic ewes that had previously undergone ovariectomy (OVX) followed by 3-months of corticosteroid administration, as well as from four (n = 4) normal control ewes. BMA was harvested at baseline and 3-months post-OVX and was processed to isolate MSCs. The viability and proliferation properties of each cell line were assessed through MTT assay and cell counts to determine population doubling time. The same cell lines were then used to compare tri-lineage cell differentiation properties between healthy and osteoporotic MSCs. Preliminary trials to determine optimal seeding density for each assay indicated that ovine MSCs should be seeded at densities of 5,000 cells/well, 100,000 cells/well, and 10,000 cells/well for MTT, population doubling, and differentiation, respectively. The results of this study will potentially establish sheep as a model for future investigative studies of progenitor cell roles in osteoporosis.

Research Grant: Preclinical Surgical Research Laboratory
Student Support: USDA Animal Health & Disease Grant

Elucidating the role of midbrain glutamate neurons in cortical memory processing

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Communication between different brain areas controls aspects of complex behavior, including learning and memory (Lisman and Grace, 2005; Liu and Kaeser, 2019; Nadel and Hardt, 2011). To better understand how the brain works, and the possible role of various brain regions in disease states, there is a rationale to trace neural circuits. I am interested in the neural circuits formed between the medial prefrontal cortex (mPFC) and the ventral tegmental area (VTA). While the mPFC governs memory formation, how excitatory (glutamate) VTA inputs modulates mPFC encoding of information is yet to be excited. Although the VTA is involved in reward and aversion learning, the significance of the VTA neural inputs to the mPFC needs to be clarified (Chau et al., 2004; Duszkiewicz et al., 2019; Hu, 2016). The goal of my summer research will be the analysis of the behavioral studies. I will use a software (Ethovision XT15) to analyze and track the behavior of mice during baseline experiments, followed by inhibition of the VTA during similar tasks. For VTA-dependent behavior, I will determine the propensity for reward location exploration when a reward is presented, followed by a sequence of reward omission. This will significantly increase our understanding of normal brain function and will have broad applications to neuropsychiatric diseases associated with cognitive deficiencies.

Research Grant: unknown
Student Support: National Institute of Health

Function of uterine epithelial estrogen receptor α in mouse uterine fluid absorption

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Uterine fluid movement is tightly regulated to support early pregnancy events. Estrogen (E2) induces uterine fluid secretion to facilitate sperm transit to the site of fertilization. Conversely, progesterone (P4) induces uterine fluid absorption to initiate intimate contact between embryo and uterine luminal epithelium (LE) for embryo implantation. Dysregulated uterine fluid movement is a major cause of infertility and early pregnancy loss. Estrogen receptor α (ERα / *Esr1*) is a transcription factor to mediate uterine functions of E2. Mice with uterine epithelial specific ERα deletion, *epiERα* (*Esr1*$^{fl/fl}$*Wnt7a*$^{Cre/+}$), have normal E2 and P4 levels but are infertile with failed semen liquefaction and defective uterine receptivity for embryo implantation. Our preliminary data show that *epiERα*$^{-/-}$ mice on day 0.5 post-coitum (D0.5) lack drainable uterine fluid and have shortened LE height, indicating blunted E2 response. To determine the transcriptome regulated by ERα, we isolate LE from D0.5 mice for mRNA-seq, which reveals the differential expression of thousands of genes, including upregulation of all the three subunits for epithelial sodium channel (ENaC, encoded by *Scnn1a*, *Scnn1b*, and *Scnn1g*), in the D0.5 epiERα-/- LE. ENaC takes in Na+ to generate an osmotic gradient for fluid absorption. Based on this preliminary data, *we hypothesize that uterine epithelial ERα* has a novel role in uterine fluid absorption. To test this hypothesis, we are determining uterine expression of ENaC and other proteins involved in uterine fluid absorption, and tracing uterine fluid absorption in D0.5 *Esr1*$^{fl/fl}$ (control) and *epiERα*$^{-/-}$ mice. Our studies will improve the understanding of mechanisms in uterine fluid absorption during early pregnancy.

Research Grant: None
Student Support: NIH T35 OD 010433 Georgia Veterinary Scholars Summer Research Program

Comparative microbiome analysis of *Myotis grisescens*

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Bats are an important part of our ecosystems and the agriculture sector, as they provide essential ecosystem services in the form of pest control. The health of bats is indispensable, yet many bat species are threatened by the spread of white nose syndrome, pesticide use, windmill turbines, and habitat loss. Bat fecal samples can be used to profile the gut microbiome to gain a better understanding of the differences and similarities of their microbial composition. Learning more about the microbiome composition of bats may give us insight into why species vary in susceptibility to certain diseases. There is not much available information about bat gut microbiomes particularly among *Myotis grisescens*. The objective of this study is to sample and analyze the fecal microbiome of wild *M. grisescens* throughout the state of Missouri. We will extract the DNA from fecal samples using the ZymoBIOMICS DNA/RNA Miniprep kit and perform 16S rRNA sequencing using standard amplicon primer sets through the Genomics Technology Core (GTC) facility. Microbiome composition data received from the GTC will be processed using QIIME2 software. We plan to analyze the microbiome data of *M. grisescens* to gain a better understanding of the microbiome composition and variation of healthy gray bats sampled over the course of a few years from multiple sites throughout Missouri. These could reveal areas of further research to determine how differences in the composition of the gut microbiome translate to functional differences in diseased versus healthy states.

Research Grant: Grant Award 0056825 to University of Missouri from Missouri Department of Conservation
Student Support: IDEXX-BioAnalytics

Development of flow cytometry panel for identifying myeloid-derived suppressor cells (MDSC) in mouse tissue

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Myeloid-derived suppressor cells (MDSCs) are a heterogenous population of myeloid cells known to proliferate in response to conditions such as neoplasia or viral infection. MDSCs have been shown to mediate the immune response through arginase-1 expression, NO production, releasing cytokines, and suppressing T-cells, making these cells a possible marker of disease progression or even a target for treatment. To better evaluate the role of MDSCs in infection and immune regulation in diseases such as tuberculosis and SARS-CoV-2, we developed and validated a flow cytometry panel to identify murine MDSCs in different tissues, including spleen and lung. An eight color cytometry panel was designed for use on the Beckman-Coulter CytoFlex benchtop cytometer to identify T lymphocytes (CD3), B-lymphocytes (CD19), and NK cells (NK-1.1), dendritic cells (CD11c), neutrophils (Ly6G), myeloid cells (CD11b, Ly6C), in addition to the intracellular cytokines arginase-1 and iNOS. The proposed panel distinguishes polymorphonuclear MDSC (P-MDSC) as $CD11b^{+}Ly6G^{+}Ly6C^{lo}$ from mononuclear-MDSC (M-MDS) as $CD11b^{+}Ly6G^{-}Ly6C^{hi}$. When stimulated ex vivo with a solution of rm-IFNg and lipopolysaccharide, MDSCs found with this phenotype are expected to show increased expression of iNOS and Arg1 enzymes known to have immune suppressive activity. We used this panel to evaluate MDSC populations in tuberculosis susceptible (B6-sst1 and S100A8 -/-) and resistant mouse strains (C57BL/6). We hypothesize that tuberculosis susceptible strains have increased numbers of MDSC limiting bacterial control. This methodology can be used to better understand the role of MDSC in infections such as *Mycobacterium tuberculosis* and SARS-CoV-2.

Research Grant: R21 AI155003-01
Student Support: T35

Comparison of hair corticosterone and ultrasonic vocalizations in offspring nursed in a shelved environment

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Early-life experiences are critical modifiers of development. Maternal interactions play a crucial role in early-life development of offspring, which can be modified by stress. Throughout nursing, mothers are typically allocated to single-level cages where they are in constant demand of the pups, a stressful situation not reflective of the natural environment. Accordingly, mothers regularly removed from the nursing environment interact differently with their offspring, leading to long-term changes in offspring physiology and behavior. Such changes commonly include modifications within the hypothalamic-pituitary-adrenal axis, of which corticosterone is a major component. Modifications in the hypothalamic-pituitary-adrenal axis may also be manifested through changes in affective behavior and assessed via ultrasonic vocalization analysis. As a means of assessing the impact of rearing in a shelved environment, we allocated mothers to standard single-level cages or cages with an integrated shelf, which allowed the mother to temporarily escape pups. Here, we show the relationship between multi-day ultrasonic vocalization analysis and hair corticosterone as a means of exploring how a shelved environment influences long-term offspring behavior.

Research Grant: Sir James Dunn Animal Welfare Centre Veterinary Summer Research Award
Student Support: Sir James Dunn Animal Welfare Centre Veterinary Summer Research Award

Assessment of β-hydroxybutyric acid as a biomarker for hepatic lipidosis in bearded dragons (*Pogona vitticeps*)

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Hepatic lipidosis (or fatty liver disease) is one of the most common non-infectious diseases of captive bearded dragons *(Pogona vitticeps)*, with a prevalence of about 38.3%. Females and older bearded dragons are at an increased risk of severe lesions. The diagnosis of hepatic lipidosis is challenging in bearded dragons as they have non-specific signs, and routine hematologic, biochemical, and diagnostic imaging techniques have low sensitivity. Currently, this disease can only be diagnosed ante-mortem by advanced imaging techniques, such as CT-scanning, or invasive techniques, such as liver biopsy and histopathology. Results from a previous pilot study using plasma metabolomics on a small cohort of 14 bearded dragons, with varying degrees of hepatic lipidosis, identified β-hydroxybutyric acid (BHBA) as a candidate biomarker for hepatic lipidosis. The purpose of this study was to further assess the use of BHBA as a plasma biomarker for the diagnosis and screening of hepatic lipidosis on a larger sample size (n = 48) of bearded dragons that were matched for age, as well as to assess the reliability of a point-of-care BHBA meter for in-hospital diagnosis. Hepatic lipidosis was scored using CT-scans, liver histopathology, and plasma BHBA obtained using a reference laboratory analyzer and a point-of-care meter. In addition, plasma samples were obtained for advanced lipoprotein profiling and metabolomics for further biomarker discovery. The association between BHBA and hepatic fat content will be evaluated using linear regression, and values between severe and mild to moderate lesions will be compared using t-tests. Other measurands will be assessed between disease severity groups using volcano plots and multivariate analysis.

Research Grant: Boehringer Ingelheim Veterinary Scholar Fellowship
Student Support: Student Support: Students Training in Advanced Research (STAR) Fellowship

Expression and localization of nephron progenitor and ureteric bud markers in the feline developing kidney

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Chronic Kidney disease (CKD), characterized by nephron loss, is second among the most common causes of mortality in cats aged five and over. Currently, there are no treatment options to prevent nephron loss and restore renal function in CKD. Nephron progenitors (NPs) cells give rise to all cells of the nephron whereas, ureteric bud (UB) cells develop into collecting ducts. NP-based therapies have the potential to stimulate nephron regeneration to address nephron loss. Molecular markers of feline NPs and UB cells are unknown. We hypothesized that the markers of NPs and UB in the developing feline kidney are highly conserved in sequence, expression, and localization between cats and humans. We utilized NCBI sequence similarity search tool, BLAST, to determine the percent identity between protein sequences of feline and human homologues of NP markers SIX2, SIX1, and ITGA8 and a UB marker, GATA3. We studied the expression and localization of these markers in the feline embryonic and fetal kidney by co-immunofluorescence (Co-IF). BLAST results showed that NP markers SIX2, SIX1, and ITGA8 shared 96%, 99%, and 91% similarity respectively, at the amino acid level between feline and human whereas, a UB marker GATA3 showed 96% similarity. Co-IF data showed that in the feline developing kidney, SIX2 and SIX1 were present in the nucleus of NPs whereas ITGA8 showed membrane localization. Besides NPs, SIX2, SIX1, and ITGA8 expression was also detectable in the NP-derived renal vesicle. GATA3 was expressed in the E cadherin positive UB and mesangial cells of the developing glomerulus. Our results determined markers to identify feline NPs and UB cells and suggest functional significance of these markers in feline renal development.

Research Grant: This research was supported by grants from NIH T35OD010432, NIGMS# 1SC2GM130475 DHHS/HRSA D34HP00001-35-00, and NIH/NIMHD RCMI grant # U54MD007585
Student Support: National Institutes of Health project # 5T35OD010432

Efficacy of equine platelet lysate on antibiotic resistant clinical bacterial isolates

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Inappropriate and prolonged use of antibiotics has placed extensive selective pressure on bacterial populations. Consequently, antibiotic resistance has evolved into a world-wide health crisis for both humans and animals. Investigation of alternative antimicrobial therapies is critical for the future of medicine. Platelet concentrate is collected from donor horses via apheresis. Platelets are lysed and release peptides with the potential to inhibit bacterial growth. Filtering the platelet releasate results in an acellular product termed platelet lysate (PL). The goal of the current study was to compare the efficacy of platelet lysate and commonly used antibiotics on clinical isolates that exhibit antibiotic resistance on microbiological sensitivity panels. Three isolates were chosen to be tested in in vitro growth curve and time kill assays: *Enterobacter cloacae* complex, *Enterobacter hormachei*, and *Morganella morganii*. We hypothesized that a 40% concentration of PL would exert a bactericidal effect on these resistant strains and would be more effective than antibiotics used commonly in a clinical setting. Exposing these isolates to 40% PL resulted in a reduction of growth over a 24-hour period. The bacteriostatic effect was confirmed in the time kill assay in which 40% PL reduced the number of bacterial colonies formed on growth agar after incubation. Redosing 40% PL at 1, 2, and 3 hours did not have a statistically significant effect on bacterial growth as compared to bacteria receiving a single dose. Ultimately, 40% PL did not have a bactericidal effect, however, PL significantly reduced the number of viable organisms when compared to treatment with commonly used antibiotics that were largely ineffective.

Research Grant: For the Love of the Horse funding, Equine Programs Research Initiative
Student Support: UGA Foundation, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Assessing behavior and nesting in laboratory mice provided with an enrichment device and supplemental heating

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Mice are routinely used as preclinical models in biomedical research; however standard laboratory housing conditions may be associated with cold stress in this species, as ambient housing temperatures range from 20-24°C, which is below the mouse thermoneutral zone (TNZ) of 29-34°C. Behavioral discrepancies have been documented between mice housed in standard laboratory conditions versus their TNZ, which has the potential to alter scientific outcomes. We evaluated behavior and nest building in mice provided with a heated floor plate and an elevated platform cradle enrichment device, either individually or in combination. Paired male mice (n = 14; CDIIc WT) were housed in individually ventilated cages on a rack equipped with heat plates below one quadrant of each cage. Each cage cycled through four treatment conditions in a randomized order: heat plate off and no cradle (Control), heat plate on and no cradle, heat plate off and cradle, and heat plate on and cradle provided. Cage side observations and videography were utilized to monitor behavior, mouse location in the cage, and nest location and quality. Preliminary results indicate that mice provided with supplemental heat display fewer nesting behaviors, and build poorer quality nests, indicating that heated plates below the cage floor provide thermoregulatory support. Further observation showed a preference to build nests directly above the heat source in mice given supplemental heating. Mice were observed to utilize the cradle enrichment platform for resting, grooming, and urination/defecation; mice did not build nests on the platform. Presence of a cradle did not appear to increase antagonistic behaviors between mouse pairs, compared to when a cradle was not provided.

Research Grant: Nathan Brewer Endowment Fund for Laboratory Animal Medicine, College of Vet Medicine, MSU; Office of the Vice President for Research and Graduate Studies, MSU
Student Support: NIH Grant 5T35OD016477-20 to Michigan State University

An investigation into plasma corticosterone levels and how they change in response to stress in Amazon parrots

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Corticosterone is a steroid hormone produced by the adrenal gland in avian species. This hormone becomes elevated during stressful stimuli such as handling and medical procedures. However, little research has been done in psittacine species to establish baseline (BL) plasma corticosterone (cort) levels and assess how those levels change in response to a stressful stimulus. The purpose of this study was to: 1) measure BL plasma cort levels in 22 Hispaniolan Amazon parrots (*Amazona ventralis*; HAP); and 2) measure how cort levels changed over the course of 1 hour in response to handling and restraint. We hypothesized that cort levels for each bird would initially be low, peak at 30 min, and decrease over the remaining time. To test our hypothesis, we caught each bird, collected an initial BL blood sample, wrapped the bird in a towel for restraint like we would in the clinic, and collected blood samples every 15 min for 60 min. To determine the concentration of cort in each plasma sample, we used enzyme-linked immunoassays (Arbor Assays, K014-H1/H5) that we validated for HAP by assessing parallelism of diluted parrot plasma samples with a cort standard curve. Preliminary results indicate that all birds, regardless of sex or presence of feather destructive behavior, significantly increased cort in response to restraint (average BL cort ± SD: 0.51 ± 0.65 ng/ml). Our initial data also suggest that there may be sex differences in cort concentrations at later time points, where females had significantly higher cort than males at 30 min, 45 min and 60 min. By establishing BL plasma cort levels and measuring how stress influences these concentrations in HAP, we can investigate new ways to reduce stress in psittacine patients.

Research Grant: LSU-SVM Avian Research Fund and LSU Start-Up Funds
Student Support: Boehringer Ingelheim

The regulation of IL-1β production by the serine-threonine kinase, Tumor Progression Locus 2 (Tpl2)

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IL-1β is a quintessential pro-inflammatory cytokine that facilitates innate immunity to pathogens but also contributes to the pathogenesis of numerous autoimmune and autoinflammatory diseases. Due to its inflammatory potency, IL-1β is tightly regulated by the multimeric protein inflammasome complex. Stimulation of host pattern recognition receptors (PRRs) (Signal 1), induces transcription of IL-1β and NLR family pyrin domain containing 3 (NLRP3), whereas activation of the NLRP3 inflammasome (Signal 2) facilitates cleavage of pro-IL-1β. The serine-threonine protein kinase, tumor progression locus 2 (Tpl2 or MAP3K8), transduces signals by host PRRs. Several lines of evidence have implicated Tpl2 in the regulation of IL-1β secretion, however the precise contribution(s) of Tpl2 to this process is unclear and is the subject of this study. We hypothesized that Tpl2 kinase activity is required for both priming and inflammasome activation. Bone marrow-derived macrophages (BMDMs) from wild type or *Tpl2-/-* mice were treated with Tpl2 inhibitor either before LPS priming or after LPS treatment but prior to inflammasome activation with ATP. IL-1β transcription and secretion were quantified. Pharmacological inhibition of Tpl2 kinase activity impaired IL-1β transcription during priming but was dispensable for inflammasome activation. Notably, Tpl2 genetic ablation completely abrogated IL-1β expression and secretion compared to pharmacological Tpl2 inhibition alone. Ongoing studies are examining how Tpl2 regulates expression and/or assembly of other inflammasome components. A better understanding of Tpl2's role in IL-1β production will help clarify its potential therapeutic use in chronic inflammation.

Research Grant: NIH-NIAID Award Number R21AI147003-01. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health
Student Support: Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Optimizing CRISPR/Cas Gene Editing in Canine Cells for Pyruvate Dehydrogenase Kinase 4

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Dilated cardiomyopathy (DCM) is the second most common form of heart disease in canines. The disease significantly affects Doberman Pinschers, and this breed has a higher prevalence rate of DCM and reduced survival time compared to other breeds. DCM is caused by two genetic mutations, one in the pyruvate dehydrogenase kinase 4 (PDK4) gene which regulates lactate and acetyl-coA metabolic flux of the heart. The gene mutation is a 16 base pair deletion in the intron of the gene which leads to altered splicing. The objectives of this study were to determine the efficacy of homology-independent targeted insertion (HITI) gene editing in primary Doberman Pinscher fibroblast cells as an approach to correcting the mutation for the PDK4 gene. We hypothesized that clustered regularly interspaced short palindromic repeats (CRISPR) and its CRISPR associated protein-9 would be effective in correcting the mutation for PDK4. Guide RNAs (sgRNA) were designed to target the mutant region and enable replacement of the deleted region by HITI. Individual sgRNAs were first tested with *in vitro* cleavage reactions using Cas9 ribonucleoproteins (RNPs) for efficacy of cutting. Candidate sgRNAs RNPs will be nucleofected into primary fibroblasts and cleavage efficiency will be assessed by direct sequencing of targeted region and analysis using Tracking of Indels by Decomposition (TIDE) and Interference of CRISPR Edits (ICE). This study investigates a novel approach to gene editing in canine cells.

Research Grant: Morris Animal Foundation (D21CA-042) (CJM, AE, CP, CV), the Doberman Pinscher Health Foundation (CJM, AE), and the ACVIM Pacemaker Fund Diplomate Grant (CJM, AE)
Student Support: Linda F. Hayward Florida Veterinary Scholars Program, UF College of Veterinary Medicine

Does disruption of FAP⁺ stromal cells by FAP-CAR T cells enhance the efficacy of immune checkpoint inhibitor?

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Immunotherapies, including both immune checkpoint blockade (ICB) and adaptive cell therapy (ACT) employing tumor antigen specific chimeric antigen receptor (CAR) T cells, have proven challenging in solid tumors, especially in pancreatic ductal adenocarcinoma (PDAC). PDAC's immunosuppressive tumor microenvironment (TME) and extensive remodeling of tumor stroma contribute to the limited success of immunotherapies. A pro-tumorigenic subset of cancer associated fibroblasts (CAFs) that express fibroblast activation protein (FAP) are major deterrents of immune cell infiltration and potent mediators of immunosuppression in the TME. Using multiparametric flow cytometry and multiplex immunofluorescence, we showed that stromal cell targeted FAP-CAR T cells, effectively infiltrate and inhibit tumor growth in mouse models of PDAC due to their capacity to deplete stromal cells and ECM that otherwise present a barrier to adoptive cell therapies. More importantly, depletion of FAP⁺-CAFs resulted in more endogenous T cells trafficking to and infiltrating into tumor nest. We are now testing the hypothesis that disruption of FAP⁺ stromal cells by FAP-CAR T cells may enhance efficacy of subsequent treatment with ICB (anti-PD1) by enhancing the function of FAP-CART cells and endogenous T cell infiltration and functionality. To date, our findings established that FAP-CAR T cell-mediated ablation of immunosuppressive FAP⁺-CAFs and disruption of the desmoplastic stroma they generate, can enhance accumulation and functionality of endogenous T cells and ongoing studies will determine if in addition, treatment with FAP-CAR T cells can enhance the efficacy of ICB therapy in the context of highly desmoplastic solid tumors.

Research Grant: TMUNITY and a grant from the PHS
Student Support: NIH T35 OD010919, Boehringer-Ingelheim, and the University of Pennsylvania

Development of a porcine oocyte collection protocol for intracytoplasmic sperm injection with stallion sperm

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Assisted reproductive technologies (ART), such as intracytoplasmic sperm injection (ICSI), are commonly utilized in clinical equine practice for mares and stallions suffering from infertility. For ICSI, a mare's oocyte is injected with a singular sperm from the desired stallion. Embryonic development to the stage of transfer remains low and research advancements are stifled due to lack of equine oocyte availability. We propose using porcine oocytes as a model to then evaluate stallion fertility and semen processing techniques. Collection of oocytes from abattoir porcine ovaries, establishment of appropriate maturation timing, and optimization of maturation media (MM) were the three main variables evaluated. Formation of a polar body (PB) indicates maturation of an oocyte to undergo ICSI and served as an end point. Follicular aspiration techniques were developed through modification of a bovine protocol and provided 760 total oocytes. There was a statistical difference in PB formation when oocytes were placed directly into control MM (cMM) post-aspiration compared to holding for 24 hours (24.4% vs. 3.3%, $P = 0.004$) and when 50% porcine follicular fluid (PFF) was added to MM compared to cMM (26.1% vs. 11.3%, $P = 0.019$). There was not a statistical difference in PB formation when 33% PFF was added to MM compared to cMM (23.7% vs. 24.2%, $P = 0.938$), or when MM contained 20% fetal calf serum vs. 10% in cMM (19.1% vs. 17.1%, $P = 0.753$). Therefore, porcine oocytes can be collected and successfully matured in an equine based MM in preparation for ICSI. Further development and implementation of this protocol provides a foundation for research opportunities devoted to improvement of a multitude of ART procedures across species.

Research Grant: None
Student Support: USDA Animal Health and Disease

Genome-wide off-target effects of antisense oligonucleotides used for the treatment of spinal muscular atrophy

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Spinal Muscular Atrophy (SMA) causes degeneration of motor neurons and is a leading genetic cause of infant mortality. SMA is caused by mutations or deletions in the *Survival Motor Neuron 1* gene (*SMN1*). The human genome contains another copy of the *SMN* gene, *SMN2*. However, due to the skipping of *SMN2* exon 7, *SMN2* cannot naturally compensate for the mutation in the *SMN1* gene. ISS-N1, a negative splicing element in intron 7, negatively impacts exon 7 inclusion in *SMN2*. Antisense oligonucleotides (ASOs) are synthetic molecules that can be used for splicing modulation. It's been shown that ASOs sequestering ISS-N1 promote *SMN2* exon 7 inclusion and increase *SMN* protein levels in SMA patient cells. One such ASO (Nusinersen) became the first FDA-approved therapy for SMA. ASOs are modified to protect against degradation, improve binding to its targets, and promote body-wide distribution. However, it is unknown if ISS-N1-targeting ASOs cause off-target effects specific to their modifications. In this study, we performed RNA-seq on transcripts isolated from SMA patient cells treated with ISS-N1-targeting ASOs with three different modifications: phosphorothioate backbone with 2'O-methyl (2OMe), a similar backbone with 2'O-methoxyethyl (MOE), and phosphorodiamidate morpholino oligonucleotides (PMOs). Analysis of the RNA-seq data revealed ASO-modification-specific off-target effects in SMA patient cells. While MOE ASO affected multiple splicing events in a sequence-specific manner, 2OMe ASOs produced the strongest off-target effect on splicing. PMO showed the least changes in splicing. We validated our findings using sqRT-PCR. Findings are instructive for future ASO-based therapies for SMA and other disorders.

Research Grant: NIH RNS R01 NS055925
Student Support: NIH T35 Training Grant T35OD027967

The Relationship Between Total Serum Magnesium Levels and Seizures in Dogs – a Preliminary Study

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The most common chronic neurological condition in dogs is epilepsy (0.5%-5%prevalence). Anti-epileptic drugs in both veterinary and human medicine, are commonly used to control the symptoms of this disease; yet the efficiency can vary amongst patients. A correlation has been found between low ionized magnesium (Mg) levels as well as high ionized calcium/magnesium (Ca/Mg) ratio in human epileptic patients. If true, Mg supplementation has the potential to be a treatment option in human epileptic patients that are not well controlled with current treatment options. The hypothesis of this study was that total serum Mg levels in canine seizure patients would be lower compared to control non-seizure canines, similar to humans. In the present retrospective study, total serum Mg level was compared between seizure patients and control dogs. Serum Mg levels were obtained from past medical records of our institution (2020-2022) for 13 dogs experiencing seizures (seven idiopathic epilepsy tier II and six structural epileptic dogs), as well as in 10 control dogs. A general linear model compared the differences between total Mg level, total Ca level and Ca/Mg ratio between groups. Results showed that total Mg was not different between control dogs and the seizure group (P-value = 0.24). As opposed to humans, Ca/Mg ratio was significantly higher in male castrated dogs in the control group compared to the seizure group (P-value < 0.5). These findings do not support the hypothesis that Mg levels would be decreased in canine patients experiencing seizures. Replication of this study using larger sample sizes with ionized Mg and Ca could yet show supporting results to the hypothesis.

Research Grant: Boehringer Ingelheim Summer Research Scholarship
Student Support: Boehringer Ingelheim Summer Research Scholarship

Investigating the role of envelope integrity protein EipA in the ovine pathogen *Brucella ovis*

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Brucella ovis is a facultative intracellular pathogen that causes Brucellosis in small ruminant males, which is characterized by reproductive issues such as epididymitis and infertility. *B. ovis* contains the periplasmic protein EipA. The molecular function of this protein is not well understood, but studies in a similar species, *Brucella abortus*, suggest it is important for envelope stress resistance. These studies have shown that when the *eipA* gene is deleted in *B. abortus*, the replication and survival of the pathogen is attenuated in macrophages. In *B. ovis*, however, *eipA* is an essential gene. When *eipA* is depleted, *B. ovis* cells form chains and present a viability defect that is hypothesized to be related to membrane instability. Based on this information, our project aims to discover the role of *eipA* within *B. ovis*. To test our hypothesis that *eipA* is important for *B. ovis* infection of macrophages, we compared macrophage entry, replication, and survival between a wildtype *B. ovis* strain and a *B. ovis* strain containing elevated levels of *eipA*. THP-1 cells were differentiated into a macrophage-like phenotype and infected with the *B. ovis* strains. Moreover, preliminary evidence suggests that the *eipA* depletion strain may overproduce an unknown polysaccharide. To characterize this putative production, we performed a plate assay containing the polysaccharide dyes Congo red and trypan blue. This study will inform our understanding of the role of *eipA* in macrophage infection and polysaccharide production by *B. ovis*.

Research Grant: NIH R25
Student Support: NIH R25 grant through the BRUSH Summer Research Program

Evaluation of quantitative MRI as a modality to assess intervertebral disc disease in canines

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Intervertebral disc disease (IVDD) is a degenerative spine disorder in dogs characterized by the herniation of the intervertebral disc into the spinal canal, causing compression of the spinal cord. This can result in chronic pain, paralysis, incontinence, and even death. Herniation can occur in any dog breed but has higher prevalence in chondrodysplastic breeds such as dachshunds. Currently, imaging can only identify herniated discs and therefore treatment only begins after the manifestation of disease. The aim of this study is to investigate whether quantitative MRI techniques can noninvasively provide measures of disc biochemical properties, with the long-term goal to identify disc degeneration and predict risk of rupture prior to injury to the spinal cord. Five initial thoracolumbar vertebral columns from canine body donations were imaged ex vivo to obtain the quantitative MRI values of T2*, T2, T1ρ, and adiabatic T2ρ in two regions of each of 10 discs (T11/T12 to L7/S1): nucleus pulposus and annulus fibrosus. Levels of collagen, glycosaminoglycan, and water content were determined by biochemical assays, and histology was performed on all of the discs. While MRI values were similar across dog breeds, there were no clear correlations between the biochemical and quantitative MRI measures within these first five vertebral columns. Ongoing data collection in additional vertebral columns may help better establish trends across dog breeds.

Research Grant: Research Grant: University of Minnesota College of Veterinary Medicine Signature Grants
Student Support: University of Minnesota Department of Veterinary Clinical Sciences

Therapeutic benefit of palliative radiation therapy assessed by an accelerator in dogs with nasal tumors

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Canine nasal tumors are locally aggressive malignancies with common clinical signs including epistaxis and respiratory distress. Palliative radiation therapy (PRT) has been the standard treatment for dogs with nasal tumors to improve quality of life (QOL). However, there are no objective measurements to assess changes in QOL in dogs with nasal tumors during and post PRT treatment. The objectives of the study are to 1) investigate the survival benefit of PRT, 2) assess QOL using sleep efficiency as a parameter, and 3) examine sleeping patterns and the owner survey. We hypothesized that patients receiving PRT will have better sleep efficiency due to the shrinkage of the tumor. Survival data analysis was performed on 135 canine patients with nasal tumors that received either no treatment (n = 117) or PRT (n = 18) at Kansas State University. We used Fitbark, a commercially available accelerator to monitor activity changes between 1 am to 5 am, and generated sleep efficiency profiles in 6 dogs, which is a subset of the 18 PRT-treated dogs. There was a significant difference between the survival curve of the no-treatment group and PRT group, with estimated median survival times of 76 and 615 days, respectively. The sleep efficiency plots show patterns consistent with owner's observation in the survey. Overall, we observed a decline in sleep efficiency during the course of PRT and an improvement shortly after finishing PRT then reached a steady level. Our study indicated a significant survival benefit for dogs who underwent PRT. The agreement between the sleep efficiency calculated by FitBark and owner survey indicate that FitBark can be a candidate monitor used to assess therapeutic response and QOL in dogs with nasal tumors.

Research Grant: Department of Clinical Sciences, College of Veterinary Medicine, KSU
Student Support: Boehringer Ingelheim Veterinary Scholars Program and Kurz Family Scholarship

Effects of ivermectin-treated bird feed on pigeon safety and mosquito mortality

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West Nile virus (WNV) is a vector-borne virus that circulates between wild birds and mosquitoes, and can cause severe disease in several species including birds, humans, and horses. With no human vaccine and current vector-control strategies facing problems related to insecticide resistance and high off-target effects, there is need for a novel approach. One potential method is to introduce ivermectin (IVM), an anthelminthic therapeutic that targets glutamate-gated ion channels in invertebrates, to birdfeed. Birds could then develop mosquitocidal concentrations of drug in their serum, causing the death of mosquitoes upon bloodfeeding. To further assess the efficacy of this vector-control strategy, we fed treated feed at 400 mg/kg IVM per kg of feed to wild-caught feral pigeons (*Columba livia domestica*) over one week. We examined their health, weight and the amount of consumed feed daily. Additionally, we drew blood which was fed to laboratory strains of *Culex tarsalis* and *C. pipiens* to evaluate mosquito mortality over the course of one week. Following completion of the diet, we necropsied all pigeons and examined the histopathology of their internal organs. The study showed that serum collected from IVM-treated pigeons conferred a marked mosquitocidal effect on *C. tarsalis*, whereas *C. pipiens* mosquitoes were only slightly affected. Furthermore, daily health assessments, necropsies, and preliminary results from histopathological examinations suggest that there are no differences between control and treated birds. Collectively these data provide further proof of principle for the impact of birdfeed-associated mosquito vector control and serve as a foundation for additional controlled pharmaceutical and field studies.

Research Grant: R01AI148633
Student Support: Boehringer Ingelheim

Evaluation of fecal carriage of antimicrobial resistant Enterobacterales and *Campylobacter* species in goats

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Enterobacterales and *Campylobacter* species are commonly found in the feces of healthy ruminants but can also harbor antimicrobial resistance of concern to human health. In this study, we hypothesize that screening for these potential zoonotic pathogens will be beneficial in assessing the current state of carriage of antimicrobial resistant pathogens of One Health concern in the gastrointestinal tract of livestock species. Fecal samples were collected from 40 goats recently procured from multiple sources; following sample collection 20 were treated with an anthelminthic drug (levamisole) and 20 were not treated based on FAMACHA score. For a broader understanding of the carriage of antimicrobial resistant pathogens, a second sample was collected from 33 of the original 40 goats approximately three weeks after treatment occurred. Samples were plated on Extended Spectrum Beta-Lactamase (ESBL), carbapenem-resistant Enterobacteriaceae (CRE), and *Campylobacter* selective media to assess the presence of antimicrobial resistant pathogens. Overall, *Campylobacter* spp. were found in 2.5% of pre-treatment and 12.1% of post-treatment samples; further antimicrobial susceptibility testing to determine if resistance is present in these isolates is ongoing. Growth of ESBL resistant Enterobacterales was found in 4.5% of pre-treatment samples and was primarily *E. coli*; no significant growth was observed on CRE plates. Post treatment samples demonstrated no significant growth in either group on ESBL and CRE media. The study has thus far revealed a minimal number of goats that carry antimicrobial resistant Enterobacterales and *Campylobacter* spp. Levamisole treatment had no observed effect on the carriage of antimicrobial resistant pathogens.

Research Grant: Foundation for Food and Agriculture Research Vet Fellow
Student Support: Foundation for Food and Agriculture Research Vet Fellow

The impact of cooked broccoli on short chain fatty acids and related gene expression in lean and obese mice

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Dietary fiber is fermented in the gastrointestinal (GI) tract by the gut microbiome (GM) to produce short chain fatty acids (SCFAs). The major SCFAs produced by the GI tract are acetate, butyrate, and propionate. SCFAs act as ligands to various receptors that affect several physiological functions including cardiovascular and metabolic health. G-protein receptor 43 (GPR43) and GPR41 are SCFA receptors of interest because of their role in insulin sensitivity and gastric emptying. Broccoli is rich in dietary fiber and this fiber content can alter the GM with frequent consumption, potentially affecting SCFA production. It was hypothesized that daily broccoli consumption increases SCFA production and alters the expression of GPR43 and GPR41 in lean and obese mice. Twenty lean mice were randomized to consume a low-fat diet (LFD) or a LFD + 10% cooked broccoli (CB, w/w), and twenty obese mice were randomized to consume a high-fat diet (HFD) or a HFD + 14% CB (HFD + 14% CB, w/w, matched broccoli content with LFD + 10% CB per calorie). The mice were fed these diets for one week. Fecal samples were collected on day 0 and 7 and analyzed by gas chromatography/mass spectrometry to measure the change in acetate, butyrate, and propionate. Colon tissue (n = 4/diet group) was collected for the analysis of GPR43 and GPR41 expression using RT-qPCR. There was no significant difference in the gene expression of GPR43 or GPR41 between lean mice fed LFD and LFD + 10% CB or obese mice fed HFD and HFD + 14% CB. The fecal SCFAs are still being analyzed. Future studies will analyze cecal SCFA concentration and additional tissues for SCFA related gene expression.

Research Grant: FSHN Innovation/Research Mini Grant Program
Student Support: Office of the Director, NIH, T35 OD011145

Impact of direct IL-27 signaling on regulatory T-cells

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Interleukin 27, an IL-12 family member, is a heterodimeric cytokine which has been shown to mediate pro- and anti-inflammatory responses. Previous work from this laboratory has shown the impact of IL-27 on various cell types, including the regulatory T-cells. Regulatory T cells (Tregs) function to mediate tolerance at steady state and quell aberrant inflammation during infection. The impact of IL-27 signaling on the Treg compartment at steady state has yet to be defined. Therefore, we have generated a mouse model which utilizes a Cre-lox system to cause specific genetic depletion of IL-27R on Treg cells. Using high-parameter flow cytometry, we were able to analyze the Treg compartment at the resolution of unique subsets. Our high-resolution approach shows that IL-27 signaling at steady state impacts the composition of the Treg compartment. Loss of IL27 signaling leads to a reduced frequency of effector Treg cells. Previous studies observed that IL27 signaling does not impact the Treg population as a whole, but our data suggests that IL27 may play a role in the development of the Treg compartment at steady state.

Research Grant: NIH R01 580697 [OR] NIH R01 805045
Student Support: NIH T35 OD010919 [or] Boehringer-Ingelheim [or] the University of Pennsylvania

Detection of *Brucella* in histologic sections of cetacean tissues using RNAScope *in situ* hybridization

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In cetaceans, *Brucella* can cause diseases including meningoencephalitis, perinatal pneumonia, and abortions. Quantitative polymerase chain reaction (qPCR) is the most-widely utilized method for detecting *Brucella* in cetacean tissues, however qPCR provides no information on location or distribution of *Brucella in situ*. Although immunohistochemistry (IHC) has been utilized for some cetacean brucellosis cases, quality antibodies are not widely available for diagnostic use. RNAScope *in situ* hybridization (ISH) is a highly specific and sensitive technique that uses uniquely designed probes for optimal signal amplification with minimal background staining. This study sought to determine if RNAScope ISH can be used to detect *Brucella* in cetacean tissues. This study also aimed to determine if qPCR cycle threshold (CT) values are predictive of the limit of detection for *in situ* assays. Formalin-fixed paraffin-embedded tissues of known *Brucella* qPCR-positive cetaceans (n = 7) were used to test *Brucella* species-specific ISH probes. Control cases negative for *Brucella* but with *Brucella*-like lesions were also examined (n = 6). To determine sample quality, a positive probe specific to *Tursiops truncates* was used, GAPDH. A negative probe (bacterial gene, *dapB*) was tested on all sections. Signal intensity using the *Brucella* probes were measured using image analysis and correlated to Ct values from *Brucella* qPCR-positive samples. Other variables including formalin fixation time and *Brucella* sequence types (STs) were also assessed in relation to quantity and location of positive signal. Histologic *in situ* visualization of *Brucella* in cetacean tissues via RNAScope will further pathogenesis research and infection control.

Research Grant: University of Illinois Zoological Pathology Program
Student Support: Office of the Director, NIH T35 OD011145

Detection of *Balantidium coli* and fecal microbiome dysbiosis in horses affected with fecal water syndrome

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Background: Fecal water syndrome (FWS) in horses is characterized by the passage of formed stool along with excessive fecal water. Satisfactory explanations for FWS are largely unknown and previous fecal microbiome studies found no differences between affected and control horses. Previous research has been focused on European horses, where feeding and management practices are different to those in North America. Objectives: The overall objective of this project is to identify any differences in fecal microbiomes of horses with FWS and determine if the protozoan *Balantidium coli* is overrepresented in affected horses. Study Design: Case-control study. Methods: Voided fecal samples were collected by owners of horses diagnosed with FWS along with paired controls when available. DNA was isolated and quantified and then used to generate 16S rRNA amplicon libraries which were sequenced. DNA will also be used to perform semi-quantitative real-time PCR using *B. coli*-specific primers. Results: We expect to see an increase in *B. coli* in the fecal samples of horses affected by FWS and a significant difference in the fecal microbiomes of affected versus control horses. Main Limitations: Since the fecal samples used were voided, there is a possibility for environmental contamination. Additionally, the fecal microbiome may not be an exact representation of the gut microbiome. Our sample size was limited to 16 affected horses. Conclusions: Anticipated results may show that *B. coli* plays a role in the development of FWS in mid-western (USA) horses. Such a result might suggest that treatment with anti-protozoal medications could be used for the management of FWS.

Research Grant: Animal Health Foundation of St. Louis
Student Support: Boehringer Ingelheim

A tale of two metabolites: an analysis of the pharmacokinetics of morphine when administered orally

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The metabolism and pharmacokinetics of intravenous morphine in the horse have been described; however, administration of therapeutic doses has also been associated with neuroexcitation and adverse gastrointestinal effects. In the current study, we hypothesized that oral administration would lead to comparable concentrations of morphine and its presumed active metabolite, morphine-6-glucuronide (M6G), without the adverse effects associated with intravenous administration. This study describes the metabolism of morphine and its pharmacokinetic parameters after oral administration, as well as the physiological and behavioral outcomes of such. Eight horses were administered a single intravenous dose of 0.2 mg/kg morphine and an oral dose of 0.2, 0.6, and 0.8 mg/kg of morphine in a four-way balanced crossover design with a minimum two-week washout period between doses. Blood samples for the determination of morphine and metabolite concentrations were collected for up to 96 hours following administration. Concentrations and pharmacokinetic parameters were determined using liquid chromatography tandem mass spectrometry. Physiological and behavioral outcomes-including the number of steps taken each minute, changes in heart rate, and gastrointestinal borborygmi-were assessed. A preliminary analysis of the aforementioned outcomes revealed evidence of a greater increase in both step count and heart rate in the horses administered the intravenous dose compared with the oral groups. M6G concentrations were higher in the oral 0.8 mg/kg dose group compared with the intravenous group. Results of the current study are encouraging for further study, specifically the antinociceptive effects of morphine following oral administration.

Research Grant: Financial support for this study was provided by the Center for Equine Health at the School of Veterinary Medicine, University of California, Davis
Student Support: National Institutes of Health T35-OD010956; Students Training in Advanced Research Program

Efficacy and toxicity of carboplatin in the treatment of macroscopic mesenchymal neoplasia in dogs

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Soft tissue sarcomas (STSs) and hemangiosarcomas (HSAs) are common mesenchymal tumors in dogs. Both are locally invasive but metastatic behavior varies based on location and grade. When local control is not feasible, doxorubicin-based palliative chemotherapy protocols are often recommended. Patients with tumors resistant to doxorubicin or who cannot safely receive doxorubicin (e.g., dogs with ABCB1-1Δ gene mutation, or with prior significant adverse events from doxorubicin, or with known cardiac disease, etc.) have limited chemotherapy options. Carboplatin is widely used in veterinary oncology but limited information regarding response for non-osseous sarcomas is available in dogs. The purpose of this retrospective study was to evaluate the response rate (RR) and toxicity profile of carboplatin in the treatment of macroscopic non-osseous sarcoma in dogs. Dogs treated with carboplatin with either macroscopic STS or HSA based on sampling or imaging characteristics with at least two evaluations of the target lesion were included. Tumor response was defined according to RECIST criteria when possible, and toxicity was assessed using VCOG criteria. Response duration [complete response (CR) or partial response (PR)] was defined as time from response to disease progression. A total of 29 dogs (17 STS, 12 HSAs) were included. Responses observed included 1 CR and 3 PR, for an overall RR of 13.8%. Median duration of response was 103 days (range 39-252). Responses were only seen in patients with HSA, for an overall response rate in these tumors of 33.3% (4/12). Most toxicities were grade 1 or 2 (65.2%), while only 12.5% were grade 3 or 4. Carboplatin was well-tolerated with variable anti-tumor activity and short response duration.

Research Grant: NIH 5T35OD010956 Student Advanced Training in Research
Student Support: NIH 5T35OD010956 Student Advanced Training in Research

Development and Assessment of a Stair Ascension Challenge in Nonhuman Primates

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Nonhuman primates (NHPs) are valuable models for studying age-related decline. Evaluation of physical function in NHPs is an essential aspect of these studies, as a decline in physical function and an increase in frailty predicts functional impairments, chronic diseases of aging, and all-causes mortality. Current physical function assessments include normal gait speed and measures of overall physical activity. While these metrics are useful, additional assessments of physical performance will provide a more comprehensive characterization of functional decline. Here we propose an additional, rigorous test of physical function: stair ascension speed. Speed of stair ascension integrates multiple components of physical function: isolated leg and back strength, proprioception, balance and range of motion. We first sought to assess the reliability and validity of stair ascension speed measurement in NHPs. Next, we assessed associations between age and speed. Our study sample consisted of ten female middle-aged to geriatric vervets/African green monkeys *(Chlorocebus aethiops sabaeus*; 10-29 years old). We observed monkeys moving naturally through their home enclosures, ascending a stair-like structure, and measured their ascension speeds via live observation and video recording (n = 5-11 observations per animal, mean speed 34.9 cm/s, CV 18-33%). Stair speed was positively correlated with previously reported gait speed data from the same animals ($r = 0.68$, $P < 0.05$) and was negatively correlated with age ($r = -0.77$, $P < 0.01$). Taken together, these data suggest that speed during stair ascension represents a valid measure for assessing age-related functional decline in NHPs.

Research Grant: P40OD010965, UL1TR001420, R24AG073199
Student Support: T35OD010946

Characterization of Carnivore Bocaparvovirus 1 (Minute Virus of Canines, MVC) distal polyadenylation site

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The parvovirus family contains linear, single-stranded DNA viruses. Carnivore bocaparvovirus 1 (Minute virus of canines, MVC) is an autonomous parvovirus in the Bocaparvovirus genus that causes respiratory and enteric disease in dogs. MVC is a unique tractable model to elucidate viral RNA processing mechanisms and host interactions geared toward the development of efficient parvovirus gene therapy and oncolytic virotherapy. It has a single promoter that generates a single pre-mRNA which is processed via alternative splicing and alternative polyadenylation to generate at least 8 mRNA transcripts. MVC contains two polyadenylation sites, one at the right-hand end (pA)d and another (pA)p that lies within the capsid coding region. Our previous results showed that parvovirus non-structural protein, NP1, suppresses the proximal polyadenylation for transcription readthrough and utilization of the distal polyadenylation for efficient capsid production. We have previously utilized RNase protection assay and 3' rapid amplification of complementary ends (3'RACE) to determine four specific cleavage and polyadenylation sites in the proximal polyadenylation region, which are preferentially utilized in the absence of NP1. The distal polyadenylation sites within the right-end hairpin are unknown. We are currently utilizing a high throughput RNA-sequencing method to evaluate the total RNA extracted 48 and 72 hours post-infection from Walter Reed Canine (WRD) cells with MVC. This will be complemented with 3'RACE to characterize and elucidate the unique MVC distal polyadenylation cis-acting elements and polyadenylation sites.

Research Grant: Iowa State University College of Veterinary Medicine Seed grant
Student Support: NIH T35 training grant awarded to Iowa State University College of Veterinary Medicine

Development of Type III Collagen Biomaterials to Improve Clinical Outcome of Canine Mammary Tumors

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Breast cancer is the leading cause of morbidity and mortality in veterinary and human medicine. Recent studies have focused on the tumor microenvironment (TME) as a promising target to decrease recurrence in patients with malignant mammary tumors. Collagen, a major component of the TME, plays critical roles in neoplastic and non-neoplastic cell behavior in cancer development. Type I collagen (Col1) expression is widely studied as having pro-carcinogenic effects and is linked to a worse prognosis. However, there has been less focus on Type III collagen (Col3). Our previous studies have shown that in contrast to the tumor permissive effects of Col1, Col3 reduced tumor growth, modulated stromal matrix architecture, and decreased aggressive cancer cell behavior in human and murine models. We hypothesize that Col3 will decrease aggressive cancer cell behavior in canine cancer cells and may be used therapeutically to decrease local and distant recurrence. To compare the effect of collagen types on canine cancer cell behavior, canine mammary carcinoma cells were grown in 3D culture supplemented with either Col1, Col3, or both. Immunofluorescence staining of e-cadherin, active caspase 3 and Ki67 was used to characterize cell adhesions, apoptosis, and proliferation, respectively. The cell line tested, CAMAC2, formed more spheroids in Col3 than in Col1 (where colonies were less organized). E-cadherin expression was increased in the presence of Col3, consistent with a more benign phenotype. Additional assays will determine the impact of collagen type on proliferation and apoptosis. This study, in addition to past murine models, will provide support for future clinical trials targeting cancer recurrence in canine and human patients.

Research Grant: Canine Health Foundation #02920
Student Support: NIH T35 OD010919, Boehringer-Ingelheim and the University of Pennsylvania

Transfer of antibiotic resistance genes by conjugative plasmids to bacteria of the human gut microbiome

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The GI tract is rich in bacterial populations that can transfer antibiotic resistance genes to each other via circular DNA molecules known as plasmids through a mating process termed "conjugation." This process can happen at alarmingly high rates within the gut, even in healthy individuals. Several studies evaluating the spread of antibiotic resistance genes through plasmid transfers from laboratory *E. coli* strains to human gut microbiota isolates have been documented in vitro. However, this plasmid transfer process has not been examined in vivo where the situation is more complex. Our study tracks the transfer of antibiotic resistance genes in isolates from fecal samples from mice carrying human-derived gut microbiotas to serve as an in vivo model rather than using lab strains of recipient and donor bacteria. Bacterial populations from C57BL/6 mouse fecal samples of 3 microbiota types were grown and isolated to act as recipients, while a commensal *E. coli* strain carrying a fluorescently labelled RP4 conjugative plasmid was used as a donor. Filter mating was performed between donor and each microbiota and transconjugants were isolated using selective media and confirmed by fluorescent microscopy. Two transconjugant colonies were isolated from one of the fecal samples grown on MacConkey agar selective for enteric gram-negative recipients. Isolating transconjugant colonies from fecal samples grown on Mueller Hinton agar to select for gram-positive recipients will be completed soon. We anticipate identifying the bacterial transconjugants and confirming the presence of the RP4 plasmid using Sanger sequencing of the 16S gene and Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF).

Research Grant: Albert C. and Lois E. Dehn Endowment
Student Support: Boehringer Ingelheim and the Graduate School at Michigan State University

Evaluating if Sex and Anatomical Location Has an Effect on Adipocyte Progenitor Concentration Within PVAT

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Perivascular adipose tissue (PVAT) is a major paracrine organ that can significantly impact the vasculature of major arteries within the body. For example, PVAT produces vasoactive adipokines which play a pivotal role in vascular function and tone. Adipose tissue hypertrophy and hyperplasia can alter the shape and function of PVAT. Dysfunctional PVAT has been linked to cardiovascular disease and hypertension in various animal species along with humans. Adipogenesis differentiates mesenchymal stem cells into adipocytes. Progenitor cells have been seen to express a protein receptor called PDGFRa. Flow cytometry has proven that sex differences and tissue location influence the frequency of adipocyte progenitor cells (APCs). However, immunohistochemistry (IHC) allows for APCs to be identified and quantified as well and can be used to show redundancy to these findings. In this study, PVAT samples were taken from *Cre-LoxP* mice. PDGFRa+ cells along with vascular smooth muscle cells (VSMCs) were stained via IHC methods. The computer program Fiji quantified the intensity of PDGFRa+ cells and their proximity to vascular cells from pictures taken microscopically. Based on preliminary data from two mice, abdominal PVAT (ABPVAT) appears to generate a higher frequency of PDGFRa+ cells as well as a closer proximity to VSMCs compared to aortic-thoracic PVAT (ATPVAT). However, proper statistical analysis will be done as more samples are analyzed. We hypothesize that changes in sex and tissue location will present a difference in both progenitor cell quantity and VSMC proximity. APCs are vital in the expansion of adipose tissue and understanding where and how they mature can provide insight on the formation of dysfunctional PVAT.

Research Grant: Unknown
Student Support: Michigan State University Biomedical Research for University Students in Health Sciences

The role of TUBB3 in neural crest cell proliferation, migration, and differentiation into craniofacial tissue

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Neural crest cells (NC) are multipotent embryonic stem cells that give rise to craniofacial cartilage, bone, and cranial nerves. For proper embryonic development, NC must proliferate, undergo epithelial-to-mesenchymal transition (EMT), and migrate to give rise to their derivatives at specific developmental time points. A novel player linked to the process of EMT is β-III Tubulin (TUBB3), a microtubule subunit. In humans and animals, mutations in TUBB3 are linked to clinical conditions including congenital blepharoptosis, congenital fibrosis of the extraocular muscles, facial palsy, and early onset peripheral neuropathy. The aim of this study is to determine if TUBB3 is both necessary and sufficient for neural crest EMT and to identify the craniofacial defects that are caused by early knockdown (KD) and overexpression (OE) of TUBB3. Unilateral KD or OE injections were performed on chicken embryos and subsequent immunohistochemistry (IHC) was used to identify changes in protein expression associated with neural crest development. Our preliminary results show that both TUBB3 KD and OE increase NC proliferation, drive premature EMT, and maintain hyper-collective cadherin-mediated migration. Specifically, TUBB3 KD and OE both increase in E-cadherin fluorescence in SOX9 (NC marker) positive cells. Our work here demonstrates interrelated programs that control rapid changes in NC cell adhesion, migration, and differentiation, and may provide potential targets for biomarkers of craniofacial anomalies and disease.

Research Grant: R03 DE032047-01
Student Support: Students Training in Advanced Research Program (NIH T35 OD010956-22 grant)

The role of aldehyde dehydrogenase-2 in modulating acrolein-mediated damage following spinal cord injury

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Spinal cord injury (SCI) is marked by primary injury (physical impact) and a secondary injury (chemical injury) that amplifies the damage and functional deficits triggered by the primary trauma. An important hallmark of secondary injury is oxidative stress. Acrolein, a key player in oxidative stress, is a toxic aldehyde that is elevated significantly following SCI. Acrolein increases reactive oxygen species (ROS) and lipid peroxidation, thus furthering the damage. Acrolein is of special importance because it has a longer half-life than known ROS and inhibits important endogenous antioxidative stress enzymes. Previous research has identified aldehyde dehydrogenase 2 (ALDH2) as an important antioxidative enzyme. ALDH2 metabolizes acrolein to suppress oxidative stress, but can also be inhibited by acrolein, especially during acrolein overload. Over 600 million people worldwide exhibit an inactive form of isoenzyme ALDH2 (ALDH2*2) that is linked to several diseases, such as Alzheimer's, Parkinson's Disease, and alcohol flushing response. The overall objective of this study was to assess the role and the potential therapeutic value of ALDH2 and the neuroprotective effect of Alda-1, an ALDH2- selective agonist, in SCI, using a transgenic mouse model with ALDH2*2. There were two central hypotheses for this study: 1) transgenic mice would exhibit a higher concentration of acrolein compared to wild-type following SCI, and 2) treatment with Alda-1 would amplify ALDH2 function in both wild-type and transgenic mice, reducing acrolein concentration in the spinal cord. Findings from this study further illustrated ALDH2 as a target for attenuating secondary injury of SCI and introduced Alda-1 as a potential treatment for SCI.

Research Grant: None
Student Support: Purdue University College of Veterinary Medicine Summer Research Scholarship

Effects of early life experiences on later problematic behaviors in homeless, rescue shelter kittens

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The effects of early familial separation have been studied in owned cats, revealing a relationship between early separation and problem behaviors, such as aggression toward humans and increased incidence of stereotypic behaviors. However, no studies to date have focused on homeless, rescue kittens. It is possible that stress experienced by their mothers may negatively affect these kittens' social development, with stress during early sensitive periods predisposing these animals to behavioral problems later in life. Behavioral and social assays provide an important window into the effects of such stress. Kittens are tested using open field, novel human approach, and novel human holding tests. These assays provide data regarding how active, social, and engaged the kittens are. At the end of the study, 60 foster kittens will have been tested at 8, 10, and 12 weeks of age. During the approach test, variables including latency to approach are measured. During the novel human holding test, variables such as duration of calm holding are measured. It is possible that there is a relationship between latency to approach and duration of willingness to be held, which would provide insight into the sociability of these kittens and whether it can be predicted. Currently, testing has been completed on 11 8-week-old foster kittens. Latency to first approach ranges from 4.66-121 seconds; and duration of first hold ranges from 1.3-20.52 seconds. Data collection is ongoing, and will provide a larger sample size. The results of this study can be used to inform guidelines for the foster program at the PEI Humane Society, as well as inform future studies with the hope to mitigate the impact of early life stress and deprivation on kittens.

Research Grant: EveryCat Feline Foundation
Student Support: AVC Veterinary Summer Research Award

Tissue and plasma enzyme activities in a managed population of golden trevally (*Gnathanodon speciosus*)

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Veterinary care of aquatic species, particularly fish, is limited by a lack of knowledge regarding their unique physiology. Tissue and plasma enzymes are used in veterinary medicine for assessing function and potential damage to specific organs and tracking disease progression. The objective of this study was to identify tissue(s) of origin and plasma concentrations for specific enzymes in healthy golden trevally (*Gnathanodon speciosus*) fish. Our hypothesis assumed enzymes would exhibit tissue specific tropisms with higher activities in one or more tissues compared to others. Six fish were obtained from a managed population to obtain antemortem blood samples. The fish were then euthanized and tissue samples were collected via gross necropsy. Activities were examined for eight enzymes in plasma and ten tissues in each fish. Enzyme activities exhibited significant organ specificities. Aspartate aminotransferase, lactate dehydrogenase, and creatine kinase (CK) levels were highest in skeletal muscle with variably high CK levels in gonads. Alkaline phosphatase levels were highest in the kidney, spleen, and liver. Alanine aminotransferase levels had high specificity for the liver. Gamma-glutamyltransferase was only detectable in the kidney and plasma. Uric acid and blood urea nitrogen were below detectable limits in all samples. This work establishes baseline tissue enzyme origins for golden trevally fish, which will aid clinicians in diagnostic interpretation of serum chemistries and improve veterinary care for understudied fish species.

Research Grant: None
Student Support: Mississippi State University Global Center for Aquatic Health and Food Security

Evaluation of classifier models to discriminate phenotypes of Salmonella enterica using FT-IR

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Salmonella spp. are associated with a broad range of infections in veterinary species and are a significant cause of human foodborne illness. Fourier-transform infrared spectroscopy (FT-IR) is an emerging tool in clinical microbiology, allowing for rapid and cost-effective generation of spectroscopic fingerprints varying based on composition of bacterial cell wall and outer membrane. Spectroscopic profiles were generated from 137 Salmonella strains of known serogroup, serotype, and species of origin. A subset (n = 105) was characterized to determine the presence or absence of antimicrobial resistance (AMR). Classifier models were generated by selecting a phenotype of interest and analyzing a training set of isolates with artificial neural networks (ANN). A serogroup classifier was generated from 118 of the available strains. The training set contained 60 strains, and the test set contained 58, which had similar strain diversity. The in-house generated classifier model had 89.6% agreement when predicting serogroups of the test isolates. Classifier models were also developed to identify multi-drug resistant (MDR) Salmonella isolates. These classifiers were developed by specifically analyzing the amide (1800-1500cm^{-1}), polysaccharide (1200-900cm^{-1}), fingerprint (900-700cm^{-1}), and mixed (1500-1200^{cm-1}) regions of the FT-IR spectrum. The classifier model generated from the amide and polysaccharide regions identified MDR Salmonella isolates with 86.27% agreement, 53.33% sensitivity, and 100% specificity. The Kappa statistic between the classifier-predicted and true phenotypes was 0.617 (substantial agreement). The results indicate FT-IR has the potential for use as a phenotypic classification tool for Salmonella spp.

Research Grant: Nebraska Experiment Station, Animal Health and Disease Research (section 1433) (accession #1017646 to JDL), Nebraska Beef Council and National Cattlemen's Beef Association (to RM)
Student Support: Foundation for Food and Agriculture Research Veterinary Research Fellowship

Phosphodiesterase inhibition treats respiratory depressed inflamed neonates by increasing breathing frequency

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Premature infants face an uphill battle due to complications that can harm their lives. Apnea of prematurity and infection are leading causes of the death in premature newborns. Bacterial infection occurs in 65% of infants during hospitalization and account for 1/3 of neonatal deaths. Inflammation increases apneas and induces irregular breathing. Caffeine is the current treatment for apneas, but has many side effects and is a nonselective phosphodiesterase inhibitor. We hypothesize that a more selective phosphodiesterase inhibitor will reverse inflammation-induced impaired breathing in neonates. To test this hypothesis, neonatal rats (P0-P3) were injected with nothing or lipopolysaccharide 3 hours prior to anesthetizing and euthanasia. The brainstem and spinal cords were isolated and placed in a recording chamber and bathed in artificial cerebrospinal fluid. These preparations produce respiratory-related motor output ("bursts") on ventral cervical spinal root C4. Preparations from LPS-injected pups had baseline burst frequencies of 5.23 +/- 0.4 bursts/min compared to 9.79 +/- 0.3 bursts/min in controls, suggesting that LPS treatment induced respiratory depression. Controls bathed with Roflumilast (a selective PDE4 inhibitor; 0.5 micromolar) acutely induced 22% increase in burst frequency above baseline that was maintained even after 60 min of drug washout. Preparations from LPS-injected pups exposed to 0.5 micromolar Roflumilast increased burst frequency by 20% above baseline that was maintained at 57% following drug washout. Thus, Roflumilast reversed inflammation-induced respiratory depression in neonates. Roflumilast may represent an alternative therapy for infected (premature) newborn infants.

Research Grant: National Institutes of Health R01 NS085226 (JJW), R01 HL142752 (JJW, TLB), and the Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin-Madison.
Student Support: Boehringer Ingelheim (BI)

Development of a new vaccine to stop feline coronavirus disease, using the replication-incompetent VSV system

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Feline coronavirus (FCoV) is a positive-stranded RNA virus that infects cats worldwide. Its symptoms are usually mild to absent, but mutations of the virus can cause a systemic acute inflammatory response, resulting in Feline Infectious Peritonitis (FIP). Once it develops, FIP is a serious disease which is typically progressive, and almost always fatal. Any cat that carries FCoV can develop FIP, but younger cats are at greater risk. Our lab recently showed that replication-incompetent VSV virions incorporating the Nipah, Hendra and Ebola glycoproteins (NHE) elicited neutralizing antibodies and protected hamsters from these viruses with 100% safety and efficacy, while all mock-vaccinated animals succumbed (Shahrzad to send citation for paper under review). We used the same method to produce a pseudotyped VSV vaccine incorporating S protein of FCoV Black and UU4 strains, to test if we could produce a vaccine to elicit an immunological response. We found that our pseudotyped VSV FCoV S vaccine is able to enter feline macrophage FCWF-4 cells. The ability to deliver unaltered viral glycoproteins, with intact immunogenic sites, is paramount in creating a potent immune response against viruses. Further development of this vaccine has the potential to help feline health, particularly related to viral infections and specifically FIP.

Research Grant: Cornell Feline Health Center
Student Support: Cornell Veterinary Leadership Program; Bostwick Family Foundation

Behavioral responses to auditory cues altered following manipulation of commissural tectothalamic projections

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Hearing impairments are a major health issue that have a considerable impact on quality of life. Central neural auditory circuits are critical to the processing of sound or auditory information. There have been many studies on the extraction of sound location, which rely on the ability to detect auditory spectral and temporal cues. This ability may be improved from the extraction of this information in higher auditory centers; notably, between the inferior colliculus (IC) and medial geniculate body (MGB). An overlooked pathway connecting these structures is the projections form the IC to the contralateral MGB. Manipulating these commissural pathways using chemogenetic techniques, may alter the ability to localize sound. This chemogenetic approach involves injecting a double-floxed virus expressing a DREDD receptor into the IC, then activating it with compound 21, injected peritoneally. This research assessed the behavioral response of inhibitory VGAT-Cre, excitatory VGLUT2-CRE and wildtype mice by utilizing the acoustic startle response using a modified pre-pulse inhibition with and without DREDD activation; followed by an electrophysiological assessment using the same stimuli. This research sheds light on the impact of the contralateral tectothalamic pathway on auditory processing.

Research Grant: NIH R01 DC 019348
Student Support: NIH T35 Training Grant T335OD012199

Gene Expression Analysis of Canine Blood and Splenic NK Cells in Resting and Activated States

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NK cells are cytokine-producing and cytotoxic innate immune cells with promising potential in immunotherapies due to their decreased risk of adverse effects compared to T cell-based therapies and their ability to be expanded from donors for off-the-shelf administration. While cellular therapies have shown promise in companion dogs, their development has been partially limited by the lack of thorough characterizations of canine immune effector cell subsets. Questions remain regarding the optimal genomic characterization of dog NK populations in resting and activated states, both from PBMC and CD5-depleted starting populations, which is highly relevant to the longevity of NK cells in vivo and their application in the clinic. Given the current lack of canine NK cell transcriptomics analyses, in-depth characterization is needed to identify factors that impact in vivo efficacy and persistence. The purpose of this study was to understand optimal NK isolation and expansion techniques for adoptive transfer through sequencing and bioinformatic analysis of canine NK cells for further clinical translation. The first aim is to identify the trajectory of genomic changes during 14-day expansions of NK cells derived from peripheral blood and splenocytes and to develop a terminal differentiation signature for canine NK cells. The second aim is to compare the differential gene expression of NK cells expanded from unmanipulated PBMCs and splenocytes versus those expanded from CD5-depleted cells. Overall, these findings will fill critical knowledge gaps in the transcriptional characterization of blood and splenic canine NK cells while additionally providing the foundation for future immunotherapy trials.

Research Grant: SVM Center for Companion Animal Health Endowment funds
Student Support: AVMA/AVMF 2nd Opportunity Summer Research Scholarship Program

Reprogramming tumor immune microenvironment of murine colon cancers using novel calreticulin nanoparticle

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Tumor microenvironment of colon cancer with aberrant immunosuppressive signaling pathways can blunt antigen presentation, cytotoxic T-cell activity, and efficacy of immune checkpoint inhibitors (ICIs). To overcome these barriers, herein, we developed a liposome-based calreticulin-nanoparticle (CRT-NP) that upon intratumoral injection transfects colon cancer cells to induce calreticulin (CRT) production. CRT is a dominant pro-phagocytic signal on the cancer cell that enhances antigen presentation and adaptive immunity. Briefly, liposomes formulated with DOTAP and cholesterol lipids were loaded with full-length clone DNA of CRT. Blank- and CRT-NP were characterized for their hydrodynamic diameter and zeta potential using dynamic light scattering (DLS), and cytotoxicity against CT26 colon cancer cells using MTT assay. To determine CRT-induced anti-tumor immunity, mice bearing CT26 colon tumors (50-100mm3) in the flank region received four intratumoral CRT-NP injections over 2-weeks. To test the hypothesis that CRT-NPs synergistically enhance ICI immunotherapy, an anti-CTLA4 agent was administered intraperitoneally 3-days post each CRT-NP treatment. Endpoints evaluated therapeutic efficacy and anti-tumor immune effects using flow cytometry ~4 wks post inoculation. Results showed that CRT-NP in physiological buffer showed a hydrodynamic diameter of ~250nm, zeta-potential of < 25mv, and PDI < 0.3 and excellent stability in physiological buffers up to several days. Characterization and analysis of CT26 growth suppression by CRT-NP vs CRT-NP and anti-CTLA4 vs. that seen in the untreated control, and immune cells present (e.g. T cells, Dendritic cells etc.) is currently ongoing.

Research Grant: The National Cancer Institute of the National Institutes of Health under award number R37 CA239150-02
Student Support: BI and the Kerr Endowed Chair at Oklahoma State University

Prevalence and Antibiotic Resistance Trends of *Enterococcus* spp. from Siluriformes within Alabama

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Aquatic environments and animals are now considered hotspots for antimicrobial resistance (AMR). Aside from this concern, foodborne illnesses from seafood and fish products have significantly increased over the last decade, overtaking poultry products. With growing public health concerns on food safety and AMR, this study aimed at (1) investigating the prevalence of *Enterococcus* spp. within Siluriformes and (2) investigating the antibiotic trends in *Enterococcus* spp. isolated from Siluriformes within Alabama. In this research, we sampled Siluriformes from RAS and pond facilities within Alabama and cultured them to isolate *Enterococcus*. Disc diffusion assay for antibiotic susceptibility profile was later conducted on these isolates. Our findings showed a percent positive prevalence of 88.29 for *Enterococcus* spp. isolated from Siluriformes. Further analysis revealed a lower prevalence of 80.48% positivity in RAS tanks and100 positivity in ponds, where Siluriformes are raised to harvest. The AMR findings revealed that *Enterococcus* spp. was resistant to rifampin. Furthermore, *Enterococcus* spp. showed intermediate susceptibility to azithromycin, suggesting gradual resistance of the bacterium to this drug.

Research Grant: DHHS/HRSA D34HP00001-35-00, NIH/NIMHD RCMI grant # U54MD007585, and USDA/FSIS/ORISE Project #OR-WD-OPPS USDA FSIS (201217259)
Student Support: Tuskegee Veterinary Scholars Program

Hepatic cytokine profile of alcohol-fed, IL-1β transgenic mice: a potential model of alcoholic steatohepatitis

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Chronic, low-dose alcohol consumption can lead to alcoholic steatohepatitis (ASH), a major cause of mortality worldwide. The absence of an animal model that fully recapitulates ASH in human patients impedes advancements of effective disease treatments. Pro-inflammatory cytokine interleukin (IL)-1β plays a major role in ASH pathogenesis by potentiating other cytokines, causing hepatic steatosis and fibrosis. Animal models that target IL-1β and its potent pyrogenic role in hepatic inflammation are important in the study of ASH. The IL-1β transgenic mouse overexpresses human(h) IL-1β solely in the upper gastrointestinal tract, serving as a model for the study of esophageal inflammation and tumorigenesis. Notably, these transgenic mice are constantly in a pro-inflammatory state, defined by high serum levels of murine(m) IL-1β and tumor necrosis factor (TNF) α. We hypothesize that the hepatic cytokine expression in IL-1β mice chronically fed low-dose alcohol will reflect the cytokine profile of human ASH. Quantitative(q) PCR was performed to measure cytokine expression in flash-frozen liver samples collected from male and female IL-1β and littermate control mice exclusively fed a liquid diet containing 0% or 2.5% (v/v) ethanol for 20 weeks. Traditionally classified pro-inflammatory cytokines of interest were mIL-1β, IL-4, IL-17A, and TNFα. Anti-inflammatory cytokine IL-10 and dual-acting cytokine IL-6 were analyzed via qPCR as well. Additionally, GAPDH expression was targeted as an internal control. Such findings allow for further molecular characterization of the IL-1β transgenic model and its potential application in ASH research.

Research Grant: NIHP30-ES002109, P01CA28842, and R35 CA210088
Student Support: The Boehringer Ingelheim Veterinary Scholars Program and NIH T35 OD033655

Lymphocyte proliferation in neonatal foals following EHV-1 vaccination

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Neonatal foal immune system development challenges the effectiveness of traditional vaccines. Little is known about the immune cell types and their activation and subsequent proliferation during early life. The aims of this study were to determine which cell types proliferate, if any, and how cell proliferation changes over time as the equine foal immune system matures. Peripheral blood mononuclear cells (PBMC) were collected from foals before and after Equine Herpesvirus 1 (EHV-1) vaccination. Cells were stimulated in vitro and allowed to incubate before being immunophenotyped and traced for proliferation using flow cytometry. Results demonstrated foal IgM+ B cells, IgD+ B cells, CD4+ T cells, and CD8+ T cells proliferate after 3 days in culture during the first weeks of life. An increased percentage of cells proliferate following vaccination at 2 days of age. Understanding lymphocyte proliferation during early foal immune system development can assist the future establishment of more effective neonatal vaccines to target specific mechanisms of these active cell types.

Research Grant: Harry M. Zweig Memorial Fund for Equine Research
Student Support: Cornell University College of Veterinary Medicine

Creating a database for Kemp's ridley sea turtle strandings in the northern Gulf of Mexico

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Kemp's ridley sea turtles are the most critically endangered species of sea turtle, and their natural range includes the northern Gulf of Mexico where they represent the majority of strandings. As sea turtles are important sentinel species and bioindicators for the health of the ocean, investigation of strandings, which remain largely unexplained, is pertinent in understanding threats these species face. The objective of this study was to construct a necropsy database for stranded sea turtles in the northern Gulf of Mexico and use the database to assess the ability to determine cause of death, discover relationships within the data, and develop recommendations for future investigators. Gross necropsy reports (n = 190) were obtained from stranded sea turtles in Mississippi and Alabama. Microsoft Excel was used to create the database, summarize system findings of each necropsy, translate findings into descriptions of general pathological processes, and identify important systems and processes associated with stranding at a population level. Of the turtles that could be evaluated, most Kemp's ridley sea turtles that stranded between 2019-2020 had no evident abnormalities based on gross necropsy. These turtles were in adequate body condition, had evidence of recent feeding, and over half also had sand present in the respiratory tract, indicating aspiration of a sediment-rich medium prior to death. Commonly affected systems or cavities included the coelomic, digestive, and respiratory. Future recommendations include collecting more histopathology samples, involving veterinary pathologists in necropsies, and collecting stranded turtles more readily to mitigate the effects of decomposition on necropsy interpretation.

Research Grant: National Fish and Wildlife Foundation under Mississippi Department of Environmental Quality – Task 3
Student Support: Global Center for Aquatic Health and Food Security

Characterizing lung injury caused by SARS-CoV-2 using a mouse adapted viral model

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Mice represent a potentially powerful animal model to study the lung injury caused by SARS-CoV-2 using a newly generated Mouse-Adapted SARS-CoV-2 (MA-SARS-CoV-2) strain. In this project, 8 week old C57BL6/J mice were infected with the MA-SARS-CoV-2 strain and lungs were collected at day 5 post-infection. Immunofluorescence imaging was used to identify infected cells (SARS-CoV-2 Nucleocapsid +). By multiplexing staining with markers of various lung cell types, we will quantify the percent of infected alveolar type 1 cells (AT1, RAGE+) and alveolar type 2 cells (AT2, LAMP3 and/or SPC+). We observed infected (Nucleocapsid +) AT2s and AT1s, as well as a number of infected cells in the alveolar space expressing neither marker. These cells may represent immune cells (e.g. macrophages) which have engulfed infected epithelial cells or epithelial cells that have downregulated canonical marker expression in response to viral infection.Next, we intend to analyze additional cell types that are infected (especially airway cells), as well as the degree of injury and cell proliferation as a surrogate for tissue repair / regeneration. If we confirm significant nucleocapsid staining in immune cells, we will pursue macrophage-specific marker staining to confirm these represent phagocytosed infected cells. It is likely we will detect viral infection of other cells in the respiratory epithelium. We are interested in expanding the experiment to explore infection of airway cells, such as club cells, tuft cells, etc. Future experiments following will analyze comorbidities, including experimental induction of Type 1 Diabetes, and SARS-COV-2 injury.

Research Grant: Pilot Grants from the University of Pennsylvania Institute for Translational Medicine and Therapeutics and the Penn Vet Institute for Infectious and Zoonotic Diseases
Student Support: NIH T35 OD010919, Boehringer-Ingelheim, and the University of Pennsylvania

Prevalence and diversity of protozoal hemoparasites in avian species on the Midwestern Glendale campus

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Protozoal hemoparasites are a diverse and prolific group of etiologic agents that result in significant morbidity and mortality in birds worldwide. Although infections are generally well tolerated in healthy, natural avian hosts, field and experimental studies have reported that chronic Haemosporida infections can result in reduced reproductive success and host fitness and increased disease susceptibility. The study objectives were to fully characterize the prevalence and diversity of hemoparasitism in birds that live in a multifactorial ecosystem with components of urban, suburban, and wild environments, and to determine the accuracy of cytology and PCR separately and in combination for the detection of Haemosporidain infection and genus identification. Cardiac tissue and impression smears were collected from recently deceased birds found on and around the Midwestern University campus in Glendale, Arizona. Cytologic evaluation was used to evaluate for parasitemia and to identify hemoparasite genus. PCR was utilized to evaluate for parasite presence, confirm genus, and identify species. *Haemoproteus* spp. was the most prevalent parasite identified, followed by *Plasmodium* spp. *Babesia* spp. and *Leukocytozoan* spp. were identified in 2 and 1, respectively, of the 250 samples collected. Once full characterization is complete, future studies will be initiated to assess the impact of human shared ecosystems on disease emergence and how anthropomorphic land use affects the dynamics between parasites, vectors, reservoirs, and hosts.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Echocardiographic assessment of apparently healthy Standardbred and Thoroughbred Racehorses

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Echocardiography is an essential component of a complete cardiac work up; it can help rule out anatomical abnormalities and provides information about overall systolic, diastolic, and valvular function. Echo was utilized, as a screening criteria, to assess the hearts of Standardbred (STB) and Thoroughbred (TB) horses to ensure there was no underlying cardiac disease, while they underwent ECG and Genetic evaluation, for evidence of Atrial fibrillation (Afib). Age, sex, breed, type of racer (pacer/trotter) and prior history were documented for on horse. Echo imaging involved acquisition of various 2D, M-mode, and Color Flow doppler views. Measurements were taken, assessing Left Ventricular (LV) wall and chamber size, Left Atrial Diameter (LAd) and qualitative assessment of valvular regurgitation, if present (Trace, Mild, Moderate, Severe). Of the 86 horses evaluated by echo, 54 demonstrated at least Trace regurgitation in one or more valves. Prevalence may be higher, as there were 8 studies where at least 1 valve was not well visualized/assessed, and the PV was only assessed on 29 studies.There was no significant difference in estimated marginal means of LAd or measurements of LV function across age, breed, or sex. STB mean LA size is 12.29 cm with a STDev of 0.76 cm; TB mean LA size was marginally smaller at 12.22 cm with a STDev of 0.72 cm. Long and short axis measurements of ejection fraction and fractional shortening were highly correlated (correlation coefficient 0.74, $P < 0.01$). P set at < 0.05. Based on available TB and STB cardiac reference measurements for the assessed values, it is suggested that the hearts of the evaluated horses are healthy and unlikely to be the cause for any detected Afib on ECG evaluation.

Research Grant: AAEP, Grayson Jockey Club Foundation, Morris Animal Foundation (D20EQ-013), Minnesota Racing Commission. Salary support (Durward-Akhurst): Morris Animal Foundation (D20EQ-403)
Student Support: Boehringer Ingelheim and the College of Veterinary Medicine

Characterisation of the cutaneous microbiome of the Northern Elephant Seal (*Mirounga angustirostris*)

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Evaluating the diverse microbial community of the skin microbiome can provide critical information about skin homeostasis, immune function, and contribute to changing perspectives in multiple fields including conservation, pathology, and medicine. However, skin microbiome data in marine mammals, especially pinnipeds, is minimal. According to current classifications by the International Union for Conservation of Nature (IUCN), 25% of marine mammals are threatened with extinction. Understanding the composition of physiological and pathological microbiomes could aid species' preservation. The aims of this project were to evaluate the impact of individuals, body sites and disease on the cutaneous microbiome composition of the Northern Elephant Seal (*Mirounga angustirostris*) focusing on characterising the prevalent microbiome. Swabs of 4 clinically normal body sites (head, under left eye, dorsum and perineum) and 1 site of alopecia were collected from 5 individuals from San Nicolas Island, California. After DNA extraction and PCR amplification, the total amplified 16S rRNA genes were sequenced. The results were compared to alopecia histopathology, which noted well-demarcated, depressed lesions of the hair shafts surrounded by algae and fungi. The overall microbiome composition comprised of 17 genera after exclusion of those present in the null samples. The perineum showed the highest number of body-site specific bacterial flora. The novel *Balneicellaceae* family (consisting of one known anaerobe) was unique to the alopecia sites, which also had highest median alpha diversity. Additional samples from a larger animal-set are currently being investigated including histopathology and culture from multiple individuals.

Research Grant: Unknown
Student Support: Fellowship from the Bostwick Family Foundation

Isolating mouse embryonic fibroblasts to assess the role of DNA repair proteins in response to damaging agents

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N-Nitroso compounds are highly pervasive, contaminating food, the environment, and commonly used drugs. These compounds are highly carcinogenic, with one *N*-nitroso compound that methylates DNA (namely, *N*-nitrosodimethylamine) having been linked to a childhood cancer cluster in Wilmington, Massachusetts. These compounds are metabolized into the methyl diazonium ion, which reacts with DNA, resulting in lesions such as 3-methyladenine (3MeA) and O^6-methylguanine (O^6MeG). Structural changes can interfere with DNA polymerases leading to toxicity and mutagenicity. The 3MeA lesion blocks replication and is repaired through the base excision repair (BER) pathway, which is initiated by the Alkaladenine-DNA-glycosylase (Aag). On the other hand, O^6MeG lesions are repaired by the O^6-methylguanine methyltransferase (Mgmt). In the absence of Mgmt, unrepaired O^6MeG can pair with thymine, triggering the mismatch repair (MMR) pathway. During MMR, repair synthesis re-inserts thymine opposite the methylated guanine, resulting in futile cycling and formation of toxic single-stranded DNA or DNA breaks. In this project, we have created primary mouse embryonic fibroblasts (MEFs) from mice that were DNA repair proficient (WT), deficient ($Mgmt^{-/-}$, $Aag^{-/-}$, and $Mgmt^{-/-}$;$Aag^{-/-}$), or overexpressing Aag (AagTg). Due to the limited lifespan of primary MEFs, the cells will be immortalized via expression of large T antigen. The final phase of this project is to assess toxicity by measuring qualitative and quantitative effects of methylating agents. This study is the first to look at the combinatorial effect of a deficiency in both Aag and Mgmt in MEFs in response to methylating agents.

Research Grant: This work was supported by the National Institute of Environmental Health Sciences Superfund Basic Research Program, National Institute of Health, P42 ES027707
Student Support: Summer fellowship funding from NIH T35 OD033655

Elucidating the mechanisms of polyunsaturated fatty acid killing of *S. aureus* small colony variants

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Staphylococcus aureus is a Gram-positive pathogen that causes 900,000 infections in the United States annually. It is the leading cause of Gram-positive sepsis and can infect every niche of the vertebrate host, emphasizing the need to develop more effective treatments. *S. aureus* grows by aerobic respiration, anaerobic respiration or fermentation. Small colony variants (SCV) can only grow through fermentation, are extremely persistent in the host and tolerate antibiotics. Arachidonic acid (AA) is an abundant host-derived polyunsaturated fatty acid (PUFA) at the host-pathogen interface. AA kills respiring *S. aureus* through a lipid peroxidation mechanism where electrophiles adduct proteins killing *S. aureus*. We tested the hypothesis that SCVs are more susceptible to PUFA killing due to their unique metabolism using NE1345, an SCV strain with disrupted menaquinone biosynthesis. AA kills NE1345 more than its parental strain, JE2. We chemically complimented NE1345 with menadione which is used to synthesize menaquinone. When NE1345 was treated with menadione, it respires and grows akin to JE2 both with and without AA. Alpha tocopherol (αTOH), an antioxidant that halts lipid peroxidation, protects NE1345 and JE2 from AA killing, indicating that lipid peroxidation is involved in PUFA killing of SCVs. I hypothesize that the cellular machinery used during fermentation may increase SCV susceptibility to PUFAs. My future aims for this project include identifying lipid electrophiles, defining the protein targets of the electrophiles in *S. aureus* SCVs, and to uncover the pathways in SCVs that are affected by PUFA. These experiments will elucidate and validate targets for future antimicrobial therapies to treat *S. aureus* infections.

Research Grant: LSU School of Veterinary Medicine Startup Funds
Student Support: Summer Scholars Research Program at LSU School of Veterinary Medicine

Predicting hatching dates for avian species using infrared cameras and machine learning models

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Artificial incubation is used in aviculture to rear rare birds in captivity. It is an important tool for rare bird conservation and can reduce the transmission of disease from parent to chick; stimulate re-nesting so birds can double reproductive output; and allow for medical support during hatching to maximize survival. Artificial incubation is imperfect and incubator settings are unknown for most bird species. As a result, eggs of many species are best left in the nest until just before hatching in order for the parent birds to provide the proper developmental conditions. In these scenarios, just before hatching, the eggs are brought in, the shells sanitized, and incubation and hatching are completed in an incubator. The problem is that egg-laying does not occur on a set schedule, requiring frequent nest box checks to track egg-laying, development, and hatching. Nest box checks can stress the nesting pair and cause reduced reproductive success. This paper proposes a method to replace nest box checks with a system to alert collections managers when hatching is imminent using machine vision from a camera mounted in the nest. Thermal imaging photos were taken of eggs (n = 41) across multiple species of Psittaciformes and Anseriform. The air cell, visible on thermal imaging, was graded at a surface area of 0%, 10%, 20%, 30%, 40%, or 50% of egg area on a 0-5 scale. A grade 5 air cell predicted imminent hatching 93% of the time. Air cell grades of 5 hatched significantly sooner than eggs in all other grades (t-test: $P < 0.001$). A machine learning model was able to detect stage 5 eggs the majority of the time. This method has great potential to monitor natural incubation and pull eggs for hand rearing prior to hatching.

Research Grant: None
Student Support: Boehringer Ingelheim VSP, Texas A&M School of Veterinary Medicine & Biomedical Sciences

Reducing dairy cow lesion-related lameness using epidemiological, genomic and extension approaches

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Lameness is a pervasive problem in the dairy industry that diminishes animal welfare and farm profitability while increasing greenhouse gas emissions-requiring further research to understand the multifactorial nature of lameness and the impact of both new and chronic hoof lesions on welfare outcomes. To understand, mitigate, and reduce the impact of hoof lesions necessitates an approach that combines field-level epidemiological, genetic, and genomic studies and an extension plan to ensure knowledge exchange with stakeholders. The long-term goals of this study are two-fold: firstly to recruit farm and hoof trimmer data to begin to develop an integrated framework technology that would allow the industry to understand and start addressing the pain, chronicity and economic costs of lameness due to foot lesions; secondly, to use the data collection framework to determine the association of hoof lesion data with more traditional animal welfare assessment methods such as locomotion scoring. Preliminary recruiting has yielded data from eight farms and three hoof trimmers with enrollment continuing into Spring 2023. Early hoof lesion data comparisons to FARM locomotion scoring will be performed to determine accuracy of the novel framework. Future research will continue to recruit farms and hoof trimmers to compile longitudinal lameness data with the goal of developing an accessible application that reduces the prevalence of hoof lesions in dairy cattle.

Research Grant: Council on Dairy Cattle Breeding (CDBC), University of Minnesota Extension
Student Support: Boehringer Ingelheim, University of Minnesota College of Veterinary Medicine

Identification of Viral Hemorrhagic Septicemia Virus in invasive Round Gobies from the Hudson River, New York

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The threat of Viral Hemorrhagic Septicemia Virus (VHSV) has continually increased across North America and the world. This piscine rhabdovirus is the causative agent of Viral Hemorrhagic Septicemia (VHS), a significant disease that has been responsible for numerous fish mortalities in the Laurentian Great Lakes and its tributaries as early as 2003. The disease's spread throughout the Great Lake Basin has been associated with the movement of invasive Round Goby fish (*Neogobius melanostomus*), endemic carriers of the disease. This species now threatens to introduce VHS to the Hudson River estuary and Lake Champlain, important ecosystems and sport fishing areas. The purpose of this study was to survey Round Gobies originating from the confluence of the Hudson and Mohawk rivers, an area yet to be associated with VHSV. RT-qPCR assays were performed on pooled liver, heart, spleen, and anterior kidney tissue, along with brain tissue, of 69 Round Gobies. A single brain tissue sample yielded an average of 19 viral particles. This finding is not conclusive of the presence of VHSV in the Hudson River. However, it is of utmost importance to continually survey nearby tributaries for the presence of the virus, especially due to its rapid dispersal and detrimental effects on local fish populations. Future directions for this project include sampling additional Round Gobies from the vicinity of the Hudson-Mohawk confluence.

Research Grant: Fish Pathology and Diagnostic services. New York Department of Environmental Conservation; 5-year MOU (2020-2025)
Student Support: NIH T35 OD010941 and the Cornell University College of Veterinary Medicine

The antihelminthic drug Niclosamide inhibits growth of acute lymphoblastic leukemia cells

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Acute lymphoid leukemia (ALL) is a cancer of the blood and bone marrow that causes excessive proliferation of lymphocytes. ALL is the most common type of childhood cancer. The 5-year survival rate for children with B-ALL is 90%, however, there are fewer effective and nontoxic therapies for relapsed disease. The cAMP-responsive element binding protein (CREB) is overexpressed in a majority of patients with ALL and acute myeloid leukemia (AML) and plays an important role in leukemia cell proliferation. Niclosamide has previously been shown to be a potent anti-leukemic agent that can inhibit CREB function in AML cells. Therefore, we hypothesized that niclosamide inhibits proliferation of ALL cells and is a potential therapeutic agent to treat ALL. We treated both B-ALL and T-ALL cell lines, including Nalm6, REH, Jurkat, and Loucy. Cells ($2x10^4$) were grown in 96-well plates and treated with either niclosamide at 10, 5, 2.5, 1, 0.5, 0.25, 0.1, 0.05, 0.025 or 0.01μM or with DMSO control. After 72 hours, CellTiter-Glo assays were performed to assess cell viability calculated as a percentage of control with a dose response curve. GraphPad Prism Software was used to calculate the concentration of drug inhibiting cell viability by 50% (IC_{50}). The IC_{50} value for Nalm6, a B-ALL cell line, was between 0.32μM and 0.58μM (N = 3) and for Jurkat, a T-ALL cell line, was between 0.18μM and 0.24μM (N = 3) with a 95% confidence interval. Our data demonstrates that both B-ALL and T-ALL cells are sensitive to niclosamide and therefore niclosamide should be considered as a potential therapeutic agent for ALL.

Research Grant: Leukemia & Lymphoma Society-Translational Research Program (#R6518-23)
Student Support: NIH T35 OD010989

An *ex vivo* model of fungal keratitis for the investigation of infection mechanics and potential therapeutics

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Fungal keratitis (FK) is a corneal infection primarily associated with *Aspergillus* and *Fusarium* fungal species. FK is treated empirically with a limited selection of topical antifungals with varying levels of success. Traditionally, improved treatments for FK are developed using *in vitro* minimum inhibitory concentration testing on antifungal agents to determine fungal susceptibility before formulating and testing the drug *in vivo*. This methodology limits treatment selection to soluble antifungal agents and demands frequent use of a painful *in vivo* model. Our objective is to develop an *ex vivo* model of FK that could be used for fungal susceptibility testing, preliminary toxicity assays, and the study of fungal organism behavior within the cornea, offering novel insight into infection mechanics. This model would improve lab animal welfare by allowing researchers to refine the proposed treatment before application in a live animal. We propose to produce this model through intrastromal injection of fungal conidia into porcine cadaver eyes to achieve a simulated FK infection. We investigate real-time fungal reproduction and behavior within the tissue, as well as fungal and corneal response to treatment with both soluble antifungal drug and exposure to cold atmospheric plasma (CAP) - an emerging treatment of choice for microbial infections. From these studies, we obtained early evidence of a differential response from the fungi to soluble drug when treated at various stages of infection progression and fungi maturation. We also demonstrated a fungistatic ability of CAP exposure. Future studies will investigate the optimization of FK treatment based on new information on fungal behavior and response to treatment within the eye.

Research Grant: None
Student Support: NIH T35OD011070 Interdisciplinary Biomedical Research Training Program

Toll-Like Receptor Activation of Mesenchymal Stromal Cells for Improved Cellular Treatment of Osteoarthritis

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Osteoarthritis (OA) represents one of the most common disorders in humans and animals. Despite high prevalence of the disease, there remains a lack of effective treatment options to reduce pain and improve quality of life without inducing adverse effects. Cellular therapies such as mesenchymal stromal cells (MSCs) are increasingly popular to treat OA but remain controversial due to lack of rigor in study design and consistency in product composition. In the presence of inflammation, as in OA, MSCs upregulate toll-like receptors (TLR) on their cell surface. Therefore, we hypothesize that preactivation of MSCs with TLR ligands will improve their effectiveness to treat OA. Osteoarthritis was induced in mice via surgical destabilization of the medial meniscus. Mice were injected in the affected joint at weeks 3 and 5 postoperatively with one of three treatments (needle insertion alone, MSCs, or TLR-activated MSCs) and monitored until week 8. Outcome parameters assessed were mouse mobility evaluated by motion activated camera (AnyMaze), histopathology of joint tissues graded via established osteoarthritis scoring systems, and gene sequencing of formalin fixed joint tissues using Nanostring technology. To further assess mechanistically the effect of TLR activation on MSC, RNA sequencing of MSC and TLR-activated MSC from mice will be performed. Preliminary analysis of gait data indicates mice treated with TLR-MSCs traveled further total distances, had greater total time mobile, and traveled at greater mean speeds compared to those receiving needle insertion alone at week 8 postoperatively. Collectively, these findings support further evaluation of TLR-activated cellular therapies for treatment of OA in both humans and animals.

Research Grant: CSU Department of Clinical Sciences, CSU, Young Investigator Grant in Companion Animal Studies, Carolyn Quan and Porter Bennett
Student Support: Boehringer Ingelheim

Rapid, low-cost, point of care population-based diagnostics to detect RNA viruses in swine

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RNA viruses are major disease players in commercial swine herds. Coronaviruses and influenza A virus (IAV) have the potential to mutate and spread quickly in susceptible populations. Porcine epidemic diarrhea virus (PEDV) and porcine deltacoronavirus (PDCoV) cause intestinal diseases and high mortality in pigs. IAV has proven to be one of the most prevalent respiratory diseases in animals and humans and remains a pandemic threat to the public health. These viruses present a great threat to the swine industry and pose additional risk through cross species transmission. Because of the potential for zoonotic disease with IAV, and the recent SARS-CoV-2 pandemic, we focused on developing rapid, point of care detection methods for common swine pathogens of concern. The objective of this study is to develop point of care assays using Loop-Mediated Isothermal Amplification (LAMP) to detect IAV, PDCoV, and PEDV. Laboratory sample RNA was extracted with Qiagen Viral RNA mini kit and Cytiva Sera-Xtracta nucleic acid extraction kit using the manufacturer's instructions. The LAMP kit was mixed with LAMP primers just prior to placing on QuantStudio 3 thermocycler for 30 minutes, with dilutions of virus up to 10 million copies were detected for influenza, PEDV and PDCoV. In the future, we hope to use our results to help design detection modalities which will assist the lay worker in analyzing tray water from packing plants to detect PDCoV, PEDV, and IAV. To our knowledge, this novel sampling type has not been used to identify these viruses. This rapid approach will allow packing plants and veterinarians to conduct surveillance for detection of viruses, and better equip them to make production decisions for disease and herd management.

Research Grant: University of Illinois School of Veterinary Medicine
Student Support: Grant provided by Foundation for Food and Agriculture Research Vet Fellows Program

The effect of hand-walking on equine gastrointestinal motility measured with electrointestinography

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Gastrointestinal disease resulting in abdominal pain, or colic, is a common cause of morbidity and mortality in horses. Hand-walking has been recommended by equine veterinarians anecdotally to increase gastrointestinal motility and prevent horses from self-harm. However, the effect of hand-walking on gastrointestinal motility has not been directly measured. Electrointestinography (EIG) is a non-invasive technique used to evaluate changes in gastrointestinal motility by capturing slow wave electrical activity produced by the cells of Cajal. This study hypothesized that hand-walking increases gastrointestinal motility in healthy, adult horses, measured by EIG, transabdominal ultrasound, and auscultation. After a 16 hour fast, a 30-minute EIG, along with transabdominal ultrasound and auscultation of borborygmi, were performed to measure baseline activity. Horses were then hand-walked (treatment group) or stall rested (control) for 15 minutes. Gastrointestinal motility measurements (EIG, ultrasound, and auscultation) were repeated immediately post-treatment and at 2 hours. A crossover study design was used so that all horses underwent both treatments, with a one-week washout. Comparisons of EIG slow wave dominant power ratios and the dominant frequency were performed within and between the horses, as well as the number of contractions measured with ultrasound and auscultation. We anticipate the horses in the treatment group will have increased motility, measured by a larger dominant power ratio compared to the controls, and that dominant frequency will correlate to contractions noted by the other methods. A positive outcome will provide support for the use of hand-walking in horses with gastrointestinal motility disorders.

Research Grant: Michigan State University Professional Development Funds
Student Support: National Institute of Health Grant #5T35OD016477-20 to Michigan State University

Detection of antibodies specific for SARS-CoV-2 antigens in animals

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In December 2019, a novel coronavirus named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) emerged in Wuhan, China. It causes a respiratory disease known as coronavirus disease-19 (COVID-19) and is responsible for the global pandemic declared in March 2020. The virus is of animal origin but can cross the species barrier and cause human infection. As of yet, the reservoir host of SARS-CoV-2 has not been identified, and the transmission events across animal species is not well understood. This study was performed to investigate the presence of SARS-CoV-2-specific antibodies in various animals across the US. Sera from 250 animals (204 bison and 46 other exotic species) were analyzed for the detection of SARS-CoV-2 antibodies against the receptor-binding domain (RBD) and the nucleocapsid (N) protein. Results showed that 4% of bison and 0% of exotic species samples tested antibody-positive using a commercial ELISA that detects antibodies specific for RBD. In addition, 2% of bison and 2% of exotic species serum samples tested antibody-positive using a commercial ELISA that recognizes antibodies against the N protein. Positive samples by these commercial ELISAs were further tested by classical serum neutralization for SARS-CoV-2. Here, 0.5% of bison and 0% of exotic species serum samples had a positive neutralizing antibody titer. In conclusion, our findings of few seropositive bison and other exotic species indicate that these animals may occasionally be infected by SARS-CoV-2 or other coronaviruses. Determining virus-specific antibody status among animals is crucial for the determination of previous virus exposure, for the detection of virus transmission among animals and for potentially identifying new hosts.

Research Grant: National Institutes of Health U18 Grant
Student Support: National Institutes of Health T35 Training Grant

Characterization of a novel LysR transcriptional regulator in *Brucella abortus*

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Brucella abortus is a Gram-negative zoonotic bacterial pathogen capable of causing brucellosis. Clinical manifestations of brucellosis include anorexia, recurrent fever, arthritis in humans and abortion in ruminants. LysR type transcriptional regulators (LTTR) are a family of well-conserved regulators in prokaryotes with diverse functions including metabolism, virulence, and oxidative stress response. We are investigating the effect of a novel LTTR (called BAB2_0857) and how it may affect the virulence and growth kinetics of *Brucella*. Additionally, we are investigating a potential binding site of the LTTR to a putative sRNA adjacent to the promoter region of the LTTR. A *lysR* deletion construct was created, purified, and then transformed into wildtype *Brucella abortus*. When a mutant strain is established in *Brucella*, growth kinetics will be compared between the wildtype and mutant on nutrient reduced and rich media, and changes in virulence will be tested within macrophages. Virulence assays will be picked based on the internal environment of macrophages and will include a comparison between the wildtype and mutant response to oxidative stress and acidic environments. As stated previously, we hypothesize that our LTTR binds to the promoter region of a putative sRNA just upstream to the LTTR. To test this, we will first confirm there is an sRNA in the area, isolate purified LTTR protein, and finally check for presence of binding. We will confirm the presence of an RNA region upstream of the LTTR using a northern blot. We will then assess binding between the purified LTTR protein and the RNA region via an electrophoretic mobility shift assay.

Research Grant: None
Student Support: NIH T35OD011887

Identification of canine cytochrome P450 enzymes involved in the metabolism of antiepileptic drugs

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Epilepsy reportedly affects 0.75% of the canine population and it is estimated that 30% of epileptic dogs have refractory epilepsy. Furthermore, antiepileptic drugs (AEDs) display considerable interindividual variation in drug disposition and are notorious for being highly susceptible to drug interactions. One cause of interindividual variation in AED disposition and response may be metabolism by polymorphic enzymes. Polymorphic cytochrome P450 (CYP) enzymes are responsible for the metabolism of many AEDs in humans and CYPs are responsible for many AED drug interactions. However, the involvement of canine CYP enzymes in the metabolism of AEDs remains unknown. The aim of this study was to identify the major canine CYPs responsible for the metabolism of primidone, zonisamide, phenytoin, imepitoin, and levetiracetam. Initial *in vitro* metabolism studies were carried out with pooled dog liver microsomes to determine if drugs were CYP substrates. If drugs appeared to be CYP substrates, reaction phenotyping was performed with a panel of recombinant CYP enzymes with results scaled to reflect the abundance of CYP isozymes in the canine liver. Metabolic stability was measured using liquid chromatography with tandem mass spectrometry. Thus far, results indicate that CYP2C21, CYP2D15, and CYP2B11 are involved in the metabolism of phenytoin. CYP2B11 and CYP2D15 are polymorphic in the dog. Studies are ongoing in our laboratory to characterize the metabolism of primidone, zonisamide, imepitoin, and levetiracetam and to screen for drug interactions with cannabidiol. Results from this study may help predict drug-drug interactions and advance precision veterinary medicine.

Research Grant: None
Student Support: NIH T35 Training Grant T35OD029981

Dogs, deer ticks, and disease: estimating infection risk from tick testing and canine serology

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Ixodes scapularis, the black-legged tick or deer tick, is a known vector for several pathogens important in veterinary and human health including *Borrelia burgdorferi* (Lyme disease), *B. mayonii, Ehrlichia muris eauclairensis* (ehrlichiosis), and *Anaplasma phagocytophilum* (anaplasmosis). *Ixodes scapularis* have a wide distribution throughout the eastern United States. However, both tick intensity and prevalence of pathogens are greater in the northern United States although the geographic range of *I. scapularis* infected with pathogens is expanding. The objective of this research is to compare prevalence of infection in ticks with seroprevalence in dogs from areas where these diseases have only recently emerged or are long established. Ticks (n = 166) collected from pets (87 dogs and 28 cats) in Ohio, Michigan, Minnesota, and Wisconsin were tested by PCR for *Borrelia spp*. (flaB), *Ehrlichia muris eauclairensis* (16S rRNA), and *A. phagocytophilum* (16S rRNA). Canine seroprevalence for each pathogen in each region was acquired from a national database (CAPCvet.org) and compared to tick infection prevalence using linear regression. Sequence-confirmed results from tick testing revealed *B. burgdorferi* in 7.4%-32.2% of *I. scapularis*, while canine seroprevalence in the same regions ranged from 1.2%-26.6%. One tick from Minnesota was infected with *B. mayonii* and PCR results for *A. phagocytophilum* and *E. muris* are pending. To date, prevalence of *B. burgdorferi* infection in ticks does not appear to predict prevalence of antibodies in dogs from the same region ($P = 0.4713$). Differences between tick prevalence and canine serology may have important implications for efforts to estimate infection risk to people and pets.

Research Grant: Krull-Ewing Foundation, Oklahoma State University
Student Support: CVM

CD4 T cell proliferation and IFN-γ production calves vaccinated with *Mycobacterium bovis* BCG by various routes

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Mycobacterium bovis bacille Calmette-Guerin (BCG) is the only approved tuberculosis vaccine for humans and the most studied tuberculosis vaccine in animals. The immune response to mycobacteria such as M. *bovis* is dominated by a T_H1 response characterized by IFN-γ production by CD4 T cells. Protection from future infections is controlled by memory T cells, including T central memory cells (T_{CM}) cells. Previous studies have indicated that IFN-γ production from T_{CM} cells is a better predictor of vaccine efficacy as compared to IFN-γ production ex-vivo. The aim of this study was to evaluate the protective and memory responses to BCG administered by various routes and different physical states (liquid and lyophilized). Thirteen Hereford or Hereford cross cattle of 24 months of age were divided into 4 groups: subcutaneously injected BCG (n = 4), liquid BCG administered orally (n = 3), lyophilized BCG inside an alfalfa-based bait consumed voluntarily (n = 3), and unvaccinated controls (n = 3). Blood was collected at 12 and 16 weeks post vaccination. Peripheral blood mononuclear cells were isolated and stimulated with PPDb. IFN-γ levels were measured by ELISA and frequency of proliferating CD4 T cells by flow cytometry. Levels of IFN-γ did not differ significantly between administration route at 12 or 16 weeks post vaccination. An increase in frequency of IFN-γ CD4 cells was observed at 12 weeks post vaccination. This percentage decreased significantly at 16 weeks post vaccination in all vaccinated groups. These findings demonstrate the proliferation of CD4 T cells capable of IFN-γ production in response to antigen stimulation. An enhanced understanding of the role of T cells will help inform future interventions for *M. bovis* infection.

Research Grant: Diagnostic and mitigation strategies to control tuberculosis in cattle and wildlife: CRIS 3625-32000-232
Student Support: USDA ARS NBAF AGREEMENT NO: ARS 59-3022-1-003

Acute developmental toxicity of the TCE metabolite DCVC in zebrafish (*Danio rerio*)

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S-[(1E)-1,2-dichloroethenyl]--L-cysteine (DCVC) is a metabolite of the toxic compound trichloroethylene (TCE). TCE was historically used as an industrial solvent and is capable of leaching into the environment from improper waste disposal sites, which raises public health concerns given that TCE is a known carcinogen and can cause developmental toxicity. Some of the toxic effects of TCE are thought to be mediated by its metabolites, and this study tests the hypothesis that DCVC contributes to the toxicity observed in zebrafish with developmental exposure to TCE. In this study, zebrafish embryos were dosed shortly after fertilization with one of four concentrations (0, 5, 50, or 500 parts per billion (ppb; μg/L)) of DCVC. Behavior tests were then performed at both 24 hours post fertilization (hpf) and 120 hpf to screen for potential toxicity. Significant alterations in behavior were observed at both 24 hpf and 120 hpf. All three DCVC exposures had altered behavioral endpoints in the 24 hpf photomotor response test while the 5 and 500 ppb exposures had significantly decreased movement in the 120 hpf visual motor response test. Further testing is needed to determine the mechanisms of action and other implications of developmental exposure; however, the results suggest that DCVC does contribute to TCE associated developmental toxicity.

Research Grant: National Institute of Environmental Health Sciences 1R15ES033361
Student Support: Boehringer Ingelheim Summer Scholars Program

Phylogenetic analysis of Canine Distemper Virus to determine directionality of cross species infections

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Canine Distemper Virus (CDV) can cause severe multisystemic disease in a wide range of carnivore hosts. Cross species susceptibility is of great concern for endangered, naive carnivore populations. In the South-Eastern United States, CDV circulates in a multi-host mesocarnivore system, but the transmission dynamics are poorly understood. We hypothesize that raccoons are the major reservoir species for CDV in the South-Eastern United States mesocarnivore ecosystem, with epizootics in raccoons driving cross species transmission events to other carnivores, particularly foxes and skunks. Samples from canine distemper (CDV)-positive wild mammal cases were submitted to the Southeastern Cooperative Wildlife Disease Study and the Diagnostic Pathology Laboratory, Athens, Georgia, US, between December 2019 and April 2022. Overall, 91 cases from 11 states were CDV positive, including 61 raccoons (*Procyon lotor*), 15 gray foxes (*Urocyon cinereoargenteus*), 10 striped skunks (*Mephitis mephitis*) and one red fox (*Vulpes vulpes*). We amplified full length H gene sequences from these samples using RT-PCR. Once H gene sequence results are available (currently pending), we will input them into BEAST software with each sample's concurrent spatial and temporal data. BEAST will reconstruct evolutionary phylogenies by creating trees with posterior probabilities to assess interspecies infections and determine directionality of infection. Identifying the pathogen reservoir with ancestral host state reconstruction via phylogenetic analysis will create a better understanding of CDV dynamics within our wildlife populations and produce an opportunity for well-designed future surveillance for cross species transmission events.

Research Grant: None
Student Support: NIH Office of Research Infrastructure Programs, Grant Number 5T35OD010433-14

Evaluation for antimicrobial resistant bacteria in U.S. captive bonobos (*Pan paniscus*)

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Bonobos (*Pan paniscus*) are an endangered great ape species native to forests within the Democratic Republic of Congo. The species is severely threatened due to habitat loss and poaching for the bushmeat trade. There are currently only 85 bonobos housed at eight Association of Zoos and Aquariums (AZA) accredited or certified facilities within the U.S. Within a zoo population, the bacteria harbored by zoo animals may be exposed to selection pressures driven by the therapeutic use of antibiotics, or sub-therapeutic doses excreted by co-housed animals receiving treatment, or by contact with wild animals that may enter zoo enclosures. Native wildlife species have been identified harboring antibiotic resistant bacteria, influenced by the relative level of exposure to anthropogenic antibiotic resistant contamination in the environment. Additional studies have found that wild raptors were more likely to shed antimicrobial resistant bacteria when found within close proximity to livestock animal facilitates. The objective of this study was to determine the presence of Extended-spectrum B-lactamases (ESBLs) bacteria, carbapenem resistant Enterobacteriaceae (CREs) and resistant campylobacter isolates in bonobos managed in human care in the United States. In addition to bonobo feces collected within bonobo enclosures, this project also sought to collect fecal samples from wildlife found within bonobo enclosures to sample for the same or different ESBLs and CREs. We hope the results of this study will provide useful data relevant to antimicrobial stewardship for the endangered bonobo, as well as add to the bank of knowledge needed for addressing antimicrobial resistance in zoological collections.

Research Grant: Dr. Roger and Marilyn Mahr Professorship in One Health and The National Institute of Antimicrobial Resistance Research and Education
Student Support: NIH T35 Training Grant

Activated clotting time in 2 different raptor species

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An astonishing 60-100% of wild raptors have anticoagulant rodenticide (AR) residues in their bodies. In our attempt to control the rodent population with ARs, humans are poisoning raptors, the environmentally friendly and natural method of rodent control. Coagulation diagnostics are a vital tool in the detection of AR poisoning in human and veterinary medicine. Diagnostics such as prothrombin time (PT) and activated partial thromboplastin time (PTT) are commercially available for use in mammals but are not validated for use in avian species. Activated clotting time (ACT) is a low-cost assay that does not require species-specific reagents, but species-specific reference intervals are needed before this test can be applied in the clinic or field setting. In a previous study, experimental Japanese quail had prolonged ACT after exposure to brodifacoum, a commonly used AR. Our aim is to establish ACT reference intervals in red-tailed hawks (Buteo jamaicensis) and great-horned owls (Bubo virginianus). These species are commonly presented to wildlife rehabilitation centers and have a high incidence of AR exposure. Blood samples were collected from these raptors after having been kept at a rehabilitation facility for a minimum of 1 month to ensure the absence of recent AR exposure. Physical exams, total solids, and packed cell volume were performed to confirm the health of each subject. The results show that red-tailed hawks have a faster ACT average of 132 seconds compared to great-horned owls at 248 seconds. Establishing normal ACT ranges for these species will aid in a quicker diagnosis of AR toxicosis, ultimately leading to faster treatment and increased survivability.

Research Grant: Western University College of Veterinary Medicine
Student Support: Western University College of Veterinary Medicine

Liver abscesses in feedlot cattle: Further delineation of the etiology and pathogenesis

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Liver abscesses are a polymicrobial infection that occur in feedlot cattle fed high-grain and low-roughage diets. The causative agents include two subspecies of *Fusobacterium necrophorum* (*necrophorum* and *funduliforme*) and *Trueperella pyogenes*. Additionally, number of other bacterial species have been isolated sporadically. Our objectives were to selectively target isolation and identification of *E. coli*, *Salmonella*, *Klebsiella*., and *Pseudomonas*, in addition to *Fusobacterium* and *Trueperella* from liver abscesses, rumen, colon and also to determine their antimicrobial susceptibilities. Ten liver abscess, ruminal and colonic epithelial tissue samples from the same animal were collected from cattle at slaughter. Samples were subjected to anaerobic and aerobic bacterial isolations. Antimicrobial susceptibilities of Gram-negative bacterial isolates were tested by Sensititre procedure. Data were analyzed using STATA v. 16.1. Overall, Prevalence of *F. necrophorum*, *F. funduliforme*, *T. pyogenes* and *Salmonella* were 100% (10/10), 40% (4/10), 50% (5/10) and 60% (6/10) in liver abscesses, respectively. The prevalence of minor pathogens *E. coli*, and *Klebsiella* spp., from liver abscesses were 60% (6/10) and 10% (1/10), respectively. The prevalence of *Salmonella* was higher in colonic tissue 50% (5/10) compared to rumen tissue 20% (2/10). No *Trueperella pyogenes* was isolated from the tissue samples. No *Pseudomonas* sp. was isolated from rumen tissue and the prevalence in colonic tissue was 20% (2/10). All *Salmonella* isolates were susceptible to the antimicrobial agents tested, whereas *E. coli* isolates showed variable resistance to tetracycline (67%) and *Klebsiella* sp. was resistant to Amoxicillin, Ampicillin, Cefoxitin and Nalidixic Acid.

Research Grant: International Consortium for Antimicrobial Stewardship in Agriculture-Foundation for Food and Agriculture Research (ICASA-FFAR)
Student Support: Elanco Animal Health

Evaluation of White Blood Cells Across the Hibernation Cycle in Captive Fat-Tailed Dwarf Lemurs (*C. medius*)

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Hibernation is an energy conservation strategy used by many mammals to reduce energetic needs during hostile conditions. In the wild, the fat-tailed dwarf lemur (*Cheirogaleus medius*) hibernates for up to seven months per year, using the fat stored in its tail to meet its metabolic needs. During the hibernation season, *C. medius* experiences two different metabolic states: multi-day torpor bouts (phases of decreased metabolism, heart rate, and body temperature), and interbout arousals (short phases of euthermia). Under the hypothesis that metabolic depression in mammals constrains immune function, we examined white blood cell patterns in dwarf lemurs under human care during the active season and hibernation season, including during torpor and arousal. Based on previous studies of squirrels, bats, and bears, we predicted a decrease in leukocytes, and specifically neutrophils, during torpor. Blood smears evaluated from 28 *C. medius* from 2020-2022 at the Duke Lemur Center, identified granulocytes (segmented neutrophils, band neutrophils, eosinophils, and basophils), monocytes and lymphocytes. Our data showed that, unlike the pattern seen in ground squirrels and bats, neutrophils were unusually increased during torpor compared to arousal and active states. Eosinophils were the only leukocyte type to decrease during torpor. Post-hibernation numbers were statistically indistinguishable from pre-hibernation levels. Unraveling leukocyte differentials will provide basic knowledge of the *C. medius* immune system and increase our understanding of how the immune system is involved in the hibernation process. This data also has potential relevance for biomedical applications and understanding the evolution of hibernation.

Research Grant: Duke Lemur Center to MBB
Student Support: VSP Summer Program

Molecular mechanisms of CHOP chemotherapy resistance in canine lymphoma

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For dogs and humans, diffuse large B cell lymphoma (DLBCL) is one of the most common lymphoma types, with dogs as a great comparative model for the activated B-cell-like (ABC) human DLBCL (hDLBCL). The current standard of care for hDLBCL is cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy together with the monoclonal antibody rituximab (R-CHOP). The five-year survival rate of ABC is 30%. The current standard of care for cDLBCL is also CHOP chemotherapy, with a two-year survival rate of only 20%. Over 90% of dogs will come out of clinical remission within 6 months after CHOP treatment. Our study hypothesizes that specific activating and inactivating genetic mutations are promoting cDLBCL cell growth leading to clonal expansion following CHOP treatment. Confirmed cDLBCL dogs at UW Veterinary Care were newly enrolled into the study, treated with standard CHOP chemotherapy, and checked monthly for cDLBCL relapse. The patient samples were collected before treatment, during clinical remission, and at relapse by either fine needle aspiration or surgical biopsy. 1 million sorted live lymphoma cells were submitted for both scRNA-seq and to extract genomic DNA for whole exome sequencing (WES). Our results show that the exhausted B cells were identified as the predominant clone in naive and progressive disease samples. At complete remission exhausted B cells were diminished, however, they re-expanded during the disease progression. In the future, we will continue to identify the deregulated gene pathway in the exhausted B cell population and will use this information to guide future therapeutic development targeting this deregulated pathway.

Research Grant: DHHS, PHS, National Institutes Of Health and Companion Animal Fund
Student Support: National Institutes of Health T35OD011078-12

Assessing T cell responses to a novel preclinical Zika virus vaccine candidate

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There is currently no FDA approved vaccine for Zika virus (ZIKV), despite it being an important public health threat in South and Central America, Africa, and Asia. We recently developed a live recombinant ZIKV vaccine for pre-clinical evaluation that uses the recently described Aripo virus (ARPV) as a viral vector. The vaccine, tentatively called Aripo/Zika virus (ARPV/ZIKV), consists of the ARPV genome with the precursor membrane (prM) and envelope (E) protein genes of ZIKV substituted in for the prM/E genes of ARPV. ARPV is an insect-specific virus; it can only replicate in insects, and not in mammals. The ARPV vector therefore confers a high degree of safety without sacrificing immunogenicity. In previous studies, ARPV/ZIKV offered complete protection against morbidity and mortality in a mouse model 4 weeks after a single un-adjuvanted dose. While ARPV/ZIKV vaccination elicits strong neutralizing antibody responses in mice, the role of T cells remains unclear. To elucidate the relative contribution of T cells in providing protection induced by ARPV/ZIKV, T cell depleting antibodies were administered to 4 week old C57BL/6 mice, which were then challenged with ZIKV after interferon blockade and assessed for clinical outcomes. Here, we will present the viremia, weight loss, and neutralizing antibody responses in vaccinated T cell depleted mice, in comparison to a sham-vaccinated group. The results will support previous studies (performed in type I interferon receptor knockout mice) to help determine the T cell contribution of the immune response to ARPV/ZIKV and further shed light on the specific immunological responses to this preclinical Zika virus vaccine, which may help in later adjuvant selection.

Research Grant: NIH Award R01AI153433
Student Support: NIH T35OD011887

Effects of confined disposal facilities on painted turtle (*Chrysemys picta*) blood lactate and WBC counts

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Dredging is commonly used to maintain navigational channels, and in Lake Erie, dredged material is often dumped into upland or in-water “confined disposal facilities” (CDFs). The long-term health implications for wildlife species living in the wetland habitats created by confined disposal facilities remain largely unknown. Due to their site fidelity, longevity, and propensity to accumulate environmental toxins, freshwater turtles can serve as sentinel species to evaluate the overall health of localized freshwater ecosystems. We compared painted turtle (*Chrysemys picta*) health assessments at two sites on Lake Erie: a managed coastal wetland and a confined disposal facility. Health assessments were performed on 51 painted turtles including physical examinations, estimated total white blood cell counts and differentials, and blood lactate levels. We hypothesized that turtles from the confined disposal facility would have higher estimated total white blood cell counts, higher heterophil:lymphocyte ratios, and higher blood lactate levels caused by anthropogenic stressors when compared to the managed wetland. Statistical analyses were performed to determine associations between blood lactate levels, white blood cell counts, and demographic parameters. Preliminary findings showed mean lactate of 8.873 mmol/L (range = < 0.3 to 17.2) for the dredge dump site and 6.853 mmol/L (range = 3.0 to 16.0) for the control (P = 0.245). Lactate is an underutilized tool in chelonian health, providing information about physiologic stress that can be measured in the field. The WBC analysis in progress will shed light on the interactions between the immune system and environmental stress as shown through lactate measurements.

Research Grant: NIH T35 OD010977
Student Support: OSU Summer Research Scholar Program

Anatomic assessment of external oblique intercostal block in rabbit cadavers

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Rabbits are the third most anesthetized small animal species. However, their overall mortality rate is significantly higher than in dogs and cats. Hypothesized reasons include their pronounced sympathoadrenal responses associated with inadequate anesthesia, sedation, and analgesia. Regional anesthetic techniques are described in rabbits, including the transversus abdominis plane block; however, it does not provide anesthesia to the cranial abdominal wall. A novel fascial plane technique, the external oblique intercostal block (EOI) may anesthetize this area and corresponding cutaneous regions. This foundational study tested whether injectate placed between external abdominal oblique and external intercostal muscles adequately spreads to nerves innervating the flank and cranial abdominal wall, an area frequently incised during surgery. An ultrasound guided EOI block was performed bilaterally in New Zealand White rabbit cadavers using either 0.25 mL/kg (LV) or 0.5 mL/kg (HV) of 1% methylene blue, randomly assigned to each hemithorax (total = 10 hemithoraces). Following immediate dissection, mean total cranial-caudal dye spread was 87 and 80 mm (range = 68-125, 59-111 mm) in HV and LV groups respectively; mean number of intercostal nerve numbers stained was 7.5 and 5.8 (range = 7-9, 3-8) in the HV and LV groups, respectively. This study may demonstrate that the external oblique intercostal block has the potential to achieve regional anesthesia in the cranial abdomen and flank regions of rabbits. Successful technique application may minimize sympathetic stimulation associated with surgery and the need for systemic analgesics and inhalational anesthetics, potentially improving patient morbidity and mortality.

Research Grant: UW Veterinary Medicine Companion Animal Fund
Student Support: UW School of Veterinary Medicine Dean's Office

Risk factors associated with canine distemper and parvovirus exposure in Mexican wolves *(Canis lupus baileyi)*

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The Mexican wolf *(Canis lupus baileyi)* is a federally listed endangered subspecies of gray wolf that is currently being recovered in the American southwest. Mexican wolves were functionally extinct until the recovery population was started with the reintroduction of 11 captive-born individuals in 1998. The population has grown over the last two decades but viral pathogens such as canine distemper virus (CDV) and canine parvovirus (CPV) pose a threat to recovery efforts. There is a persistent gap in knowledge regarding disease dynamics in the wolf population. Here we use historical serological data collected by the United States Fish and Wildlife Service to better understand the factors that affect the seroprevalence of CDV and CPV in Mexican wolves. The dataset consists of blood antibody levels collected from wolves captured for routine monitoring between the years 2002 to 2020. Seroprevalence and variables including sex, age class, and year were statistically analyzed to determine the relationship between predictive risk factors and pathogen exposure. Additionally, the association between seroprevalence and the proportion of wolves vaccinated in each pack was explored, as well as, seroprevalence and the annual recruitment of puppies. Preliminary results suggest that year and age class are important variables in CDV exposure. However, CPV exposure appears to remain relatively uniform across years and age classes. Results from this study will provide guidance for future species recovery planning and help reduce negative species outcomes related to infectious disease.

Research Grant: None
Student Support: Morris Animal Foundation

Proteomics identifies A2M, gelsolin and lubricin as synovial fluid biomarkers for equine osteoarthritis

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Osteoarthritis (OA) accounts for up to 60% of equine lameness. While OA is multifactorial, disease progression is defined by irreversible cartilage damage and chronic pain. Synovial fluid (SF) provides lubrication and nutrition for articular cartilage but also can serve as a sentinel fluid for biomarkers in early joint disease. The objective of this study was to identify differentially expressed proteins between OA and healthy equine carpal joints. An untargeted proteomics analysis using nano LC-MS/MS was performed using SF from equine carpal joints (n = 8 healthy, n = 8 OA), and Proteome Discoverer 2.3 was used to identify proteins. Statistical analysis was performed using MetaboAnalyst, and proteins were classified using PANTHER pathway analysis. A total of 120 proteins were detected in SF, of which 32 were differentially expressed in OA joints (14 upregulated, 18 downregulated, non-FDR adjusted $P < 0.05$). Pathway analysis revealed that 42% of upregulated proteins, including alpha 2 macroglobulin (A2M), were classified as protein-binding activity. A2M and lubricin were both significantly upregulated (1.54- and 1.66-fold change, respectively), while gelsolin was downregulated (0.70-fold change). Gelsolin, an intracellular actin-binding protein, has been implicated as a potential inflammation and tissue injury predictor. This study identified several differentially expressed proteins between OA and healthy joints. Some of the candidate biomarkers, including A2M and lubricin, have been proposed as potential therapies for joint disease in horses and humans. Future studies will elucidate how these candidate biomarkers are involved in the dynamic OA pathogenesis and may identify patient-specific therapeutic strategies.

Research Grant: Weill Cornell Medical College/CTSC NIH Pilot Award 1 UL1 TR002384 and ACVS Foundation Research Grant
Student Support: Boehringer Ingelheim, Cornell University College of Veterinary Medicine

Synbiotic-IgY effects on ileal and colonic mucosal microbiota in dogs with chronic enteropathy

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Normal microbial balance is essential for preserving intestinal health by increasing immune defenses, regulating gastrointestinal (GI) motility, and maintaining epithelial barrier function. When dysbiosis occurs, as seen with canine chronic enteropathy (CE), therapies are needed to reduce intestinal inflammation and restore beneficial microbial communities. One example therapy includes the administration of synbiotics, a combination of prebiotics and probiotics, to induce remission of GI signs. The purpose of this study was to investigate the effects of diet and a synbiotic-IgY dietary supplement on the mucosal microbiota in dogs with CE using fluorescence in situ hybridization (FISH) techniques. Endoscopic biopsies of the ileum and colon from 20 dogs having active CE-10 receiving the synbiotic-IgY supplement and 10 receiving placebo-were obtained for intestinal microbial analysis. Representative three-color FISH images were examined and captured. Mucus adherent bacteria comprised of *Clostridium*, *Bacteroides*, and *Enterobacteriaceae* species were quantified both pre- and post-treatment using Metamorph software. We hypothesize that clinically significant changes in beneficial and harmful bacterial species would occur in dogs administered the dietary supplement as compared to dogs administered placebo. Furthermore, comparing favorable changes in microbiota compositions to clinical disease indices, histopathology, and biomarkers of intestinal inflammation will provide a comprehensive overview of the potential beneficial effects of synbiotic therapy in dogs with CE.

Research Grant: Synbiotic treatment of canine chronic enteropathy; IG Biosciences, Inc.
Student Support: Iowa State University College of Veterinary Medicine Summer Scholar Research Program

Variance in pathogenicity of *Naegleria fowleri* isolates in mouse model of primary amoebic meningoencephalitis

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Naegleria fowleri, colloquially known as "brain-eating amoeba", causes an acute, fatal disease called primary amoebic meningoencephalitis (PAM). *N. fowleri* is commonly present in warm freshwater and soil, yet PAM is a rare disease producing a > 97% fatality rate, with only 4 survivors out of 151 known U.S. cases. One persisting question is why few people succumb to disease when so many are potentially exposed? *In vivo* studies utilizing mouse models of PAM often produce sporadic untreated infected controls who do not succumb to infection. To date, no studies have compared pathogenicity of numerous isolates. We hypothesized that clinical isolates vary in inherent virulence and these variances affect the minimum infectious dose required to induce PAM. Utilizing a mouse model, we intranasally inoculated 5 clinical isolates: Nf69, V067, V631, Villa Jose, and V596, at 3 concentrations per mouse: 100, 1000, and 5000 amoebae. Results showed significant variance in acuteness of disease and fatality rates among isolates and within genotypes. The highest infectious dose induced 100% disease from all isolates except V067(87.5%), with a large variance in onset of endpoint symptoms: V596(4dpi) > V631(4-6dpi) = Villajose(4-6dpi) > Nf69(7-12dpi) > V067(11- > 35dpi). Only V596 produced 100% fatality at the 100 and 1000 inoculums. Concurrently we assessed *in vitro* pathogenicity, comparing feeding rates among isolates seeded onto mammalian cells. While in progress, initial findings indicate potential correlation between an increased feeding rate *in vitro* and virulence *in vivo*. Overall, these results support our hypothesis of inherent differences in pathogenicity between isolates, with variance in minimum infectious doses.

Research Grant: NIH R03AI141709; Georgia Research Alliance
Student Support: NIH T35 OD 010433 Georgia Veterinary Scholars Summer Research Program

Effect of systemic dehydration and ovariectomy: a histopathology pilot study of the rat larynx

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The integrity of the laryngeal vocal folds is important in the creation of the voice. They are a known target organ for estradiol, and their function is negatively affected by systemic dehydration. Estradiol in humans has been shown to increase capillary permeability by vasodilation, modulate vasopressin release, and respond to changes in blood osmolarity. The objective of this study was to determine if the presence of estradiol influences vocal fold histology during systemic dehydration. Twelve female Sprague Dawley rats were divided into dehydrated (n = 6) and euhydrated groups (n = 6). Each hydration group had intact (n = 3) and ovariectomized (n = 3) rats to simulate the loss of sex hormones. Rats were ovariectomized 14 days prior to the start of the study to allow hormone levels to stabilize. Serum estradiol levels and packed cell volume (PCV) were determined at the start of the study. During the 5-day baseline period, all rats were given ad lib food and water; the rats were weighed, and water intake was measured daily. Following the baseline period, a 5-day dehydration period in which the dehydrated group received 4 ml water/100 g of baseline body weight daily and euhydrated continued to receive ad lib water. At the end of the dehydration period all rats were euthanized, blood was collected to measure PCV and estradiol levels, and the larynx was dissected and placed in 10% neutral buffered formalin for histological processing. Larynges were sectioned at 4um in the coronal plane and stained with hematoxylin and eosin for routine morphological assessment. Immunohistochemical labeling of laryngeal estrogen receptors will be quantified using digital software.

Research Grant: Research Supported by NIH R01DC020179
Student Support: Boehringer Ingelheim Animal Health and Purdue University College of Veterinary Medicine

Maternal gut microbiota influences offspring physical activity levels

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Obesity is the largest comorbidity affecting 41.9% of adults older than 20 in the United States. Increased food intake and decreased activity levels are key contributors to the development of obesity. The gut microbiome is known to modulate factors such as behavior, weight gain, and metabolism. Recent investigations have linked the maternal microbiome to lasting changes in offspring body weight. However, the mechanism is largely unknown. The objective of the current study is to determine if physical activity levels contribute to this change. In this experiment, CD1 mice have either a low diversity microbiome, GM1, or a high diversity microbiome, GM4. The pups of these mice are cross-fostered to the opposing microbial profile at birth and develop the microbiome of their foster dam. At 47 days of age, the cross fostered mice are single-housed in a cage with a mouse wheel to measure their voluntary activity levels for a total of 14 days. GM1 or GM4 mice that were not cross-fostered serve as controls and fecal samples for all mice are collected to characterize the microbiota via 16S rRNA sequencing. Preliminary results suggest that the mice born from a dam with a more diverse microbiome have higher activity levels even after their base microbiome has changed. Based on this finding, we believe that the maternal gut microbiome elicits a permanent neurodevelopmental effect on offspring behavior.

Research Grant: Supported by a grant from the American Society of Laboratory Animal Practitioners Foundation (funding from ASLAP Foundation) and an endowment established by IDEXX-BioAnalytics
Student Support: ASLAP Foundation Grant

Mutational burden of the carcinogen *N*-Nitrosodimethylamine in drinking water in the gpt-Delta mouse

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N-Nitrosodimethylamine (NDMA), a probable human carcinogen, is a toxicant found at multiple Superfund sites, in cured meats and, recently, in contaminated pharmaceuticals. NDMA contamination of water near the Superfund site in Wilmington, MA forced the closure of several municipal wells supplying thousands of residents. A recent study by the Massachusetts Department of Health associated in utero exposure to NDMA with a cancer cluster in children whose mothers received water from the affected wells. NDMA induces methylation of the genome, producing mutations that eventually might lead to tumorigenesis. To exert its mutagenic effects, NDMA must first be metabolized by the phase I enzyme cytochrome P450 isoenzyme 2E1 (CYP2E1). Sulforaphane, a cancer-preventive compound, has been shown to be a competitive inhibitor of CYP2E1. We hypothesize that sulforaphane can reduce the number of NDMA-induced mutations by preventing CYP2E1 metabolism. The purpose of this study was two-fold. First, a dose-response study determined a chronic low-dose at which NDMA induces mutations when administered to mice in drinking water. Second, the ability of sulforaphane to prevent mutations of NDMA is being evaluated. To evaluate mutations, *gpt* Delta C57BL/6J mice were administered increasing doses (0.5, 1 and 5 ppm) of NDMA in drinking water for 2 weeks. The *gpt* assay, which enumerates mutations induced *in vivo*, was performed on liver tissue. Results show a 4-fold increase in mutation frequency with a 5 ppm dose of NDMA over a vehicle control. Future studies will determine if sulforaphane-containing diets decrease the NDMA-induced mutations, which would suggest a possible route toward mitigation of cancer risk from unavoidable exposure to NDMA.

Research Grant: NIEHS Superfund Research Program Grant P42-ES027707; NIEHS Core Center Grant P30-ES002109; National Cancer Institute Grant CA-80024; Jameel Water and Food Systems Laboratory Seed Grant
Student Support: NIH T35 Training Grant T35OD033655

Prevalence of *P. tenuis* in Invertebrate Vectors from Ohio Counties with Clinical Disease in Camelids

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Parasites pose a significant public health and animal welfare concern globally. The purpose of this study is to investigate the prevalence and transmission of meningeal worm (*Parelaphostrongylus tenuis*), a neurotropic nematode, in domestic ungulate species in Ohio counties. This will provide critical information for parasite management to reduce infection in domestic camelid and other susceptible species. It is hypothesized that there is another route of transmission, involving the shedding of infective L3 larvae from terrestrial gastropod intermediate hosts via slime trails into the environment rather than direct ingestion of infected gastropods by vulnerable hosts. This would lead to the contamination of grasses, hay, and other feedstuff by the gastropod intermediates. Collected gastropods from camelid farms in different counties (Madison, Perry, Warren, Portage, Lawrence so far) were identified for genus and species based on microscopy. Slime trails were collected for nematode larvae detection followed by analysis of nematode stages in the gastropod tissues. Gastropods were humanely euthanized with 5% MgSO4 solution and digested using acid-pepsin solution with larvae detected via microscopy. Larvae recovered were further processed for molecular analysis. Ohio lacks a contemporary terrestrial snail inventory which this study initially addresses. To date, the total number of gastropods collected is 138. Nematodes were visually detected in 4% of the slugs/snails and are undergoing molecular characterization. GIS mapping was used to visualize the environmental factors present on Ohio properties that have reported clinical *P. tenuis* cases in livestock.

Research Grant: NIH T35 OD010977
Student Support: OSU Summer Research Scholar Program

Colostrum depletion in the post-partum mare

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It is vital that a newborn foal ingest high quality colostrum via its dam or through supplementation shortly after parturition. This is due to there being no transplacental transfer of maternal antibodies from the mare to the fetus in utero. Newborn foals acquire IgG following ingestion of colostrum in the first 24 hours of life. Repeated bouts of nursing by the newborn foal decrease the IgG concentration of antibodies in the mammary fluid over time. The goal of this project was to evaluate the depletion of IgG concentration in mammary fluid over a 48-hour period post-partum. Colostrum samples were collected from mares immediately after foaling and subsequently every 2 hours up to 12 hours post-partum. Additional samples were collected at 24 and 48 hours post-partum. Samples were analyzed using a Brix refractometer and also evaluated for the depletion of IgG antibodies through the use of single radial immunodiffusion (SIRD) assay. Utilizing the results of these two tests can help better understand when colostrum IgG concentrations decline to baseline levels. This knowledge is helpful in determining when to allow a foal considered to be at risk of neonatal isoerythrolysis to nurse from their dam and for when the best time to collect and store high quality colostrum for future use.

Research Grant: United States Department of Agriculture
Student Support: Unknown

Assessment of equine fibroblastic wound healing *in vitro* after exposure to stimulated Ad-MSC supernatant

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Equine cutaneous lesions or wounds can have life-threatening implications for horses. Mesenchymal stem cells (MSC) have been shown to advance tissue regeneration, but limited information exists regarding the ability for MSC to improve healing rates. The objective of this study was to determine if supernatant from stimulated adipose-derived MSC (Ad-MSC) would enhance fibroblast migration and proliferation thereby leading to more rapid wound closure. Equine Ad-MSCs were stimulated in vitro with various mediators (lipopolysaccharide, transforming growth factor-beta 1, or interleukin-1b) followed by collection of the supernatant. Scratch wounds were created in cultured equine fibroblast using the Woundmaker tool. Stimulated Ad-MSC supernatant or the respective control media were added to the wounded fibroblasts. Wound gap area over time will be measured using Incucyte live cell imager and compared between groups. We anticipate that stimulated Ad-MSC supernatant will enhance closure of the wound gap compared to controls. Results from this study could be further used to develop in vivo therapies to improve wound healing in the horse.

Research Grant: This research is supported by the LSU Equine Health Studies Program
Student Support: National Institute of Health T35 (LSU # 47349, AWD-002018)

SDFT structure-function adaptation and athletic training in Thoroughbred racehorses

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The pathophysiological mechanisms responsible for superficial digital flexor tendon (SDFT) injuries in Thoroughbred (THB) racehorses are poorly understood. SDFT tensile strength is primarily attributed to the structural hierarchy comprising of collagen fibers that are tightly packaged into fascicles and separated by elastin-rich interfascicular matrix (IFM). This research investigates if the biomechanical function and the structural biochemical constituents of Thoroughbred racehorse SDFT undergo adaptations reflective of the horse's age and athletic training. Mid-metacarpal SDFT (n = 42) from 2-, 3-, 4- year old THB racehorses necropsied through CAHFS were preserved within 48 hours of death/euthanasia. Total tendon elastin (FASTINTM), collagen (SircolTM), and proteoglycan (PG via DMMB) contents were quantified using colorimetric assays. Load-to-failure tensile biomechanical analyses of whole tendons and fascicle-IFM units were conducted. Logistic regression ($P < 0.1$) was used to assess differences in the age groups for biomechanical indices (yield strain, maximum strain, elastic modulus), biochemical composition and athletic training/exercise variables. Tendon elastic modulus ($P = 0.09$) and training variables - layup time ($P = 0.003$), average layup length ($P = 0.012$) and events since last layup ($P = 0.006$) were identified as significant factors. Subsequent one-way ANOVA ($P < 0.05$) did not find that SDFT elastin, PG contents and elastic modulus significantly differed among the age groups. Further investigation of training variables, fascicle/IFM histomorphometry and their impact on SDFT mechanical strength can aid in designing training regimens that could reduce the incidence of SDFT injuries in THB racehorses.

Research Grant: Grayson Jockey Club Research Foundation and NIH NCATS KL2 Research Career Development Award
Student Support: OSU College of Veterinary Medicine NIH T35 Training Grant OD010977

Understanding myocardial metabolic changes in a rodent model of stress cardiomyopathy

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Stress cardiomyopathy is characterized by acute contractile dysfunction in response to sudden physical or emotional distress. While chronic cardiomyopathies have linked significant metabolic alterations to contractile dysfunction, these mechanisms are unknown in acute cardiomyopathy. The aim of this study was to evaluate for metabolic changes associated with stress cardiomyopathy. A cohort of rats (N = 133) were injected with 75 mg/kg IP isoproterenol and underwent hemodynamic assessments at prespecified time-points after treatment (Day 0, Day 1, Day 3, and Day 7). Animals were anesthetized, and transthoracic echocardiography and left heart PV loop catheterization were performed. Hearts were then rapidly harvested, frozen, and subject to additional analyses. Transthoracic echo revealed peak apical hypokinesia at Day 1 after injury that improved by Day 7 (0.45 $\pm$ 0.27 vs. 0.21 $\pm$ 0.26, $P < 0.05$). Invasive hemodynamic assessments confirmed parallel reductions in stroke work, cardiac output, ejection fraction, and dP/dt that largely recovered by day 7. Metabolomic analysis via LC/MS demonstrated a significant increase in acylcarnitine and decrease in lactate concentrations that correlated with the pattern of injury. A colorimetric plated assay demonstrated markedly decreased intracellular triglyceride concentrations that did not recover by day 7. Fluoro-respirometry showed no difference in electron transport chain activity with disease (P = ns). In our rat model of stress cardiomyopathy, an apparent defect in fatty acid oxidation was correlated with reduction in function without permanent deficit to the electron transport chain. Further assays are underway to better characterize this deficit and explore opportunities for treatment.

Research Grant: Unknown
Student Support: NIH T35 OD010919, Boehringer-Ingelheim, and the University of Pennsylvania

Characterization of disease progression in a mouse model of COVID-19

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the agent of coronavirus disease 2019 (COVID-19), remains a threat to public health. In severe cases, a bilateral pneumonia may develop driven by a proinflammatory cytokine storm. The transgenic K18-hACE2 mouse expressing human angiotensin-converting enzyme 2 (ACE2) has been used to study SARS-CoV-2 pathogenesis. Initial studies have shown that mice develop interstitial pneumonia during acute disease; however, the full scope of disease is not yet understood. The objective of this study was to characterize disease progression in SARS-CoV-2 infected K18-hACE2 mice over an 8-week period, with a focus on lung and brain cytokines and histopathologic changes. Mice were inoculated intranasally and cohorts were euthanized weekly for collection of lungs, brain, and serum. Half of each tissue was preserved for histology and half was preserved for multiplex cytokine analysis. Initial histologic analysis has revealed that acute disease (6-8 days post-inoculation) is characterized by a multifocal mild to moderate lymphohistiocytic interstitial pneumonia and mild meningoencephalitis, with the latter being most evident in mice exhibiting clinical signs at this time point. In mice 8-weeks post-inoculation, lung lesions have progressed to primarily mild multifocal lymphoplasmacytic aggregates suggestive of ongoing disease resolution. Preliminary cytokine analysis revealed increased levels of the proinflammatory cytokine IL-6 in lungs of all mice and brains of mice exhibiting clinical signs. These results will offer insight to disease progression in this COVID-19 model, improving the translatability of studies and ultimately providing a better understanding of human disease.

Research Grant: NIH U42 OD010918, Mutant Mouse Research and Resource Center
Student Support: Kent Tomazi Memorial Research Fund in Veterinary Medicine and an IDEXX BioAnalytics endowment

Determining the effects of four semen extenders on spermatozoa motility in corn snakes (*Pantherophis guttatus*)

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Reptiles are in decline, and it is expected that more than 50% of all herpetological species may be lost to anthropogenic influences by the end of the century. To ensure that these animals can survive, it is vital that we develop appropriate assisted reproductive methods. Unfortunately, to date, this research has been limited in reptiles. While the use of semen extenders has been documented in a variety of mammalian species, strategies for preserving snake semen samples are largely restricted to simple refrigeration. This greatly hinders our ability to share genetic material between captive breeding programs for threatened and endangered species without incurring significant declines in spermatozoa motility after portable cooling systems have expired (typically after 48 hours). This study evaluated four different semen extenders and crystalloids (INRA 96, Andro ProChill LT, Ham's F-10, and Hank's balanced salt solution) in corn snakes (*Pantherophis guttatus*). Our hypothesis was that INRA 96 and Ham's F10 would maintain spermatozoa motility > 50% for longer than 48 hours. Semen samples were manually collected from four corn snakes weekly in a complete cross-over study design. The semen samples were diluted directly into 1 mL of extender and a baseline motility was measured. Samples were refrigerated and evaluated at 24-hour intervals until spermatozoa motility reached 0%. The results of this study suggest that there is a high degree of individual variability in semen motility in corn snakes.

Research Grant: Fluker Farms
Student Support: Boehringer Ingelheim Summer Scholars

Genome wide association study of feline diabetes mellitus

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Insulin independent diabetes mellitus (DM) is a commonly occurring disease in cats. Diabetes mellitus is a complex trait impacted by many genes and with many risk factors- including obesity, pancreatitis, and advanced age. Previous genome-wide association studies (GWAS) mapping complex feline traits have used the 63k single nucleotide polymorphism (SNP) Illumina array. We previously used a one-time high density proprietary 340k SNP array and identified a significant association with DM on chromosome D4 (P = 1.62x10-7). Genes of interest within this interval are *prostaglandin synthase G/H isoform 1* (*PTGS1*) and many genes in the olfactory receptor family. The goal of this study is to replicate this association using a new proprietary Affymetrix array of 2 million SNPs developed by Hill's Pet Nutrition. Cats are both purebred and random-bred and were mostly phenotyped at Cornell University Hospital for Animals. We performed quality control on the initial data from the first batch of cats genotyped, including a principal component analysis to assure breed identity and appropriate matching of cases and controls and a preliminary GWAS. After quality control, there were approximately 1.7 million SNPs across 40 cases and 45 control cats. We will continue to genotype a total of 960 cats on this new array. With a larger array and greater sample size, we aim to identify additional loci associated with DM. These results will be useful in genetic screening, development of precision medicine, and as a model for human diabetes mellitus through future comparative studies.

Research Grant: Cornell University Feline Health Center, Everycat Health Foundation Miller Trust Grant (MT21-014), arrays designed and donated by Hill's Pet Nutrition Inc
Student Support: Cornell University College of Veterinary Medicine and NIH T35 OD010941

Screening sheep bred for research for a mutation responsible for Dermatosparaxis type Ehlers-Danlos syndrome

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Dermatosparaxis type Ehlers-Danlos syndrome (dEDS) is an autosomal recessive connective tissue disorder found in humans, cattle, sheep, dogs, and cats. The disorder is caused by a mutation in ADAMTS2 which encodes for procollagen I N-proteinase, an enzyme responsible for the extracellular cleavage of the N-terminal-propeptide of type I, II, III, and V procollagen. Dorper Sheep have been noted to be carriers of a nucleotide substitution within ADAMTS2 that results in a premature stop codon resulting in an abbreviated peptide. The goal of this project is to determine if any of the sheep in the existing Gray-Edwards Lab research flock are carriers of this ADAMTS2 mutation. The flock has both Jacobs Sheep and Dorper genetic lines. To accomplish this DNA is isolated from anticoagulant treated blood and polymerase chain reactions are utilized to amplify ADAMTS2 for sequencing. Geneious software is used to compare the amplified sequence to the documented ADAMTS2 mutation in sheep. If the mutation is present in the flock, it invites the possibility of selectively breeding for a large animal model of dEDS that could be used to investigate the efficacy of AAV gene therapy as treatment for the disease.

Research Grant: unknown
Student Support: T35

Postoperative sequelae for cats treated with dilute epinephrine to prevent scrotal hematoma

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Scrotal hematomas are a common and distressing complication of feline castrations. Dilute epinephrine is a simple and inexpensive tool used in human medicine to reduce bleeding, but it is not widely employed in veterinary medicine due to concerns for peripheral necrosis and cardiac effects in small patients at high concentrations. In a triple-blind trial, cats neutered through Midwestern University's Trap-Neuter-Return program were randomly assigned to a scrotal wash of 0.2 mL control (saline), 1:120,000 epinephrine, or 1:40,000 epinephrine. Systemic effects were evaluated by comparing median and maximum heart rate (HR) in beats per minute (BPM) between groups after treatment. Peripheral necrosis was evaluated by surveying caregivers regarding signs of scrotal necrosis 3-5 days after surgery. Of a calculated sample size of 500, 82 cats have been recruited; 18 have had complete HR monitoring and responses have been collected from 45 caregivers. Median HR after treatment was 133 BPM (IQR 112, 152; max 180; n = 8) for group A, 149 BPM (IQR 131,153; max 185; n = 6) for group B, and 151 BPM (IQR 128, 164; max 164; n = 4) for group C. Compared to group B, there was no difference in median or maximum HR for A (P = 0.256; P = 0.231) or C (P = 0.437; P = 0.528). Proportional reporting ratio (PRR) for A:B was 0.71 (95%CI = 0.07,7.66), A:C 0.67 (95%CI = 0.06,7.23), and B:C 0.94 (95%CI = 0.13,6.71). One cat in B and one in C that were typically seen by caregivers daily had absences in the 3-5 days postoperative, and no cases of scrotal abscessation were noted. There were no differences in HR between groups after treatment. PRR showed no difference between any group for postoperative sequelae, but many more cats are required to reach full enrollment.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program and Federal Work Study

Hematological comparison of cage-side and sedated blood collections in Japanese Macaques (*Macaca fuscata*)

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Growing interest in Japanese Macaques (*Macaca fuscata*) as models for malaria research presents unique challenges, as animals must be sedated for frequent, small volume blood sampling from the femoral vessels to monitor parasitemia and potential onset of anemia. Cage-side blood sampling from the pinna is a more convenient and less invasive method of sampling and mitigates the need for frequent sedation and removal from the primary enclosure. However, the use of cage-side collections as a clinically-relevant proxy for monitoring the hematological parameters and overall health of Japanese macaques has not been formally assessed. Based on preliminary use of this technique in rhesus macaques, we hypothesized that hematologic parameters are comparable between pinna and femoral blood sampling as well as alert and sedated pinna sampling. To assess this, ten healthy adult male Japanese macaques were trained via positive reinforcement to present their pinnae for cage-side blood sampling for complete blood count analysis. The animals were then sedated for additional comparative pinna and femoral blood sampling. Full data set and results pending.

Research Grant: Bill and Melinda Gates Foundation, University of Georgia
Student Support: Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Effect of COVID-19 on the dog populations in Mississippi animal shelters

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Anecdotal reports indicated that many animal shelters received less dogs, with an increase in adoptions throughout the COVID-19 pandemic. However, current literature supporting these claims is lacking. The aim of this study was to determine if there is a relationship between the COVID-19 pandemic, changes in operational procedures, intake rates and outcomes of dogs. Dog outcomes were categorized into euthanized, transferred, returned to owner, and adopted for each year between 2017 and 2021. A survey was constructed and administered to 48 qualifying Mississippi animal shelters resulting in a 31.25% response rate. 93.33% reported at least one operational change during the pandemic with 13.33% reporting changes in source of funding. Using Tukey Kramer adjustment for multiple comparison, a statistically significant difference during the COVID 19 pandemic was seen for intake, transfer, and euthanasia. However, no statistical significance was present for adoptions. The rate of intake significantly decreased during 2020 along with euthanasia numbers, whereas transfer numbers significantly rose during 2020. Overall, shelters experienced many significant changes in operation, intake and outcome during the COVID-19 pandemic that were not seen prior to it.

Research Grant: National Institutes of Health T35 OD010432
Student Support: None

Prevalence of *Brucella* species in small cetacean pulmonary nematodes along the coast of California

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Brucellosis is a zoonotic disease caused by *Brucella* spp., an intracellular bacteria affecting mammals, including humans. Brucellosis occurs in several marine mammals, including cetaceans, with infection often resulting in abortion, meningoencephalitis, and pneumonia. Marine *Brucella* strains can also cause human disease and there is concern over zoonosis amid populations in contact with cetaceans. A potential route of *Brucella* infection is by pulmonary nematode infection. *Halocercus* and *Pseudalius*, common cetacean nematodes, harbor *Brucella* and cause disease, however their vector potential is unknown and cetacean brucellosis remains understudied. To determine the prevalence of *Brucella* in cetacean nematodes, as well as their vector potential, nematodes from small cetaceans with and without clinical brucellosis stranded over a five year period off the coast of California were collected and identified by pathologists with The Marine Mammal Center. These nematodes were archived at the National Marine Life Center and then shipped to Ohio State where RT-PCR was used to detect the presence of *Brucella* spp. within each nematode sample. Results are still ongoing for this study. It is the hope that study results may be used to explore vector-borne transmission of marine brucellosis and provide background for future studies in order to determine the impact of *Brucella* on vulnerable cetaceans, as well as potential public health risks.

Research Grant: OSU Parasite and Pathogen Ecology Lab
Student Support: Dr. Thomas Mack Global Health Fund

Characterization of the wing microbiome in bats afflicted with White Nose Syndrome

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White Nose Syndrome (WNS) is a disease caused by the fungus *Pseudogymnoascus destructans*, which has resulted in massive population losses in bat species across the United States. WNS is transmitted by direct contact in a seasonal pattern, impacting bats during their hibernation period. Fungal growth on the wings of bats afflicted with White Nose Syndrome is not uniform. We hypothesized that the composition of the wing microbiome might contribute to the restriction of fungal growth in some areas, a notion supported by evidence from in vitro studies where bacteria isolated from bats showed inhibitory properties in co-cultures with *P. destructans*. Utilizing shotgun metagenomics and RNA-seq analysis of bat wing biopsies, this project seeks to test this hypothesis by profiling the bat wing microbiome and characterizing the host immune response in both *P. destructans* affected and unaffected areas on the wing of individual bats. The data collected will help further our understanding of how the fungal infection develops with potential implications for future conservation and control efforts.

Research Grant: University of Pennsylvania
Student Support: NIH T35 OD010919, Boehringer-Ingelheim

Investigation of NCAM1 as a potential biomarker of feline infectious peritonitis

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Feline infectious peritonitis (FIP) is a devastating infectious disease of felids. The etiologic agent, feline infectious peritonitis virus (FIPV), is thought to arise from a genetic mutation of the innocuous feline enteric coronavirus (FECV). The current gold standard for FIP diagnosis is immunohistochemistry (IHC) for Feline Coronavirus (FCoV) antigen on biopsy/autopsy tissues. Given the invasive nature of this test and the poor condition of the patient, most IHC diagnoses are made postmortem. Traditional virological and serological methods are rarely able to diagnostically differentiate FECV from FIPV. Proteomic analysis is a powerful tool which can measure/compare large sets of plasma proteins to identify diagnostically relevant biomarkers. We previously used the SOMAscan assay to quantify over 1000 different plasma proteins between FIP and non-FIP cats and identified 18 proteins which best differentiated the two groups. The SOMAscan assay is designed for human proteins and each protein-specific aptamer must be validated for recognition of the feline homologue. The purpose of this study was to validate the specificity of one of those 18 proteins, neural cell adhesion molecule 1 (NCAM1), using mass spectrometry (MS). Briefly, streptavidin plates were coated with SOMAmer ; feline plasma samples were added to the wells, and then the SOMAmer :protein complexes were released from the plate by UV photocleavage. Protein identification was confirmed by gel electrophoresis, excision, and MS. Future studies will validate additional SOMAmers on their respective feline proteins followed by testing their diagnostic potential to identify cats with FIP.

Research Grant: EveryCat Health Foundation (W19-024)
Student Support: Mumford Feline Foundation

Effect of administration of NSAID's following racing guidelines on furosemide-induced diuresis in horses

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Exercise-Induced Pulmonary Hemorrhage (EIPH) is common problem in racehorses. In the United States, furosemide is administered 4 h prior to racing to attenuate EIPH. Non-steroidal anti-inflammatory drugs (NSAIDs) are also commonly administered to racehorses. Current regulations for Quarter Horse racehorses allow administration of either phenylbutazone, flunixin meglumine, or ketoprofen 48 h before competition. Previous research has demonstrated that NSAID administration, an hour prior to furosemide administration, can limit the furosemide-induced diuresis by 25-30%. To determine whether current regulations for administration of NSAIDs to Quarter Horses affect furosemide-induced diuresis, we measured urine production (volume) during the 4 h after furosemide administration after pre-treatment with these NSAIDs. We hypothesized that the 48 h withdrawal period after NSAID administration would have no impact on subsequent furosemide-induced diuresis. To test this hypothesis we studied eight healthy mares in a replicated 4 x 4 Latin Square design. Horses were administered a single dose of each NSAID or saline as a control treatment 48 h prior to furosemide administration. In the hour before furosemide administration, mares were instrumented with bilateral ureteral catheters. Following a baseline urine collection period, furosemide (1 mg/kg, IV) was administered and total urine produced in the subsequent 4 h was collected. We found no difference (mean ± SD) in urine production between the four treatments: control 20 ± 4 mL/kg; phenylbutazone 18 ± 3 mL/kg, flunixin 20 ± 4 mL/kg, and ketoprofen 20 ± 2 mL/kg (P = 0.19). In conclusion, administration of NSAIDs 4 h prior to furosemide administration had no effect on furosemide-induced diuresis.

Research Grant: AQHA
Student Support: Boehringer Ingelheim and MSU Graduate School

The effect of combined senolytic and NaR supplementation on activity and attention in aging dogs

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As the molecular basis of aging is becoming better understood, new targeted therapies are being developed in both human and veterinary medicine. Cellular nicotinamide adenine dinucleotide (NAD) levels decline with age and senescent cells accumulate, causing inflammation and further depletion of NAD. There is growing evidence that oral senolytics and nicotininc acid riboside (NAR) can improve healthspan. We hypothesize that a combination of a senolytic and NAR administered orally will improve activity and attention in aged dogs. This hypothesis will be examined in a placebo controlled, randomized, blinded 3-arm clinical trial (placebo, doses 1 and 2). The trial is designed to have 80% power to detect a 20% change in cognitive scoring. Primary outcomes include owner scoring of cognition and quality of life, and activity levels quantified using an activity monitor. The first aim is to recruit 60 dogs > 10 years of age with cognitive and mobility deficits and randomize to a treatment group. The second aim is to quantify the health status of dogs through owner questionnaires, physical exam, cognitive and mobility tests, activity monitors and blood work at baseline, 1, 3 and 6 months after starting the supplements. The final aim is to compare outcomes between groups. We have enrolled 39 dogs and one dog has completed the protocol; an interim analysis will be performed when 60 dogs have reached the 3 month time point. This clinical trial will provide objective data on the clinical course of activity and cognition in elderly dogs, will quantify the placebo effect in this population of dogs in aging trials, and will allow careful comparison of the effect of senolytics and NAR on activity and attention in dogs.

Research Grant: Animal Biosciences
Student Support: NC State CVM Veterinary Scholars Program

Refining sample size recommendations for PQA Plus and CSIA audit tools

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The Common Swine Industry Audit (CSIA) is a national audit program to safeguard consumer trust by verifying animal welfare standards and pre-harvest food safety measures are being met. The CSIA is conducted on all stages of production and evaluates animal-based measures as a means to assess animal welfare. CSIA utilizes a pre-calculated sampling method to identify the subpopulation of animals on-farm that will be evaluated during the audit. This sampling method is based on previous epidemiological work used to detect disease and has not been verified as an appropriate sampling method for animal-based measures. Therefore, the objective of this study was to evaluate accuracy of prevalence of animal-based measures using the CSIA sampling size compared to the total farm population. A total of five farms, nursery or finisher farms, were enrolled on this study. CSIA sample size was determined prior to on-farm visits by collecting information on total farm capacity, buildings, pens per building, and pigs per pen. On-farm, all pigs were evaluated on nine animal-based measures and information on their pen location was recorded. Measure prevalence was calculated by taking the total number of animals identified with each measure divided by total sub-sample population (CSIA sampling method) or total population (all pigs). Approximately 13,000 pigs were observed on farms with minimal benchmark measures found. One farm was found to have a total of forty-five hernias (1.24%), with its respective CSIA subpopulation containing only two (0.70%). These results indicate a need for further data collection for validation of this sampling method.

Research Grant: None
Student Support: NC State University Fluoroscience Endowment

Gastrointestinal helminths of wild canids in Pennsylvania

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Southeastern Cooperative Wildlife Disease Study (Simpson, Garrett, Haynes, Yabsley, Cleveland), University of Georgia, Athens, GA; Department of Veterinary and Biomedical Sciences (Brown), Pennsylvania State University, University Park, PA; USDA Wildlife Services (VanWhy), Harrisburg, PA

Urban expansion increases interactions between wildlife, domestic animals, and humans, resulting in the transmission of gastrointestinal parasites from wild canids to domestic dogs and humans. Therefore, there is a need to evaluate the helminth species present in wild canids across urban-rural gradients. The aim of this study was to compare the diversity, prevalence, and infection intensity of gastrointestinal helminths in wild canids in urban and rural Pennsylvania. Gastrointestinal tracts were harvested from coyotes (*Canis latrans*; n = 28), red foxes (*Vulpes vulpes*; n = 10), and gray foxes (*Urocyon cinereoargenteus*; n = 2) from urban counties (Allegheny, Bucks; n = 20) and rural counties (Cambria, Cameron, Clearfield, Elk; n = 20) in Pennsylvania (2019 - 2020). Parasites isolated from gastrointestinal contents were morphologically identified to Class (nematode, trematode, cestode) and, when possible, to Order, Family, or Genus. Individual infection intensity was quantified by counting nematodes, trematodes, and cestode scolices. We found 100% of urban coyotes and 78% of rural coyotes were infected with at least one species of helminth. More investigation is needed to evaluate helminth prevalence in red and gray foxes. The most common helminths recovered were ascarids, hookworms, and cestodes. Rarely, trematodes and whipworms were identified. Individual canids had 0-8 ascarids, 0-65 hookworms, 0-97 cestode scolices, 0-2 trematodes, and 0-1 whipworm. More ascarids, whipworms, and cestodes were found in urban individuals, while more hookworms and trematodes were recovered from rural individuals. Our preliminary results highlight the impact urbanization has on helminth diversity and infection intensity in wild canids.

Research Grant: None
Student Support: Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Effects of ororuminal forced feeding in severely dehydrated calves

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Diarrhea is the most common disease in bovine neonates and is a significant financial and welfare issue in the cattle industry. Diarrhea in calves can result in severe dehydration, metabolic acidosis, increased incidence of other diseases, and death. Intravenous fluid therapy (IV) and oral electrolytes have solved some clinical signs. However, there is little scientific evidence available in combining these two therapies and using force-fed milk through an ororuminal tube to treat dehydration in diarrheic calves, despite this being a common practice in cattle operations. Therefore, we hypothesized that severely dehydrated calves treated with IV combined with force-fed oral electrolytes will have lower mortality, and number of treatments than dehydrated calves treated with IV combined with force-fed milk. Calves (n = 356) were randomly enrolled when the farm personnel diagnosed mild or severe dehydration into one of the four groups: healthy control (HC), IV only (Mixture of Ringer Lactate 1L, Vitamine B12 10,000 IU, and $NaHCO_3$ 14 g), IV plus oral force-fed electrolyte (2.0 L; ELE), and IV plus oral force-fed milk (2.0 L; MILK). Linear and logistic regression were used to analyze differences in the number of treatments and mortality, respectively. We did not observe a difference in mortality among the groups. The mortality rate for HC, IV only, ELE, and MILK were 1.12%, 8.99%, 5.62%, and 6.74%, respectively (P = 0.23). The average number of IV treatments given to HC, IV only, ELE, and MILK were 0.26, 1.65, 1.53, and 2.28, respectively ($P < 0.0001$). Finally, treatment with IV plus oral force-fed electrolytes may aid in shortening recovery times for dehydrated calves and financial costs associated with treatments.

Research Grant: WSU CVM Summer Research Fellowship
Student Support: Francisco Leal Yepes

Risk Assessment to Reduce Chronic Wasting Disease Transmission in Farmed Cervids

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Chronic wasting disease (CWD) poses an existential threat to cervid farms due to continued disease spread. Current CWD preventive practices are focused on postmortem surveillance and preventing transmission through regulatory policies and implementation of biosecurity practices focused on preventing direct contact with infected farmed or wild cervids. These regulatory policies and biosecurity practices alone have not been adequate to contain further spread of CWD, because they fail to address other means of transmission. In addition to direct contact transmission, CWD can be spread through other pathways such as indirect contact through scavenger species and contaminated soil which are not addressed through current policies or biosecurity practices. The purpose of this study is to address the critical need to develop and evaluate on-farm educational information and tools to facilitate adoption of biosecurity practices on cervid farms to reduce risks of CWD introduction, based on the evaluation of the most likely pathways determined on each individual farm. To accomplish this study objective, we developed a risk assessment tool for use on cervid farms and are beginning to evaluate its usefulness and feasibility to improve biosecurity against CWD through use on study farms. Preliminary results indicate that use of this risk assessment process can identify CWD transmission risks not previously considered by cervid producers and producers are willing to consider adopting improved biosecurity practices to minimize CWD risks.

Research Grant: USDA APHIS - Veterinary Services 2021 Farmed Cervid Chronic Wasting Disease (CWD) Management and Response Activities
Student Support: University of Minnesota, Department of Population Medicine

Dietary advanced glycation end-products in homemade diets using different protein-processing methods

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Dietary advanced glycation end products (dAGEs) are intermediate by-products of the Maillard reaction. These compounds induce protein-cross linking and intracellular oxidative stress and are associated with the pathogenesis of human diseases. Given the association of increased consumption of dAGEs with disease in humans, a similar association is possible in dogs.Production of dAGEs occurs during cooking when proteins and sugars are exposed to dry heat. The current pet food market is predominantly composed of diets that undergo either extrusion for kibble or retorting for canned foods. These processing methods are associated with high dAGE content based on previous studies. Given the popularity of cooked homemade diets for dogs, this study's objective was to compare dAGE quantity in homemade diets prepared using 6 different protein-processing methods. A balanced homemade diet was formulated by a Board-Certified Veterinary Nutritionist with the primary protein being boneless, skinless, chicken thighs. The chicken was prepared in a crockpot, a pressure cooker, boiled in water, pan fried, oven roasted, or grilled. Two additional composite samples were analyzed that had been prepared a year prior and stored at -20°C. We hypothesized there would be different levels of dAGEs, specifically carboxymethyllysine (CML) and carboxyethyllysine (CEL), based on method used to process the chicken. We hypothesized the diet using chicken prepared in a crockpot would have the lowest levels of dAGEs and the highest levels of dAGEs would be found in the diet using grilled chicken. Samples of each diet were analyzed for CEL and CML via ultra-high-performance liquid chromatography-tandem mass spectrometry. Results of this study are pending.

Research Grant: This project was funded through both a research grant from the Companion Animal Nutrition & Wellness Institute (CANWI) and funding from the Department of Small Animal Medicine and Surgery
Student Support: UGA Foundation, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Comparison of two freezing techniques on equine spermatozoa

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Since the discovery of glycerol in 1948 and the first foal from frozen semen in 1957, equine semen cryopreservation has been rapidly developing different techniques to produce the most viable sperm. Currently semen is most commonly frozen with either automated programmable freezers or via the commonly accepted conventional method where semen is cooled in a styrofoam cooler 15 cm above liquid nitrogen for 20 minutes to reach -196°C at a rate of 20 to 50°C per minute. The current cryopreservation technique used at the Iowa State University Cryopreservation Laboratory is to slowly cool the semen 5 cm above liquid nitrogen in a 3,482 L MVE cryogenic stock tank beginning at -100°C to -120°C at 2°C per minute over 10 minutes. Cooling is then continued by 5°C per minute to -196°C prior to submerging into the liquid nitrogen. The purpose of this study is to compare the slow-cool technique to conventional method by assessing subjective and objective motility, morphology, viability, and membrane integrity of six stallion semen samples. These will be evaluated using a phase light microscope, computer-assisted sperm analysis (CASA), a nucleocounter SP-100, and the hypoosmotic swelling test (HOST) respectively. To date, nine stallions of various breeds have been enrolled ranging in ages from 2 to 15. Successful ejaculates were collected from seven stallions using a Missouri Artificial vagina on a dummy mount with six subsequently frozen based on acceptable semen quality. Preliminary statistical analysis showed no difference in subjective post-thaw motility ($P = 0.59$) or post-thaw viability ($P = 0.77$). Objective motility assessment and comparisons based on initial semen quality remain pending.

Research Grant: Boehringer Ingelheim
Student Support: NIH T35 Training Grant

Clinical and histopathologic features of feline gastrointestinal eosinophilic sclerosing fibroplasia

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Feline Gastrointestinal Eosinophilic Sclerosing Fibroplasia (FGESF) is an entity found in the alimentary tract and lymph nodes of cats. Common clinical findings include eosinophilia and masses at the ileocecocolic junction or pyloric sphincter. The cause and pathogenesis of FGESF is undetermined, but the role of eosinophils suggests genetic predisposition to eosinophilic inflammation or potential bacterial or parasitic infection. Previous studies have demonstrated intralesional bacteria in FGESF, but the mixed bacteria were thought to be secondary to the lesion and not the cause. Nematode migration has been associated with sclerotic and eosinophilic gastritis in non-domestic felids. We hypothesize that a common nematode of cats, *Toxocara cati*, plays a role in the pathogenesis of FGESF. In this study, we determined common clinical and histopathologic features of FGESF in 40 cases of suspected FGESF. Clinical data was available for 34 cases; all cats presented with gastrointestinal signs of varying duration. Twenty-two cats had complete blood counts performed and 10/22 (45%) cats presented with eosinophilia. Hematoxylin and eosin-stained sections of affected tissues were scored for the presence of collagen, necrosis, and inflammation. Expansion of the submucosa and muscularis was measured for each case. Histopathologic features of FGESF included expansion of the submucosa by trabeculae of collagen, eosinophils, plump fibroblasts, and mixed mononuclear inflammatory cells. The average number of eosinophils within lesions of FGESF was 40.75 eosinophils per 10 x 10 grid at 40x. In this study we will also have determined the role of *T. cati* in the pathogenesis of FGESF via investigation of *T. cati* DNA within 14 cases of FGESF.

Research Grant: Research funding provided by EveryCat Health Foundation EC22-018
Student Support: Research funding provided by no. 5T35OD016477-20 from NIH Grant

Evaluation of additively manufactured bioresorbable trauma implants in a rat femur fracture model

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Many veterinary orthopaedic interventions rely upon metallic implants to bear mechanical loads during healing. However, this approach can lead to stress shielding and metal implants remain within the body permanently. 3-D printed bioresorbable polymers, such as PLGA, can be tuned to change mechanical properties and alter degradation kinetics to improve healing. However, little is known about the temporal mechanical behavior of such implants. The aim of this study is to determine how the degradation of novel, 3-D printed, implants affect bone healing at clinically relevant timepoints (4-16 weeks) using a rat femur osteotomy model. We hypothesize that the bone formation will be accelerated in the resorbable implant group compared to controls. In this ongoing experiment, we expect to see substantial callus formation beginning in the resorbable group by the 4-week timepoint, but do not expect to see these changes until 8-weeks in the control group. We expect to see early cartilage bone formation in addition to high levels of osteoblastogenesis beginning in the resorbable group at the 4-week timepoint. We also expect bones treated with resorbable implants to demonstrate higher torsional strength than the control group at the 8 and 16-week timepoints. Preliminary results from in vitro testing demonstrate that mechanical strength of the bioresorbable implants is maintained from the 0 to 8-week timepoints but significantly decreases at the 16-week timepoint. Results from this study will guide the design and development of patient specific PLGA devices that may shift the paradigms of veterinary orthopaedic surgery.

Research Grant: NIH K25AR078383, NIH/NIAMS P30AR069619
Student Support: NIH T35 OD010919

Diagnostic techniques identifying bovine infectious anemia associated with *Theileria orientalis* Ikeda genotype

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The hemoprotozoan parasite *Theileria orientalis* Ikeda genotype is transmitted via *Haemaphysalis longicornis* and has been associated with bovine infectious anemia. There is currently no treatment for theileriosis and cattle become persistently infected. *Theileria orientalis* Ikeda genotype and *Anaplasma marginale* are indistinguishable in their clinical signs and are both associated causes of bovine infectious anemia in Virginia. Determining anemia in cattle is an important early diagnostic step for a definitive diagnosis, therefore, it is necessary to have practical alternative methods of detection like a colorimetric hemoglobin assay. Packed cell volume (PCV) has been the primary method used to determine anemia; however, this diagnostic technique declines in reliability as processing time is delayed such as in a diagnostic laboratory following sample shipping. A time series was conducted for four weeks to evaluate the reliability of results for PCV and hemoglobin concentration. A correlation between low hemoglobin concentration and high presence of *Theileria orientalis* Ikeda genotype DNA in blood samples was conducted. An examination of Diff-Quik and Wright- Geimsa stained slides was conducted to identify the presence of piroplasm in red blood cells of anemic cattle suspected of theileriosis. We anticipate that a colorimetric hemoglobin assay will detect anemia in older blood samples more effectively than PCV. Anemic cattle will have a significantly greater parasitemia of *Theileria orientalis* Ikeda genotype DNA present in blood. Additionally, the presence of *Theileria orientalis* is not easily identifiable on cytology making it a poor choice for detection, particularly in persistently infected cattle.

Research Grant: Cooperative agreement with the U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS)
Student Support: Virginia-Maryland College of Veterinary Medicine

Evaluating the relationship of mechanical allodynia and *Chrna6* downregulation in transgenic mouse models

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Chronic pain management is a major concern in public health. Chronic pain may be characterized by spontaneous pain or mechanical allodynia. The mechanical allodynia mechanisms are not entirely understood, but it is known that mechanical allodynia occurs following injury. Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand gated ion channels composed of α and β subunits which are a target for analgesics. There is little known about the function of $\alpha6\beta4^*$ nAChRs in the central and peripheral nervous systems. The *Chrna6* gene, which encodes the $\alpha6$ nAChR subunit has been associated with the development of allodynia. The current focus of nicotinic receptor analgesics has been $\alpha4$ nAChRs, but $\alpha6$ nAChRs may be a promising drug target. The goal of our study is to further investigate the mechanisms between *Chrna6* downregulation and allodynia that were previously reported. We hypothesize that increased mechanical allodynia associated with neuropathic and inflammatory injuries is correlated with the downregulation of the *Chrna6* gene in transgenic mouse models, including those with GFP labelled $\alpha6$ nAChRs ($\alpha6$GFP mice). To test this, we induced neuropathic or inflammatory pain in $\alpha6$GFP mice with a spared nerve injury (SNI) or injection of complete Freund's Adjuvant (CFA), respectively. Our current findings from behavioral assays utilizing an electronic Von Frey machine suggest mechanical allodynia develops in the $\alpha6$GFP mice following either a SNI or CFA injection. The gene expression of *Chrna6* following development of allodynia will then be measured in mouse dorsal root ganglion using RT-qPCR and the $2^{-\Delta\Delta Ct}$ method. Ultimately, this study will help characterize the role of nicotinic receptors in chronic pain.

Research Grant: Virginia-Maryland CVM, iTHRIV Program by NCATS of the NIH UL1TR00315 and KL2TR00316
Student Support: Boehringer Ingelheim Veterinary Scholar

Super-resolution analysis of chromatin nanostructure in bovine oocytes obtained by follicular aspiration

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Chromosome instability is a leading cause of pre-implantation embryo loss in humans and cattle. Embryo loss in cattle is also of high economic impact and improved methods to identify oocytes for healthy pregnancies are highly sought after. Currently, oocyte quality is assessed only by morphological criteria. Here, we aim to understand the regulation of nanoscale chromatin structure and its role in maintenance of chromosome stability and developmental potential in bovine oocytes. We conducted the first comprehensive analysis of chromatin nanostructure in bovine oocytes obtained by follicular aspiration. Using a combination of 3-D confocal microscopy, high-resolution epifluorescence and super-resolution structural illumination, we analyzed the nuclear localization of heterochromatin domains at different scales and resolved sub-chromosomal compartments down to ~100nm. Tri-methylation of lysine 9 of histone H3 (H3K9me3), a marker of heterochromatin in mouse cells, exhibits a conserved centromeric localization in bovine somatic metaphase chromosomes and oocyte chromocenters suggesting an important role for chromosome stability and oocyte quality. Notably, in contrast with mouse oocytes, H3K9me3 is enriched at GC-rich sequences detected with the nucleic acid dye YO-PRO-1, while it is absent from AT-rich DNA stained with DAPI in the bovine oocyte genome. Super-resolved 3D-image renderings revealed critical spatial information on the localization of the nucleolus and different chromatin domains during dynamic transitions of chromatin configuration. Our ongoing studies are focused on computational image analyses and correlations with morphological parameters to establish novel criteria of bovine oocyte developmental potential.

Research Grant: NIH (HD093383), NSF CMaT (164805), and AFRI-USDA grant (2020-67015-30882)
Student Support: UGA Foundation, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Wildfire effects on Sin Nombre hantavirus prevalence and *Peromyscus maniculatus* abundance in Red Clover Valley

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Wildfires have been steadily increasing in frequency and severity globally due to climate change and fire suppression. This has led to a dramatic loss of land causing a surge in cross-species pathogen transmission. *Sin Nombre orthohantavirus* (SNV; genus: *Hantavirus*) is maintained by *Peromyscus maniculatus* rodent reservoirs in North America and causes hantavirus cardiopulmonary syndrome (HPS), with human case fatality ratio of 35-40%. Climate change has been linked to an increased prevalence of human hantavirus infections, but it is still unclear how wildfire affects SNV prevalence in deer mice. The aim of this study is to understand the effects of wildfire on rodent populations and SNV prevalence at recently burned (August 2022) and unburned sites. Oral, fecal, urogenital, and blood samples were collected from each captured rodent. RNA was extracted from oral and urogenital samples and used in RT-qPCR to test for SNV. Preliminary data has shown a decrease in SNV prevalence in both burned and unburned areas one month post-fire. Seven months post-fire, more rodents were trapped in the burned site than in the unburned site, suggesting a resurgence of rodent populations following new vegetation growth. It is expected that as rodent populations increase in the burned site, SNV prevalence will also increase. This may indicate that as wildfires become more common, viruses such as SNV will become more prevalent in their reservoir hosts, posing a greater threat of pathogen spillover to humans.

Research Grant: California Department of Fish & Wildlife GGRP, California Conservation Wildlife Board Grant
Student Support: Students Training in Advanced Research Fellowship: NIH T35-OD010956

Identification of miRNA biomarkers associated with the severity of spinal cord injury

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Acute spinal cord injuries (SCI) such as intervertebral disc herniations are a common neurological issue in both animal and human patients that involve mixed compressive and contusive damage to neural tissue. Circulating miRNAs are small non-coding RNAs involved in post-transcriptional gene regulation that may also play a role in the physiological/pathological response to spinal cord injury. As of now, there are few diagnostic biomarkers available to help establish effective therapeutic strategies for patients with SCIs. Therefore, establishing profiles of specific miRNA biomarker expression associated with injury severity may serve as a potential diagnostic tool for distinguishing the severity of SCI while also allowing for the prediction of the long-term clinical outcome of a patient. To generate these profiles, miRNA was isolated from peripheral blood samples of canine patients with SCIs of varying severities (mild, moderate, and severe) along with healthy canine patients without SCIs as control. miRNA candidates for each severity were then quantified using real-time PCR to determine if there were any correlations between the expression of specific miRNAs and the severity of SCI. It is expected that circulating miRNA profiles from the peripheral blood of dogs with acute SCI will reflect the injury severity. These results will ideally potentiate the use of miRNA biomarkers as a monitoring tool for neuroprotective therapies while also providing new avenues for precise therapeutic treatment of SCI in both canine and human patients.

Research Grant: None
Student Support: University of Missouri College of Veterinary Medicine Office of Research

A synthesis of the implications of sea level rise on zoonotic diseases

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Sea-level rise poses a threat to the habitat of humans and animals that reside in coastal regions across the world. As a result, sea-level rise is expected to impact the mass movement of humans and animals, which in turn has a direct impact on zoonotic disease potential. Although islands and coastal areas are the first to feel the consequences of sea-level rise, inland populations will also be affected. In particular, lower-income communities already impacted by climate change and infectious diseases will be disproportionally affected. While climate change and its impacts on zoonotic disease risk have been examined, further understanding of the role that sea-level rise might play is needed. This synthesizing review aimed to fill this knowledge gap and explore the One Health connections that will aid future transdisciplinary approaches to reduce zoonotic emergence and transmission. We conducted four literature searches across three databases (PubMed, CAB Abstracts, and Scopus) using search terms aimed at identifying literature associated with a.) sea-level rise and human movement, b.) sea-level rise and wildlife movement, c.) human movement and zoonotic disease, and d.) wildlife movement and zoonotic disease. In total, we identified 2,747 papers, with 502 resulting from search a, 954 from search b, 492 from search c, and 799 from search d. Preliminary findings of the returned literature have shown 64% of the papers were excluded based on content, 7% based on format, 10% that require further review based on the full text before deciding, and 19% included in the synthesis. Future work will continue to examine patterns in the existing literature and identify the gaps in knowledge for future transdisciplinary study.

Research Grant: None
Student Support: Boehringer Ingelheim and the College of Veterinary Medicine

Widespread pathologic disease and vertical transmission of *Mycobacterium avium* subsp *paratuberculosis* in goats

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Johne's disease (JD) is an economically devastating disease of ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). MAP infection causes chronic, progressive malabsorptive enteritis and can result in malnutrition, chronic diarrhea, muscular wasting and production losses. Infection is difficult to detect until disease is severe and environmental contamination contributes significantly to endemnicity within a herd. While the pathogenesis and transmission of JD is best studied in dairy cattle, the underlying mechanisms of disease, infectivity, and transmission is not well described in small ruminants. The goals of this study were to determine the extent of MAP dissemination and disease in infected goats and to identify potential mechanisms of vertical transmission. Various tissue samples were collected from MAP ELISA+ / fecal PCR+ goats (14 does, 1 buck) as well as available fetuses (n = 7) and kids (n = 2). MAP cultures were confirmed from fecal samples and tissues were processed for MAP DNA detection using RT-PCR, histopathology, and Fite's staining. MAP DNA was detected in intestinal sections and associated lymph nodes, but also in several tissues "outside" the gastrointestinal tract with associated granulomatous disease. Additionally, RT-PCR indicated likely vertical transmission from infected does to fetal tissues in several animals. Results from this study provide evidence of transmission and elucidation of disease that can be utilized to develop improved control and detection strategies in goat herds.

Research Grant: Oklahoma State University CVM
Student Support: Oklahoma State University CVM and OSU CVM Department of Veterinary Pathobiology

Investigating the role of thyroid hormone in ovine placenta using trophoblast cell lines

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It has been reported that maternal nutrient restriction models of intrauterine growth restriction (IUGR) pregnancies result in a decrease of thyroxine (T4) levels in maternal and fetal circulation. In humans, thyroid hormones (THs) are provided to the fetus by transport of maternal THs through the placenta. In particular, T4 plays an important role in the activation, differentiation and maturation of the fetal central nervous system (Patel et al.). Transthyretin (TTR) is a TH binding molecule produced by trophoblast cells that binds and moves T4 through the placenta. In vitro experiments use trophoblast cells for testing of TTR expression since they are the cells involved with the placenta in utero. After growing cell lines of immortalized ovine trophoblasts (IOTR) we distributed them equally into treatment groups. This experiment involved IOTR cells plates incubating in either 3% or 21% oxygen. It has been suggested that a lower oxygen concentration may mimic in vivo conditions better than our standard 21% oxygen incubation. Since TTR is secreted by cells we added Brefeldin A (BA) to each plate after they had incubated in their respective treatment group for 24 hrs. 6 hours after BA application cells were collected and processed for protein collection. Then a Western Blot for TTR was performed for both treatment groups. The TTR protein amount detected in the 3% oxygen was significantly higher than the 21% oxygen. This data alone is not sufficient enough to draw any conclusions. However, some possible experiments include, rtPCR on the RNA from the previous experiment to investigate if there is RNA related to TTR and using serum free media with T4 added to stimulate TTR production.

Research Grant: USDA
Student Support: USDA and Dr. Quinton Winger

Alterations in the ocular surface immunome and microbiome in horses with corneolimbal squamous cell carcinoma

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Squamous cell carcinoma (SCC) is the most prevalent ocular tumor encountered in equine ophthalmology. Response rates vary widely among the different treatment modalities suggesting the presence of multiple factors that may regulate tumor responses, including the microenvironment of the ocular surface. Therefore, understanding the immune and microbial constituents of the ocular SCC tumor microenvironment (TME) using next generation sequencing technologies should help with the development of more targeted therapies. The goal of this study therefore was to evaluate the ocular microbiome and the ocular immunome between eyes with corneolimbal SCC and clinically normal eyes in the same animal to identify important differences. The study will also use in vitro assays to help elucidate the impact of the ocular surface microbiome and secreted factors on tumor immune responses. Ocular surface gene expression will be evaluated using next generation RNA sequencing, while microbiome analysis will be performed using 16S bacterial rRNA and internal transcribed spacer (ITS) gene sequencing respectively. Correlations between ocular surface immunomes and microbiomes in healthy versus affected eyes will be determined using appropriate statistical analyses. Use of these next-generation sequencing technologies will provide greater ability to identify new connections between the microbiome and surface immunome of the eye, and how ocular cancer may impact those connections. Furthermore, this research will provide important translational insight across species because the horse serves as an excellent spontaneous model for ocular squamous cell carcinoma in humans.

Research Grant: Research Grant: Young Investigator's Grant and Shipley Family Foundation
Student Support: Student Support: Boehringer Ingelheim

Effects of serum starvation and seeding density on equine tenocyte recovery and viability

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Tenocytes are utilized in cell culture assays to study tendon pathophysiology and novel treatments. Serum starvation is implemented to synchronize cell growth; however, no studies have examined its effect on equine tenocytes. The objective of this study was to determine the optimal duration of serum starvation and tenocyte seeding density for tenocyte migration assays. Equine tenocytes were seeded in 12-well plates (surface area 3.5 cm^2) at three densities (1.0, 1.5, and $2.0x10^5$ per well), grown in standard media with 10% fetal bovine serum, and grown to confluence. All three seeding densities then underwent growth in serum free media for 6, 12, or 24 hours. Assays were performed for a total of 6 replicates. Following serum starvation, total cell count, percent cell recovery (seeded minus recovered x 100), and tenocyte viability (live-dead staining) were determined. Data were analyzed with a two-way ANOVA in GraphPad Prism. Following 12 hours of serum starvation, a significantly lower percentage of cells were recovered from wells seeded at $2x10^5$ compared to $1.5x10^5$ ($P < 0.05$) and $1.0x10^5$ ($P < 0.01$). However, no significant difference in percent cell viability of adherent cells at the end of serum starvation was noted for any group. From these data, the surface area of 12-well plates likely limits the number of cells that can attach when a density greater than $1.5x10^5$ is used as noted by the lower percent cell recovery rate for some groups. However, it appears that once attached, serum starvation for up to 24 hours does not affect adherent tenocyte viability.

Research Grant: Fund for Orthopedic Research in honor of Gus and Equine athletes (F.O.R.G.E), The Barton & Marie-Claude White Equine Orthopedic Research Endowment
Student Support: NIH T35OD011070 Interdisciplinary Biomedical Research Training Program

Evaluation of transmission potential of SARS-CoV-2 through surveillance of companion and exotic animals

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There are over 89 million human cases of COVID-19 in the United States, and with 70% of households owning a pet, millions of companion animals have potentially been exposed to SARS-CoV-2 through their owners. SARS-CoV-2 has been reported to infect both wild and domestic animal species after contact with infected humans, and transmission from infected animals to humans has been reported in rare cases after close contact. Veterinary professionals have an increased likelihood of exposure to zoonotic diseases like SARS-CoV-2, so improved understanding of the transmission risk of this virus in animals and the veterinary community is warranted to better protect their health. Our goal is to measure the prevalence of SARS-CoV-2 in companion and exotic animals to assess transmission risk of the virus. We collected oral swabs, nasal swabs, and opportunistic blood samples from domestic mammal species seen at the CSU Veterinary Teaching Hospital. Oral and nasal samples were tested using polymerase chain reaction (PCR) to detect nucleic acid, while serum was tested for antibodies against SARS-CoV-2 using a plaque reduction neutralization test. Study enrollment is ongoing with the goal to obtain 340 samples with a range of species. PCR tests detected SARS-CoV-2 definitively in 1 nasal swab of 133 canine oral/nasal samples and 1 feline oral swab in 74 feline oral/nasal samples. Data derived from this study will provide infection rates of domestic and exotic pets with SARS-CoV-2 and contribute to a better understanding of its transmission risk in a veterinary setting. Study results will support informed decision making by veterinary professionals regarding biosafety and use of personal protection equipment during animal handling.

Research Grant: NAHLN Farm Bill
Student Support: NIH Grant Number T35 OD015130

Quantitative analysis of stress following student palpation per rectum in the mare

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Cognizance for the welfare of institutionally-owned teaching animals used for the instruction of veterinary students exists amongst educators. However, removal of live animals from a veterinary curriculum would ablate school provided experiential education. As educators and advocates, we strive to prepare students for careers as veterinarians while remaining vigilant of stress in animals secondary to use in veterinary curriculums. Our objective was to evaluate stress levels of horses, a prey animal, by quantifying the physiological response of mares as students perform palpation per rectum (PPR) examinations. In a randomized crossover study, we utilized the Horse Grimace Scale (HGS) and salivary cortisol to assess discomfort and the hypothalamic-pituitary-adrenal axis response. Salivary samples were collected with a Salivette cotton swab and a left facial profile picture for HGS scoring was obtained from all mares at 5 and 20 minutes. Thirteen mares served in both Control (C) and Treatment (T) groups. The C mares were left standing and unhandled between sample collections, while T mares also stood unhandled for 5 min before student interactions and PPR were initiated. Salivary cortisol concentrations were measured via ELISA; HGS was scored by assigning a number to each of the six facial action units. Two-way repeated measures ANOVAs with Bonferroni corrections indicated the treatment condition resulted in lower transformed cortisol readings, $F(1,12) = 6.62$, $P = 0.024$, $\beta 2 = 0.356$. Unexpectedly, HGS ratings significantly increased from 5 to 20 min for C and T groups, $F(1,12) = 7.89$, $P = 0.016$, $\beta 2 = 0.397$. These findings reveal that student PPR does not increase mare cortisol; perhaps increased social student contact is beneficial.

Research Grant: Auburn University College of Veterinary Medicine, Department of Clinical Sciences startup funds for Dr. Lyman
Student Support: Boehringer Ingelheim Veterinary Scholars program 2022

Role of sigma factor (*btrS*) in innate and adaptive IFNγ responses to *Bordetella pertussis*

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Bordetella pertussis (*Bp*), the causative agent of whooping cough, has evolved complex mechanisms to modulate and avoid the host's immune response, making it both a difficult pathogen to study and to develop a protective vaccine for. One of the immunomodulatory mechanisms that is believed to be critical in the pathogenesis of *Bp* is its ability to survive within macrophages. It has previously been shown that *btrS*, a *Bordetella*-specific sigma factor, plays an essential role in immunomodulation of innate and adaptive responses in *Bordetella bronchiseptica*, a close ancestral relative of *Bp*. The role of *btrS* in *Bordetella pertussis* was therefore investigated in THP-1 human-derived monocytes. Intracellular survival of a *Bp* mutant lacking *btrS* (*Bp*ΔbtrS), inflammasome activation, and cytotoxicity of the mutant were analyzed in comparison to wild type *Bp*. Due to the relationship between IFNγ and the recruitment and activation of macrophages, colonization of both wild type and mutant *Bp* in IFNγ -/- mice was also evaluated. To explore the role of *btrS* in adaptive responses, T-resident memory (TRM) cell populations recovered from convalescent mice were also analyzed via flow cytometry for their expression of IFNγ or IL-17. Conclusions generated from this study will aid in the understanding of the broad role that *btrS* plays in immunomodulation and its relation to IFNγ in *Bp* infections. Full data set and results are pending.

Research Grant: NIH, Grant Numbers AI149787, DC018496, AI156293, AI159347, AI139449
Student Support: NIH Office of Research Infrastructure Programs, Grant Number T35 OD 010433

Resultant vector acceleration to measure time spent performing activities of various intensity in dogs

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The treatment of chronic pain secondary to osteoarthritis (OA) in companion animals is limited by our lack of effective objective measurement tools. Inertial measurement units (IMU) are effective for the objective measurement of daily activity when worn in a home environment. However, IMUs have not been validated for the measurement of chronic pain in dogs nor have open-source algorithms, both of which are requirements for regulatory studies and quality assurance. Our objective is to validate IMU output in a clinical setting for the evaluation of chronic pain in dogs. We utilize acceleration vector magnitude collected from an IMU to compare time spent performing activities of varying intensity in orthopedically "normal" dogs and in dogs with chronic pain secondary to OA. We also compare activity in dogs with chronic pain before and after treatment with an NSAID. An IMU will be placed on 40 normal dogs for 2 weeks, and 40 dogs with OA for 4 weeks. The OA group receives a placebo for 2 weeks followed by an NSAID for 2 weeks. The IMUs were calibrated to report a resting IMU resultant vector acceleration as g = 0.95 to 1.05, a collection frequency of 25Hz with output limits at 8g, and with no additional filters added. Activity intensity is measured by IMU acceleration vector magnitude where g = 1 is no motion. We test the null hypotheses that 1) normal dogs and dogs with chronic pain secondary to OA spend the same amount of time in various activity intensity zones and 2) time spent in various activity intensity zones in dogs with chronic pain will be the same when treated with a placebo and an NSAID. Twenty normal dogs and 3 OA dogs have completed the trial. We do not yet have an analysis of the data that has been collected.

Research Grant: None
Student Support: NIH, Office of the Director, Award Number T35OD011118

Characterization of immunomodulatory property of *Mycobacterium avium* subsp. *paratuberculosis* novel antigens

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MAP (*Mycobacterium avium* subspecies *paratuberculosis*) is a causative bacteria of the chronic and highly contagious enteritis known as Johne's disease. Johne's disease does not currently have a cure, and only available vaccine merely limit the progression of the disease but do not protect animals from infection. The discovery of bacterial antigens that can stimulate the strong protective immune responses in animals will help in the development of effective vaccines. In an investigative study of MAP secreted proteome during bacterial exposure with the bovine intestinal and mammary gland cells and milk, ten MAP proteins were found to be commonly synthesized. Some of these proteins have high similarity to *M. tuberculosis* proteins known to provoke immunostimulatory activity and/or protective innate immunity in phagocytic cells in vitro or in vivo. To investigate if selected MAP proteins are potential antigens, at first, the recombinant proteins were made by cloning selected MAP genes into pET6xHN_C expression vector and purified with His-tag system. Purified MAP proteins were tested for cytotoxicity in RAW264.7 macrophages through titration, and the working concentrations were established. Currently, recombinant proteins are tested for ability to stimulate innate immune responses in macrophages that can attenuate intracellular growth of MAP. In addition, the immunogenicity properties of candidate proteins are investigated to determine if they can stimulate secretion of pro-inflammatory cytokines such as TNFα, IL-1β, IL-6, IL-12, and MCP1, which are relevant to MAP infection. This new knowledge will contribute toward the development of MAP vaccine.

Research Grant: The Agricultural Research Foundation (ARF) and Carlson College of Veterinary Medicine Biomedical Sciences Internal Grant
Student Support: Boehringer Ingelheim and Oregon State University

Analysis of Transcriptional Changes in Peripheral Leukocytes from Dairy Calves with Respiratory Disease

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Bovine Respiratory Disease (BRD) is one of the leading causes of preweaning mortality in dairy calves. Difficulties in managing BRD include diagnosing calves affected with lung pathology. This diagnostic insufficiency results in increased morbidity and mortality of calves and subsequent monetary losses to producers. Transcriptomic analysis of peripheral leukocytes has been utilized to identify genes associated with disease resistance and resiliency. This study followed two cohorts of 30 calves over the course of 11 weeks on two dairies in central WA. Blood samples were taken along with clinical assessments using the Wisconsin clinical respiratory scoring chart (CRSC) as well as thoracic ultrasonography to categorize calves into four populations: heathy, clinical respiratory signs positive (CRSC+), thoracic ultrasound positive (TUS+), or both CRS+ and TUS+. CRSC+ calves were required to have a 2 or greater in CRSC parameters of nasal or cough. TUS+ calves were so defined if they possessed a minimum of one fully consolidated lung lobe. Peripheral leukocytes were extracted using a ficoll paque separation method and subsequentially analyzed by Novogene Corporation. Transcriptome analysis was conducted via the GEMmaker workflow, which orchestrated bulk sample raw reads through quality assessment (FastQC), mapping (Kallisto) , and the construction of a gene expression matrix (GEM). The reads were mapped to the Bos taurus reference genome (ARS-UCD1.3), and the resulting GEM was analyzed using the Bioconductor packages EdgeR and DESeq in R, producing a preliminary list of differentially expressed genes of interest. These genes of interest may indicate biomarkers associated with BRD resiliency or susceptibility.

Research Grant: Research Grant: Agriculture and Food Research Initiative Competitive Grant no. 2019-68008-29897 & 2021-68014-34144 from the USDA National Institute of Food and Agriculture
Student Support: Student Support: WSU CVM Summer Research Fellowship Program

Evaluation of antimicrobial resistance of Pasteurellaceae in the respiratory tract of goats

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Global food security and sustainable agricultural animal production relies heavily on antimicrobial stewardship in food producing species due to a continual increase in antimicrobial resistance. The literature for antimicrobial resistance in caprine respiratory pathogens is limited compared to bovine pathogens. In this study, double guarded nasopharyngeal swabs were collected randomly from goats recently procured from multiple sources across the United States. When available, lung swabs were also collected from animals that died or were euthanized prior to antimicrobial treatment. Streaking for isolation was performed on each swab and evaluated on Sheep Blood agar plates, 5% Bovine Blood agar plates, and *Pasteurella* selective plates followed by aerobic growth at 35°C. Isolates of interest to this study including *Mannheimia haemolytica, Pasteurella multocida* and *Bibersteinia trehalosi* were identified using colony morphology and confirmed via MALDI-TOF mass spectrometry. Antimicrobial susceptibility testing was then performed using standardized broth microdilution plates to determine minimum inhibitory concentration values of commonly used antimicrobial classes. Using clinical breakpoints established for cattle, little resistance to ceftiofur, enrofloxacin, danofloxacin, florfenicol, gamithromycin, spectinomycin, tildipirosin, tilmicosin, tetracycline or tulathromycin was noted for isolates of *M. haemolytica* or *P. multocida*; resistance to ampicillin, tetracycline, and penicillin was noted in at least one isolate of *B. trehalosi*. In conclusion, our results demonstrated minimal antimicrobial resistance present in bacterial pathogens from this population of goats obtained prior to antimicrobial treatment administration.

Research Grant: Foundation for Food and Agriculture Research Vet Fellow
Student Support: Foundation for Food and Agriculture Research Vet Fellow

Diagnosis of incipient condylar stress fracture in Thoroughbred racehorses using virtual mechanical testing

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Condylar stress fractures make up ~25% of catastrophic injury in racing Thoroughbreds, where euthanasia is performed due to injury. Prodromal pathology associated with these fractures consists of an accumulation of subchondral fatigue damage in the parasagittal groove, seen as focal subchondral lysis with diagnostic imaging. Screening for fetlock subchondral bone injury (SBI) is primarily done via digital radiography (DR) but it is prone to false negatives. Currently, there is an unmet need for an approach to detect racehorses with imminent risk of condylar stress fracture, given its high prevalence. Our hypothesis is that concerning fetlock SBI cannot be reliably identified using DR, and that a diagnostic computational pipeline can be developed using standing computed tomography (sCT). We have developed a pipeline by building finite element (FE) models of the distal cannon bone using *ex vivo* sCT scans of limbs from Thoroughbred racehorses that experienced fatal injury. DR images were made to compare imaging specificity for prodromal pathology, compared with Equina ™ sCT. Virtual mechanical testing of the distal cannon bone used patient-specific 3D FE models. Our research will validate the FE model approach before testing its predictive capability clinically with racehorses in training. We anticipate that bones with concerning SBI will exhibit substantial stress concentration and a low failure load in the FE model. indicating a high fracture risk clinically, and that DR has lower accuracy than sCT at detecting concerning fetlock SBI. Our long-term goal is to reduce the incidence of catastrophic injury in Thoroughbred racehorses by fetlock screening using sCT which can be performed without disruption to training.

Research Grant: Grayson-Jockey Club Research Foundation
Student Support: National Institutes of Health (NIH) T35 Training Grant OD011078-12

Association of reproductive acyclicity and insulin resistance in female African elephants at the Cleveland Zoo

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Unless trends in reproduction of African elephants are reversed immediately, the North American population of African elephants will be reproductively dead within a few decades. More importantly, poor reproduction in captive African elephants may represent an early warning sign of a significant systemic health threat. Up to 29% of female African elephants in captivity have abnormal reproductive cycles. Ovarian inactivity is a significant reproductive problem for African elephants housed in zoos and cystic ovaries have been identified in many captive African elephant females. Many of the acyclic females are hyperprolactinemic. In human females polycystic ovarian disease and hyperprolactinemia are associated with insulin resistance and reduced glucose tolerance. These clinical findings in human females represent warning signs of a systemic health threat, including an increased risk of type 2 diabetes and cardiovascular events.There is a critical need to determine whether a similar systemic health threat exists in acyclic African elephants. The objective of this pilot study is to determine whether the reproductive acyclicity seen in CMZ African elephants is associated with insulin resistance, as measured by serum insulin and insulin to glucose ratio. Information gained through this study will be used to improve the reproductive and overall health of captive African elephants.

Research Grant: Research Grant: NIH T35 OD010977
Student Support: None

Gastrointestinal release site for delayed release and gelatin capsules in healthy dogs and cats

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Clinical use of fecal microbiota transplantation (FMT) is expanding in veterinary medicine. FMT is the delivery of fecal material from a healthy donor into a diseased recipient with the goal of altering the gut ecosystem to confer a health benefit. FMT is routinely delivered as a slurry via enema but can also be encapsulated for convenient oral administration. Traditionally, in veterinary medicine capsular FMT is uncommon and if performed clinicians have utilized gelatin capsules. However, this likely results in delivery of fecal material into the stomach, due to the rapid dissolution of gelatin capsules in the low pH environment in the stomach, instead of the desired location in the small intestines. Delayed release capsules (Lonza; DRCaps) are an alternative to gelatin capsules (GelCaps), which are designed to resist pH dependent dissolution which could allow for release of the FMT in the small intestine. The utility of using DRCaps for FMT in veterinary medicine remains unexplored. This randomized, blinded crossover clinical trial aims to determine the effectiveness of DRCaps compared to GelCaps to deliver contents into the optimal location in the small intestines. Healthy dogs and cats (n = 6 each) undergo baseline abdominal radiographs and then are given DRCaps or GelCaps containing barium impregnated polyethylene spheres (BIPS: ten 5-mm and thirty 1.5-mm spheres) as single oral dose. Immediately after serial radiographs are preformed to track dissolution of capsules, and location of BIP release is noted. Animals undergo a 7-day washout prior to crossover. This ongoing study will be the first to provide evidence-based recommendations for utilizing DRCaps and/or GelCaps for capsular FMT delivery in dogs and cats.

Research Grant: Research Grant: Discretionary start-up funds provided by the Ohio State University College of Veterinary Medicine
Student Support: Student Support: NIH T35 OD010977

Campylobacter Prevalence and Antimicrobial Resistance in Animal Shelters

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Campylobacter is a zoonotic pathogen that can easily spread to susceptible individuals, including children. *Campylobacter* is one of the most common causes of bacterial enteritis in humans and is associated with direct animal contact. The prevalence of animal shelter dogs shedding *Campylobacter* was previously estimated between 50-73%. Potential adopters should be aware of the risks of *Campylobacter* and the symptoms associated. The purpose of this study was to determine asymptomatic shedding of *Campylobacter* by dogs in animal shelters and the risk of zoonotic disease transmission to new pet owners. Shelter dogs in central Ohio were screened for the presence of *Campylobacter* in fresh feces. At each shelter twenty fecal samples were collected from the ground. One shelter was sampled weekly and other shelters were sampled cross-sectionally throughout the summer. For *Campylobacter* culture, each 1g fecal sample was mixed with 9 mL of supplemented Bolton broth and incubated at 42°C in microaerophilic conditions. After 24 hours, samples were plated on CVA and Campy-Cefex and again incubated overnight. Isolated colonies were grown on TSA blood agar and speciated using MALDI-TOF. A total of 108 samples have been collected to date, with two samples (2%) positive for *Campylobacter* spp. Additional sampling throughout the summer is expected to provide data to better estimate the risk of *Campylobacter* shedding in shelter dogs, and the risk of zoonotic transmission to new pet owners.

Research Grant: Residual research funds
Student Support: NIH T35 OD010977

Seasonal and Sexual Differences in Liver Histomorphology of Fish Exposed to Hg and Se

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Mercury (Hg) is a metal contaminant that is found globally throughout our environment. When deposited in aquatic systems, Hg methylation is facilitated primarily by sulfate-reducing bacteria under anoxic conditions, warm water temperatures, and low pH (Fuhrmann et al 2021). The methylated form of Hg is the toxic form to both fish and those consuming them. The amount of Hg found in fish can vary by size, season, and the watershed environment. An essential nutrient, Selenium, plays a role in redox reactions and physiological functions within the body and may relate to the effects of Hg on aquatic species but its role in protecting against Hg toxicity is under debate. This study will document potential effects that Hg and Se have on different aspects of the fish systemically. Data will support the use of hepatosomatic index (HSI) as a biomarker of hepatotoxic effects and identify confounding factors that should be considered when interpreting relationship between contaminant concentrations and HSI. The study used a collection of 31 largemouth bass liver samples were collected, fixed with H&E and PAS staining. By observing cellular changes of the liver, we identify morphometric characteristics of the nutritional and reproductive status of fish and differences in potential toxicity among sexes. The study is underway and there are pending results. It is expected that changes in liver histomorphology will relate to HSI but will differ depending on sex, season, and metal concentrations of fish. Inflammatory indicators and oxidative stress in the samples will be positively related to HSI and reduced Se concentration, while indicators of necrosis and reduced glycogen content will be negatively correlated to the Se:Hg molar ratio.

Research Grant: Funds for this work were provided by Missouri Department of Conservation
Student Support: Stipend for Ashley Sturgeon is supported by an endowment established by IDEXX-BioAnalytics

Biomechanical Comparison of Three Tibial Tuberosity Transposition Stabilization Methods *Ex Vivo*

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Medial patellar luxation (MPL) is a common pathology among canines that can cause lameness and discomfort to affected patients. Tibial tuberosity transposition (TTT) procedures used to surgically correct MPL currently have a 18-43% rate of postoperative complications, and many of these complications can be attributed to a pin or screw being placed through the tuberosity. A newly proposed TTT technique involves the use of a partial osteotomy with a spacer pin to avoid the placement of a pin through the tuberosity. This cadaveric study compared the biomechanical outcomes of a complete osteotomy with a 2 pin and tension band wire fixation (group 1), a partial osteotomy with a 2 pin fixation (group 2), and a partial osteotomy with a spacer pin fixation (group 3). There were no significant differences between groups in respect to stiffness or maximum failure force. Group 1 failed via patellar ligament failure, tension band wire untwisting, and tibial fracture. Group 2 failed via patellar ligament failure and tuberosity segment fracture, while group 3 failed via patellar ligament failure, tuberosity segment fracture, and tibial fracture. As the spacer pin fixation held similar loads to the complete osteotomy with 2 pin and tension band wire fixation and partial osteotomy with 2 pin fixation groups, our study suggests that pins inserted through the tuberosity segment are not necessary during TTT procedures. By utilizing this technique, the complications associated with inserting a pin through the tuberosity segment can be avoided. This cadaveric study described a new technique for the stabilization of TTT and demonstrated that the technique can withstand forces similar to commonly performed stabilization methods.

Research Grant: None
Student Support: None

Search for the genetic cause of Norwich terrier upper airway syndrome

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Purpose: Norwich terrier upper airway syndrome (NTUAS) is an inherited syndrome consisting of anatomic abnormalities that obstruct airflow through the pharynx, larynx and infraglottic opening. The condition appears unique to Norwich terriers and varies in severity. We hypothesize that there is a single, major genetic variant necessary for NTUAS. Methods: A genome sequencing-based approach was used to search for variants of interest. The whole genomes of 15 Norwich terriers, 4 controls and 11 affected, were sequenced to a 30x depth of coverage. Sequences were aligned to the canine reference genome CanFam6.Variants unique to the affected dogs were further filtered using haplotype-based variant detection software to identify variant types and effects. Variants of interest were defined by presence in the coding region of genes predicted to have effects on collagen structure and formation within the upper respiratory system. Selected variants will be genotyped (PCR amplification and Sanger sequencing) to investigate segregation in an additional 93 NTUAS cases. Results: Over 4.5 million variants from CanFam6 were identified per sample, and filtered down to 351 variants that differed between the controls and affected. Of these 351 variants, some variants of interest have been identified within the coding regions of potential candidate loci. These are being genotyped in NTUAS cases outside of the initial sample. Data mining of the whole genome sequencing data is also continuing. Conclusions: While identified variants of interest show promise due to their relation to the systems affected by NTUAS, more analysis is still required to identify the genetic cause.

Research Grant: Myers Dunlap Endowed Research Fund
Student Support: NIH Grant 5T35OD016477-20 to Michigan State University

Effect of Zylkene supplementation on stress-associated findings in cats

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Visits to the veterinary clinic are extremely stressful experiences for cats. Methods to decrease stress in cats primarily involve the usage of pheromones and/or medications such as gabapentin. For short-term stress management in cats, use of a natural substance with stress-relieving properties could be preferred over drug usage. The objective of this pilot study was to evaluate if a nutraceutical containing the natural stress-relieving substance alpha-casozepine (AC; Zylkene; Vetoquinol) could alter physical parameters including mean arterial blood pressure, heart rate, respiratory rate, body temperature, select behavioral stress scores, and serum cortisol concentrations. In this study, twelve healthy cats were randomly assigned to two equal groups. Once daily for five days, the cats in the experimental and control groups were fed canned food with or without the AC product, respectively. Twelve hours following the final feeding, the cats were taken in groups of three to measure routine bloodwork and physical parameters following a 12-minute drive to the veterinary clinic and again on return to the research facility. Cats fed the AC product were more likely to enter the transport carriers without struggle (6 of 6 cats) than the control cats (3 of 6 cats), but the difference was not significant. The mean serum cortisol concentrations for the cats fed AC were numerically lower than for the control cats, but the difference was not significant. The mean arterial blood pressure on arrival to the veterinary clinic was statistically higher (p-value 0.0204) for the cats supplemented AC. After a washout period, the groups will be alternated and the measurements repeated to increase the statistical power.

Research Grant: Vetoquinol
Student Support: Center for Companion Animal Studies

Investigation of the avian oral microbiome

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Understanding the microbiome of peridomestic species is important to evaluate the risk of zoonotic disease transmission. The oral microbiome can differ between life stages and can be disrupted by oral and respiratory infections. To assess the natural range of variation in the oral microbiome of the House Sparrow (*Passer domesticus*), we tested for sex, age, and body condition-related differences. Wild House Sparrows (n = 60) were caught in a mist net to swab the oral cavity and measure body parameters. Next generation sequencing and microbial species identification were performed by MiDog by targeting the 16SrRNA bacterial gene and ITS-2 region for fungal DNA analysis. If differences between conditions are found, it will be essential for future studies to include variations in sex, age, and body condition to fully understand the oral microbiome. House Sparrows are a successful invasive species in North America, and across much of the globe outside of their native range. The close proximity of House Sparrow populations to human populations creates a potential for zoonotic disease transmission and the transfer of antibiotic resistance genes.

Research Grant: Payne County Audubon Society Research Grant, Department of Integrative Biology Oklahoma State University Waters Grant-in-Aid of Research
Student Support: Oklahoma State University College of Veterinary Medicine

The future of food: the use of wild-caught mosquitoes as a quality feed protein

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Interest has increased for the use of insects as a high-quality protein source in livestock production. At present, insect protein is used in feedstuff from farm-raised insects such as black soldier fly larvae, crickets, and mealworms. But what if there was a way to harvest wild-caught insect pests from natural environments to convert into feed protein? This would not only serve to provide a sustainable protein alternative but also as a pest control tactic where pest populations are abundant. However, using wild insects as a food source raises concern for potential pathogen transmission to animals, and in turn, presents zoonotic risks. To address these concerns, samples were treated with low-intensity drying (79.4C for 6 minutes) or high-intensity heat (204C for 45 seconds) to quantify surface and gut microbial disinfection methods. Whole mosquitoes were used as the control treatment. Disinfectant treatments were plated and incubated to determine residual bacterial presence and microbial growth was observed on plates inoculated with whole mosquito (6/12), drying (8/12), and heat treatment (0/12). Shotgun sequencing plates prior to plating will allow broad-spectrum pathogen screening of wild mosquitoes and the use of and Sanger sequencing of plated colonies will help identify specific bacterial risks present in each treatment. This strategy provides the framework for additional research to optimize collection methods, further pathogen detection, and nutritional analysis of dried and heat-treated samples for industrial consideration.

Research Grant: USDA ARSX
Student Support: USDA ARS FABADRU AGREEMENT NO: 3022-32000-024-00D

Determining the relationship between cell size and tracheole density in *Drosophila melanogaster*

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Cells have a wide variety of physical characteristics and physiologies, but all rely on diffusion to maintain function. Therefore, smaller cells should be able to have higher relative diffusive rates due to their larger surface area-to-volume ratio. This means an organism with smaller cells should be able to produce energy more efficiently. Research on this hypothesis, however, remains sparse. Current evidence suggests that development of *Drosophila melanogaster* in hypoxic conditions increases the density of tracheoles, but it is unknown if these changes are related to variation in cell size. Therefore, the adaptive significance of cell size remains unclear. Here, we investigated the relationship between oxygen delivery and cell size in four populations of flies that had their cell sizes modified by varying methods: (i) genetic modification via CRISPR/Cas, (ii) rapamycin-induced alterations to molecular pathways that control cell size, (iii) development under three selective thermal regimes, or (iv) development under controlled temperature and oxygen conditions. In all groups of flies, flight muscle cell sizes were measured histologically, while the tracheoles were imaged using confocal microscopy combined with Amira software to determine tracheole density in muscle tissue. Overall, there was a significant difference in tracheole density between the four treatment groups (one-way ANOVA; $F = 6.338$; $P = 0.0005$). This relationship suggests an adaptive advantage of variation in cell size dependent on oxygen delivery and thermal regime.

Research Grant: Polish National Science Foundation Award (Nardowe Centrum Nauki)
Student Support: Boehringer Ingelheim Veterinary Scholars Program and Federal Work Study

Coculture of nontoxigenic *Clostridium difficile* RT416 and toxigenic RT078 and effects of p-cresol on RT416

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Clostridium Difficile (*C. difficile*) is a pathogenic spore-forming anaerobic bacterium which colonizes the intestinal tract of humans and other animals. *C. difficile* infection (CDI), characterized by symptoms such as diarrhea, inflammation, as well as tissue necrosis, can be potentially lethal in elderly individuals and those with gut dysbiosis, many times caused by antimicrobial treatments. *C. difficile* virulence ability has been determined to be mediated by secretion of toxins classified as toxin A (TcdA), toxin B (TcdB), and the binary toxin CDT, depending on the strain. This study focuses on ribotyptes (RT) 416 (non-toxigenic) and 078 (toxigenic). Apart from the inability to produce clostridial toxins, previous data has also shown that RT416 is unable to produce para-cresol (p-cresol), a secreted molecule thought to aid in colonization by inhibiting growth of other bacteria in the intestinal tract. The aim of this study is to analyze the growth patterns of the two mentioned *C. difficile* RTs separately as well as 1:1 ratio of the two RTs. A comparison of the same analysis will also be conducted under the influence of p-cresol. Preliminary data suggests possible upper hand of RT416 over RT078 based on colony numbers and growth curves displaying a pattern more similar to RT416 while analyzing the 1:1 ratio. To confirm these results, we intend to perform multiple co-cultures and growth curves as well as performing toxin ELISAs for different timepoints at which toxin is known to be produced. P-cresol will also be used to simulate in vivo conditions and make sure there is cell viability in the prescence of p-cresol production from other RTs.

Research Grant: NIH R01 GM6685
Student Support: NIH T35 Training Grant T335OD012199

Evaluating the impact of shared decision making on pet-owner decisions

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Objective – To evaluate what impact shared decision making (SDM) during small animal wellness visits has on pet owner decisions and how it can be improved.
Design – Qualitative study of visit footage in a university small animal primary care setting.
Participants – 37 pet-owning clients and 8 small animal primary care veterinarians.
Procedures – Thirty-seven small animal wellness visits seen at Mississippi State University College of Veterinary Medicine's Primary Care service containing decisions for non-core vaccinations and fecal flotations were audio- and video-recorded. Each decision was categorized depending on the decision type (fecal floatation or non-core vaccination) and decision outcome. The recordings of the conversation leading up to each decision were then individually coded using a veterinary-adapted Observer OPTION5 instrument to evaluate the level of shared decision making.
Results – The mean Observer OPTION5 score for non-core vaccination and fecal float decisions was 1.85 (0-20), indicating the use of very little SDM, and was lower than previous studies. The mean Observer OPTION5 score was higher for non-core vaccine decisions than fecal float decisions indicating more SDM surrounding non-core vaccination decisions. There was no statistically significant relationship between SDM level and decision outcome.
Conclusions – The results indicated that there is significant room for increased SDM for fecal floatation and non-core vaccinations during wellness visits. This is especially evident in the fecal floatation Observer OPTION5 scores. SDM is an important communication method that is underutilized in small animal wellness visits.

Research Grant: None
Student Support: Boehringer-Ingelheim

Uncovering mechanisms of miR-375-mediated control of host resistance to helminth infection

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Soil-transmitted intestinal helminths pose a serious threat to human health worldwide. The intestinal epithelial layer serves as a barrier between the host and the helminth. MicroRNAs (miRNAs) are small regulatory molecules that play a crucial role in proper intestinal mucosal immunity and epithelial cell differentiation. However, it is not known whether miRNAs are involved in regulating gut response to helminth infections. Two important intestinal epithelial cell types that play an essential role during host response to helminth infections are goblet and tuft. Like other intestinal epithelial cells, goblet and tuft cells originate from intestinal stem cells (ISCs). Based on our previous miRNA profiling studies, miR-375 is highly enriched in ISCs. Moreover, miR-375 is predicted to target key drivers of the goblet and tuft lineages. We hypothesized that miR-375 modifies host response to helminth infection in part by regulating the allocation and/or function of tuft/goblet cell lineages. To test this hypothesis, whole-body miR-375 knockout (375 KO) mice were inoculated with *Heligmosomoides polygyrus*, a parasitic helminth of the mice. Small intestinal tissue was obtained 14 day post-infection and worm burden analysis along with single-cell RNA-seq was performed. The results of the study indicated a significant reduction in worm burden in the 375 KO mice relative to WT. In ongoing histologic and immunofluorescence studies, we are assessing whether 375 KO mice exhibit increased numbers of goblet or tuft cells post-infection. Overall, the findings demonstrate for the first time that miRNAs can play a regulatory role in the gut response to helminth infection.

Research Grant: R21HD104922 (awarded to PS)
Student Support: NIH T35 OD010941 and the Cornell University College of Veterinary Medicine

Chronic social defeat stress in group housed swine: effect on behavior and immunity

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The chronic social defeat stress (CSDS) model of major depressive disorder (MDD) developed in rodents has contributed greatly to our understanding of the role of chronic stress in psychological well-being, but it has seldom been applied to production animals. In this study the CSDS model was adapted to breeding swine, and its effects on behavior and immunity were evaluated. In production settings, mother sows are increasingly housed in large groups that allow for aggressive interactions. These agonistic behaviors help establish a strict social hierarchy and likely create chronic social stress for the low ranking animals. In this study, a total of 16 naive gilts (stressed "S" group) were introduced to an existing pen housing approximately 60 pigs. The not stressed "NS" group was identified based on their daily feed order as 16 high ranking animals already in the pen. Anxious behaviors and anhedonia (the inability to experience pleasure) were compared between the two groups over a 4 week period. A sucrose preference test was used to evaluate anhedonia based on the animals' sucrose solution consumption relative to water. Anxious behaviors were examined with a combined open field/novel object test that assessed mobility in the open field, latency to interact with, and total duration of contact with the novel object. The severity of skin lesions were also assessed pre- and post-introduction. Saliva samples were collected from all animals at the end of the study to measure IgA concentrations. Applying the CSDS model to pigs is a novel approach that has the potential to be a valuable tool for assessing animal welfare as well as furthering our understanding of MDD beyond what has been learned from the current rodent model.

Research Grant: NIH T35 OD010919
Student Support: University of Pennsylvania NIH/Boehringer Ingelheim Summer Research Program

Validating an anti-CD80 antibody as a marker for monocytes and neutrophils in the dog

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The B7 family molecules play a key role in the regulation of the immune system and prevention of autoimmunity. CD80, also known as B7.1, is a costimulatory molecule expressed on the surface of antigen-presenting cells, such as monocytes and macrophages, during pathological processes in humans. There is a need for new myeloid markers that can differentiate canine hematopoietic neoplasms on flow cytometry. We hypothesise that CD80 may be a useful diagnostic marker for monocytic variants of acute myeloid leukaemia in dogs. In this study, our aim was to characterise the expression of CD80 antigen on leukocytes of healthy dogs to better inform the research of CD80 expression in hematopoietic malignancies. Peripheral blood mononuclear cells were isolated from venous blood of healthy dogs and double-labelled with antibodies against CD80 and the monocyte marker, CD14. Cells were sorted based on positive or negative antibody reactions using a flow cytometric sorter. Cytological analysis was then performed on each sorted population. Blood monocytes, T cells and B cells were also isolated with immunomagnetic beads, using antibodies against CD14, CD5 or CD21, respectively, and then labelled with the anti-CD80 antibody. Flow cytometric and cytological analysis were performed on the isolated populations. We found that CD80 was expressed on monocytes and neutrophils, but not on lymphocytes and eosinophils, in the blood of healthy dogs. In ongoing studies, we are evaluating CD80 expression on tumour cells in dogs with acute leukaemia and lymphoma to explore its potential as a diagnostic marker for myeloid cells.

Research Grant: This study was funded by a Cornell Canine Health Centre and an American Kennel club Canine Health Foundation grant (#02987)
Student Support: Cornell University College of Veterinary Medicine

Epidemiology of canine B cell leukemia and lymphoma

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Lymphoproliferative disorders affect many dogs across the U.S., but the frequency, clinical signs, and breeds associated with various subtypes are not well characterized. This study aims to identify genetic predispositions and other traits associated with various B cell neoplasms in the blood and lymph nodes. Data on 22,201 B cell neoplasms were obtained from Colorado State University's Clinical Hematopathology database from 1/1/2015 to 5/1/2022. Odds ratios comparing breed-specific risk for each B cell subtype were determined using population data from Banfield Pet Hospital (n = 9,928,122 unique dogs) and the Dog Aging Project (n = 33,172 unique dogs) and data from 16,857 nodal large cell B cell lymphoma, 1,959 nodal small cell B cell lymphoma, and 3,385 small cell B cell leukemia cases were analyzed. Compared to mixed breed dogs, the odds ratios suggest breed trends associated with the different subtypes. Large breed dogs have greater odds of developing nodal large B cell lymphoma and many small breed dogs have decreased odds. Conversely, many small breed dogs have greater odds of developing small cell B cell leukemia while large breed dogs have decreased odds. A mix of both large and small breed dogs were identified to have greater odds of developing nodal small cell B cell lymphoma. Researchers and practitioners can use this information to better recognize and diagnose these disorders in their canine patients, and to identify breed-specific genetic risk factors for different types of B cell neoplasms.

Research Grant: None
Student Support: Boehringer-Ingleheim

Paradoxical increase in inhibitory GABAergic synaptic input found in CA sea lions with temporal lobe epilepsy

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The global rise in harmful algal blooms, notably those of the domoic acid (DA)-producing *Pseudo nitzschia* spp., is causing an increase in strandings and mortality in California sea lions. DA, a neuroexcitatory toxin acting as a glutamate receptor agonist, bioaccumulates in the diet of sea lions resulting in neuron loss (sclerosis) in the hippocampus similar to human patients with temporal lobe epilepsy. We hypothesized that temporal lobe epilepsy in CA sea lions is caused by a loss of inhibitory GABAergic synaptic input to cells in the dentate gyrus of the hippocampus. Hippocampi from 7 sea lions were sectioned then stained with two antibodies tagging GABAergic synaptic boutons: vesicular GABA transporter (VGAT) responsible for packing GABA into synaptic vesicles and glutamic acid decarboxylase (GAD), the enzyme that synthesizes GABA from glutamate. Stereology was used to estimate the number of granule cells and GABAergic terminal boutons in the dentate gyrus. In controls, the dentate gyrus contained 530 $\pm$ 50 million GABAergic boutons. Consistent with the hypothesis, the number of boutons in the sclerotic group decreased by 41% (P = 0.004, t test). A reduction was observed in all layers of the dentate gyrus. However, epileptic sea lions display substantial granule cell loss so the number of boutons per granule cell was calculated for all hippocampi to account for this loss. In controls, the median was 194 (25-75%: 168-267) GABAergic boutons per granule cell. Surprisingly, the relative number of boutons per granule cell was 4.7 times higher in the sclerotic group (P = 0.002, Mann-Whitney rank sum test). These findings suggest a mechanism other than decreased GABAergic terminals causes temporal lobe epilepsy in sea lions.

Research Grant: NSF ES021960, NIH NS107290
Student Support: T35 OD010989

Using gemcitabine-loaded thermosensitive liposomes to treat pancreatic ductal adenocarcinomas

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Pancreatic ductal adenocarcinoma (PDA) is a highly lethal malignancy with poor prognosis attributed to late detection and limited success of systemic therapy for those who do not qualify for resection. Gemcitabine is an anticancer drug used to treat a variety of metastatic diseases including PDA. Gemcitabine alone has a one-year survival rate of 17-23% and produces general myelosuppression. This study will characterize the efficiency and release of encapsulated gemcitabine via triggering the liposomal phase transition temperature (Tm) with various heat modalities. Future integration of liposomal chemotherapy and radiofrequency ablation into cancer treatment plans has the potential to reduce systemic effects by initiating targeted release of the anti-cancer agent in the tumor vasculature. Liposomes synthesized with a Tm of 41°C are remotely loaded with gemcitabine via an ion gradient created with ammonium sulfate. Gemcitabine release is characterized via Uv/vis spectroscopy and relative concentration is measured. Gemcitabine-loaded liposomes are applied in a 100mM concentration to two pancreatic cell lines; BXPC-3, a human model, and KPC, a mouse model. Each cell line is subjected to rapid heating in a water bath reaching temperatures of 37°C, 39°C, 41°C, 43°C, and 49°C and maintaining each temperature for 10, 20, 30, and 60 minutes. MTT reagents are added 24 and 48 hours post heat treatment. The formazan absorbance is spectrophotometrically quantified using a wavelength of 550 nm. The increase in absorption directly correlates to metabolic activity in the sample thus demonstrating viability. Data collection is currently in progress.

Research Grant: SUCCESS FYI Kansas State University College of Veterinary Medicine
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Role adjustments and identity norms affiliated with interprofessional collaboration across DVM and MD students

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Interprofessional collaboration is integral to tackle the latest challenges in public health. The readiness of professionals for collaboration is rooted in programs of study that shape orientations to such work and socialize students into their professional roles. Previous work has demonstrated differences among medical (MD), veterinary (DVM), and dual degree Master of Public Health (MPH) students in aspects related to identity, group work, and role expectations. However, little is known about how role adjustments and identities, which are key factors in facilitating each of these known differences, foster collaboration among these groups. This project describes readiness for and adjustments within interprofessional learning across DVM and MD students to better understand those identity norms that are influential on readiness for interprofessional collaboration. DVM and MD students at medical institutions at the University of Missouri will be invited to take the Readiness for Interprofessional Learning Scale (RIPLS) instrument and participate in supplemental interviews to develop insight around deliberations affiliated with the practice of collaborating with other professionals. We expect a deeper understanding of differences in readiness for collaboration among DVM and MD students to positively impact educational programs and thus future interprofessional collaborations.

Research Grant: None
Student Support: Stipend for Jessica Topka is supported by an endowment established by IDEXX-BioAnalytics

Changes in serum haptoglobin concentration in cattle administered LPS

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Haptoglobin (HPT) is an acute phase protein that is produced in the liver. Lipopolysaccharide (LPS) is a component of the cell wall in gram-negative bacteria. Also known as endotoxin, it binds to toll-like receptor 4 (TLR-4) and CD14 on immune cells to initiate an inflammatory response. Lipopolysaccharide can therefore be used to model an inflammatory response similar to when cattle are infected with a complex of viral and bacterial pathogens as found in bovine respiratory disease complex. Previous studies in our laboratory indicated that HPT remained elevated in blood post-administration of LPS, even after clinical signs reverted to normal. Therefore, we designed a study to follow serum concentrations of HPT in LPS-treated cattle over the course of 4 weeks. This study will determine the time required for HPT to return to baseline concentrations post LPS administration. Thirty cattle will be included in our experiment. Fifteen will be given an intravenous injection of LPS (0.25 mg/kg in 1-3 ml saline) and 15 Control animals will receive saline only. Cattle will be observed over the course of 28 days. The data collected will include clinical signs, rectal temperature, respiration rates and HPT concentration measured in serum collected from whole blood. These measurements will be taken prior to LPS treatment (D0) and 24 hours post-treatment (D1) as well as once weekly for 4 weeks (D7, D14, D21, D28). We hypothesize that serum HPT concentration will return to baseline concentration between 7 to 14 days. Results will be presented.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Behavioural habituation following repeated stimulation of dorsal raphe serotonin neurons

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Serotonin (5-HT) is a neuromodulator involved in a vast array of behaviours and emotions, with first-line therapeutic interventions for depression and anxiety targeting the serotonin system. Despite the widespread use of selective serotonin reuptake inhibitors (SSRIs) as antidepressants, the function and mechanism of action of 5-HT still remain incompletely understood. The acute response to SSRI administration can manifest as an initial increase in anxiety, with a shift to emotional stabilisation following long-term exposure. One prominent side-effect of chronic SSRIs is emotional blunting, which can prompt medication discontinuation. Pharmacological approaches have been used in the past to study the functional role of 5-HT at the timescale of minutes to hours. However, more recent studies have shown that brief optical stimulation of 5-HT neurons can induce emotional behaviours on the timescale of seconds. We hypothesize that 5-HT stimulation may become less effective in inducing emotional behaviours over time as stimulation is repeated and becomes chronic. To test this hypothesis, we used optogenetics to stimulate 5-HT neurons in the dorsal raphe nucleus (DRN) in mice and measured the associated locomotor response in an open field environment. Prior studies have demonstrated the link between 5-HT and behavioural inhibition, with acute short-term 5-HT stimulation in low-threat environments resulting in a freezing behaviour. Stimulation was repeated daily over several weeks to observe the long-term effects of repeated 5-HT stimulation. The results of this experiment have important implications for understanding the link between serotonin and behaviour and suggest a possible mechanism for SSRI-induced emotional blunting.

Research Grant: Cornell University
Student Support: Cornell University Veterinary Leadership Program/Bostwick Family Foundation

Infectious diseases of farmed deer *(Odocoileus virginianus)*

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Captive white-tailed deer (*Odocoileus virginianus*) farms face several health challenges including fawn diarrhea and more recently, possible infection with SARS-CoV-2. The purpose of this study was to screen fecal samples for deer parasites and blood samples for SARS-CoV-2 antibodies from a deer farm in Kentucky. Centrifugal fecal flotation procedures were used to identify deer gastrointestinal parasites. Strongyle-type eggs were identified in two out of sixteen fawn samples (12.5%, 95% CI: 1.6%-38.4%). One adult deer with diarrhea was positive for both *Eimeria* sp. oocysts and Strongyle-type eggs. A serum neutralization assay was used to test for SARS-CoV-2 antibodies at Cornell University Animal Health Diagnostic Center. Two related fawns were positive for antibodies out of eighteen total fawns tested (11.1%, 95% CI: 1.4%- 34.7%). Results show a low prevalence of parasitic disease on this farm, suggesting that fawn diarrhea could stem from bacterial infections, such as *Escherichia coli* or *Clostridium* sp., or by stress associated with handling and husbandry. Antibodies for SARS-CoV-2 were detected in only two fawns so far in this study, which is low compared to other published studies; however, additional samples have been collected with results pending. Future goals of this study include SARS-CoV-2 PCR testing to identify active infections and resampling positive deer to track the influence of time on SARS-CoV-2 infections. A limitation of the study was that feces and blood from fawns and adult deer were subject to selection bias. With continued sampling on this farm, and additional farms in Kentucky, we hope to establish a truer prevalence of gastrointestinal parasitism and SARS-CoV-2 within captive deer farms.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Evaluation of *Pasteurella multocida* antibody titers in free-range brown laying hens

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Pasteurella multocida (*PM*) vaccines are a valuable disease prevention tool for the organic, free-range layer industry due to restricted antibiotic use. Industry standard is to vaccinate at 8 and 12 weeks of age for *PM* with expectation that protection lasts the duration of a hen's life at 75 weeks. However, *PM* remains a concern in late production among vaccinated hens. It is unclear what this is due to since antibody baselines for *PM* are rarely established. We hypothesized that antibodies would decrease with age and be unprotected at 65 weeks of age. In this cross-sectional study, antibody titers for 1140 commercially owned free range Bovan and Hyline brown laying hens were measured using an IDEXX *PM* enzyme-linked immunosorbent assay (ELISA). Hens were vaccinated at 8 and 12 weeks of age with one of two prime-boost protocols. One group was primed with a commercial killed *PM* vaccine (Avipro108 FC3 Platinum) and boosted with a modified live *PM* vaccine (Poulvac CholeraPM-1). The other group was primed with a modified live *PM* (Poulvac CholeraPM-1) and boosted with killed *PM* autogenous vaccine (Ceva). Age points for sampling in flocks occurred at 15, 40, 45, 50, 55, 60, 65 or 70 weeks of age and was done by collecting 1.5 mL of blood from 30 hens per flock. In total, 20 flocks; 5 at each age point were sampled. Using a SPECTROMAXx the average optical density of the samples that have been run is 0.163 absorbance with a range of 0.064 to 1.08 absorbance. Determination of antibody levels, coefficient of variation of each age group is forthcoming. This study will help vaccination strategies for *PM* and an understanding of antibody levels at different ages allowing for insight where minimum protection occurs during late production.

Research Grant: Kalmbach Feeds and Department of Veterinary Preventive Medicine Internal Funds
Student Support: NIH T35 OD010977

Surveying sialic acid receptors to understand the cell and species tropism of canine influenza virus

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Canine influenza virus (CIV) can cause severe diseases in dogs, caused by frequent outbreaks including in the USA in 2021. As influenza viruses frequently cross species barriers, this project is meant to examine the diversity of sialic acid (Sia) receptors across species that may impact canine influenza viruses. The influenza hemagglutinin (HA) is responsible for attaching to Sia and entering the cell during an infection. Different species express different Sia, most frequently in the form of N-acetylneuraminic acid (Neu5Ac). Canines (and humans) lack of the enzyme cytidine monophospho-N-acetylneuraminic acid hydroxylase (CMAH), so that they can only express the Neu5Ac form. Whereas many other species express CMAH and make N-glycolylneuraminic acid (Neu5Gc), including laboratory mice. To determine the differential display of Neu5Ac or Neu5Gc, or other Sia forms, we are using a variety of molecular probes that bind Sias. These probes often derive from viral proteins of influenza viruses and coronaviruses. We are using these probes to screen cell culture cells of varied animals, as well as tissue sections to determine the display and distribution of the Sia receptors, with particular interest in respiratory tissue and salivary glands. A novel aspect of this project is the generation of Neu5Gc-binding probes derived from influenza HA forms. These are expressed in human embryonic kidney 293 (HEK 293) cells as HA trimers with a fluorescent tag. Our goal is to map Sia receptor variation in dogs and other species to inform how influenza crosses host barriers and causes disease with specific tissue tropism.

Research Grant: Supported by CRIP (Center of Research in Influenza Pathogenesis), an NIAID funded Center of Excellence in Influenza Research and Surveillance (CEIRS) contract HHSN272201400008C to Colin Parrish
Student Support: Leadership Program for Veterinary Students at Cornell University / Canine Health Center

Modulation of immune cell recruitment by CT226 during *Chlamydia trachomatis* infection

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Chlamydia trachomatis, an obligate intracellular bacterial pathogen, is the most common cause of preventable blindness and sexually transmitted bacterial infections. Chlamydial infections can lead to chronic conditions, such as pelvic inflammatory disease, tubal infertility, ectopic pregnancy, and HPV-associated cervical cancer even after treatment. Despite ongoing research there are still major gaps in understanding immune cell recruitment during Chlamydial infections. Recent studies and work in our lab, have shown that a Chlamydial protein CT226 interacts with key host signaling pathways that regulate the inflammasome. Using the murine cervicovaginal model, we plan to characterize immune cell recruitment to reproductive tracts infected with *Chlamydia trachomatis* L2 wildtype and L2-ΔCT226 (uninfected tracts, negative control). C3H/HeJ mice (n = 18 per group x 3 groups) were treated -7 and -3 days to infection with 2.5mg of medroxyprogesterone to synchronize estrous. Each mouse was then intravaginally infected with 1x106 EBs of their respective Chlamydial strain. On days 3, 7, and 21 reproductive tracts were harvested, digested manually and with collagenase, and then cells were assessed by flow cytometry to characterize cells present during infection in the reproductive tract. No results have been collected at this time.

Research Grant: National Institutes of Health, R15, 1R15AI149439-03
Student Support: Oklahoma State University Summer Research Training Program

Pharmacokinetics and pharmacodynamics of codeine in combination with acetaminophen in horses

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Codeine and acetaminophen in combination have proven to be an effective analgesic treatment for moderate to severe and postoperative pain in human medicine. Studies have demonstrated that both codeine and acetaminophen, when administered as sole agents are well tolerated by horses, however, a recent study in horses failed to demonstrate a significant effect on thermal nociception at doses up to 1.2 mg/kg. In the current study we hypothesized that administration of the combination of codeine and acetaminophen would result in a significant thermal anti-nociceptive effect compared to administration of codeine by itself. To that end, the current study describes the pharmacokinetics and pharmacodynamics, including effects on thermal nociception of the combination product.. Six horses were administered oral doses of codeine (1.2 mg/kg), acetaminophen (20 mg/kg) and codeine plus acetaminophen (1.2 mg/kg codeine and 6-8 mg/kg acetaminophen) in a three-way balanced crossover design with a 2-week washout period between treatments. Plasma samples were collected up to 96 hours, concentration of drug and metabolites determined via liquid-chromatography mass spectrometry and pharmacokinetic analyses performed. Pharmacodynamic data obtained included effect on thermal threshold, step counts as an assessment of excitation, heart rate and rhythm, gastrointestinal borborygmi, and defecation incidence and consistency. Codeine was metabolized into C6G, norcodeine, morphine, M3G and M6G. Concentrations of morphine metabolites were higher in the combination group compared to the codeine group. All drugs were well tolerated with no significant adverse effects noted, warranting further study of this combination in horses.

Research Grant: This study was supported by the K.L. Maddy Pharmacology Lab program funds
Student Support: Boehringer Ingelheim Veterinary Scholar Program

Survey for prevalence of *Batrachochytrium dendrobatidis* in the LSU Lakes

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Batrachochytrium dendrobatidis, a fungal organism, is responsible for significant amphibian die offs that have led to the extinction of multiple species. (Galt et al.,2021; Searle et al., 2011) This fungus interferes with gas exchange and electrolyte levels leading to death of some amphibians while it is non-lethal in others. (Pare,2021) Although *Batrachochytrium dendrobatidis* has been documented around the world in a variety of ecosystems, in Louisiana, reports are limited to the Gulf Coast Water Dog (*Necturus beyeri*) and crayfish (*Procambarus* spp). (Brannelly et al.,2015; Glorioso et al., 2017) The LSU lakes are artificial bodies of water in an urban environment. They are also being targeted for a restoration plan to improve water quality and their utilization by the community. Our goal was to determine the prevalence of *B. dendrobatidis* in anurans inhabiting the LSU lakes in their current state. If *B. dendrobatidis* is identified, it would be the first report for this habitat. Future studies after the LSU lakes restoration project are completed, could help determine if an improvement in habitat quality can have a positive effect on anuran health by decreasing the prevalence of this organism. Surveying of the lakes and data collection is currently underway, and results are pending.

Research Grant: None
Student Support: Boehringer Ingelheim; Louisiana State University Summer Scholars Research Program

Impact of apitherapy on canine, equine, and chicken lymphocytes, *in vitro*

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Apitherapy is a form of alternative medicine that utilizes products from the western honeybee (*Apis mellifera*), including honey, propolis, and honeybee venom, to improve the health status of patients by modulating host immunity. Major gaps in the data exist regarding the impact of apitherapy on immune cells, and minimal studies exist in veterinary species. In this *in vitro* pilot study, honey, propolis, and honeybee venom were cultured with enriched canine, equine, and chicken peripheral blood lymphocytes. Following 72hr culture (92hr for chickens) with honeybee by-products, the PBLs were assessed for cell proliferation, cell viability/apoptosis, and cell morphology. In chickens, lymphocyte proliferation was significantly increased with honey (0.1 & 0.2%) and venom (0.1 & 1.0 μg/mL) but decreased with propolis (1, 10, & 100 μg/mL). Lymphocyte proliferation increased in horses with propolis (1, 10, & 100 μg/mL) and venom (0.1, 1, & 10 μg/mL). Viability was significantly increased and apoptosis was decreased in chicken lymphocytes with venom (0.1, 1, & 10 μg/mL). Horse lymphocyte viability increased and apoptosis decreased in the presence of venom (1 & 10 μg/mL). Horse lymphocyte viability decreased and apoptosis increased in the presence of propolis (1, 10, & 100 μg/mL) and honey (0.1 & 0.2%). Canine results are currently being evaluated. These preliminary results suggest that honeybee by-products can differentially alter peripheral blood lymphocyte function based on veterinary species.

Research Grant: The Georgia Beekeepers Association, The Office of the Dean of UGA College of Veterinary Medicine, The Department of Small Animal Medicine and Surgery
Student Support: Morris Animal Foundation

The effect of increased growth with selective breeding on promoting cardiovascular insufficiency in pigs

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Selective breeding of pigs has favoured increased growth to enhance meat quantity for economic gain. However, the cardiovascular system may not have developed proportionally to support the larger body size. We hypothesized that compared with pigs immediately post-weaning (growers), pigs at market weight (sows) will have impaired cardiovascular responses to a stress evidenced by lower baroreflex sensitivity (BRS) during a modified Oxford test. Beat-by-beat blood pressure (femoral catheter) and heart rate (ECG) were evaluated in female growers (n = 7; 12.5 ± 0.4 kg) and market pigs (n = 7; 80.9 ± 2.3 kg) under baseline conditions then during a modified Oxford test. The modified Oxford was performed by infusing phenylephrine (PE; α1 adrenergic agonist; 6μg/kg/min) followed by sodium nitroprusside (SNP; nitric oxide donor; 10μg/kg/min) to increase and decrease blood pressure, respectively. To assess cardiac response when under direct stress stimulation PE and SNP infusions were repeated following dobutamine infusion (β1 adrenergic agonist; 5, 10, and 20μg/kg/min for 2 min at each rate). BRS without and with dobutamine was determined as the slope of the linear regression between systolic blood pressure and the ECG RR interval. Preliminary results indicate that the BRS is similar between growers and market pigs but reduced during dobutamine conditions in market pigs. Reduced cardiovascular regulation during β1 adrenergic stimulation may reveal impaired cardiac stress responses in market age pigs. Such cardiovascular insufficiency may impact the health and welfare of swine, particularly during transportation or other stressful conditions.

Research Grant: ADF Student Experience Grant
Student Support: Boehringer Ingelheim veterinary scholars program & WCVM Research and Graduate Studies

Prevalence of *Borrelia* spp., *Babesia* spp., and *Ehrlichia* spp. in Ixodid ticks of the Cumberland Gap Region

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Due to increasing tick abundance, shifting habitats and hosts, and introduction of exotic species, identified cases of tick transmitted diseases are rising. This research aims to understand current prevalence of tick-transmitted pathogens (e.g., *Borrelia spp*., *Babesia spp*. and *Ehrlichia spp.*, among others) in the Cumberland Gap Region of Virginia, Kentucky, and Tennessee. Ixodid ticks (n = 1940) were collected between 2016-2020 including *Amblyomma americanum* (n = 390), *Ixodes spp*. (n = 494), *Dermacentor variabilis* (n = 747), and *Rhipicephalus sanguineus* (n = 231) as well as the invasive *Haemaphysalis longicornis* (n = 11). We hypothesized that *A. americanum* and *Ixodes spp*. would have the highest prevalence of pathogens as they are known vectors of *Ehrlichia spp*., *Borrelia spp*., and *Babesia spp*. Tick DNA samples were screened with pan-genus primers for *Ehrlichia spp*. and *Babesia spp*., *Ixodes spp*. were screened with qPCR for *Borrelia burgdorferi sensu lato*. Positive amplicons were bi-directionally sequenced. For piroplasms, including *Babesia spp*., 732 samples have been screened with PCR resulting in 142 positives. Sanger sequencing was performed on 26 so far, with 10 positive for *Babesia spp*. For *Ehrlichia* and *Anaplasma spp*., 45 samples have been screened, with 3 positive for *Ehrlichia canis* (6.7%), 4 positive for *Ehrlichia ewingii* (8.9%), and 6 positive for *Anaplasma spp*. (13.3%). For *Borrelia spp*., 371 *Ixodes spp*. were screened and 28 were positive (7.5%). Screening and sequencing for *Babesia spp*., *Ehrlichia spp*., and *Borrelia spp*. are still ongoing. Going forward these data will be essential to understanding prevalence and will help in monitoring changing levels of disease presence as well as the risks they present to the community.

Research Grant: Lincoln Memorial University College of Veterinary Medicine
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Stop worrying, we have you covered! Physical barriers significantly reduce fly worry behavior in horses

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Stable flies (*Stomoxys calcitrans*) are obligate blood feeding muscid flies, the painful bites of which cause significant fly worry for both cattle and horses. On-horse fly control is primarily achieved through chemical or physical means, although no comparative study evaluating their relative effectiveness has been published. This study aimed to evaluate the efficacy of physical barriers (fly sheet, mask, and leg coverings), pyrethroid-based spray, and bio-based spray composed of plant essential oils in pasture-boarded horses. Pasture-boarded horses were evaluated for fly worry behavior (skin twitch, leg stamp, tail swish, and head movement) before and after intervention by filming for 5-minute intervals and counting the number of fly worry events. Physical barriers were more effective than chemical sprays (t test, $P = 0.0426$) and while bio-based sprays were more effective than pyrethroid sprays (71.71% $\pm$ 12.21 vs 54.32% $\pm$ 19.86 respectively), this effect was not significantly different (t test, $P = 0.1085$). The reduction in fly worry behavior was negligible within 4 hours of application for either spray application. It is recommended that physical barriers be used for all day protection while chemical sprays be used as an alternative for short durations when physical protection cannot be used such as while horses are being ridden or groomed.

Research Grant: None
Student Support: National Institutes of Health T35 Training Grant

Comparable placement characteristics and flow rates of two intraosseous devices in canine and feline cadavers

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Intraosseous (IO) catheterization enables rapid access to systemic circulation in critical patients. An automatic IO catheter placing device (EZ) utilized by veterinarians has 1000 uses per device, and the price of the device and supplies can be costly. A new manual IO device (SAM) has a lower price, and each device can perform over 10000 actuations. Placement characteristics (time to placement attempt and successful placement rate) and flow rates were compared for each device (EZ and SAM) from 72 catheterization events performed by 6 novice users (veterinary students) in the humerus and tibia of 12 dog and 6 cat cadavers. Flow rate determinations were limited to successfully placed IO catheters. The order of user, cadaver, device, and site of first placement was randomized. Placement was attempted in the contralateral site with the other device. We hypothesized that placing IO catheters with EZ would be faster than with SAM, and that both devices would have similar placement success and flow rates. Users had overall limited successful placements. In dogs, the median placement attempt time was faster with EZ (34s EZ, 72s SAM, $P < 0.001$) but similar between devices for successful catheter placement (33s EZ, 45s SAM, $P = 0.156$). There was no difference in successful placement attempts (50% EZ, 46% SAM, $P = 0.775$). Infusion rates were similar (1.62L/hr EZ, 2.03L/hr SAM, $P = 0.566$). In cats, successful placement was similar between devices (17% EZ, 25% SAM, $P = 1.000$) but not enough successful placements occurred for further statistical comparison. Overall, this is the first study to examine the SAM device in animals, providing valuable preliminary data for future IO studies and potential direct clinical veterinary use of the device.

Research Grant: SAM Medical sponsored
Student Support: Supported by NIH Grant Number T35 OD015130

Development of a modified live attenuated influenza vaccine against H9N2 avian influenza

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Influenza A viruses (FLUAV) of the H9N2 subtype are enzootic in poultry in parts of Asia, the Middle East, Europe, and Africa, causing significant economic damage to the poultry industry due to high morbidity and associated mortality. Due to their zoonotic potential, the World Health Organization (WHO) places H9N2 FLUAVs among those with pandemic concerns. In addition, H9N2 FLUAVs have contributed the internal gene segments to more virulent zoonotic strains such as H5N1/N6, H7N9,and H10N8/N3. Current vaccination platforms, consisting of inactivated vaccines, produce only partial protection with little cellular immune activation. On the other side, modified live attenuated influenza vaccines (MLVs) mimic a natural infection generating humoral and cellular immune responses, which make them promising candidates for the development of broadly protective vaccines against FLUAV. In this study, MLVs of the H9N2 subtype based on genome rearrangements in PB1, carrying molecular diagnostic markers and immunomodulators were generated. The results show that the MLVs generated are stable and replicate to similar levels in comparison with wild-type strains *in vitro*. Studies conducted in chickens show that MLVs are immunogenic, generating similar levels of NP- and neutralizing antibodies as an inactivated vaccine. Additionally, the inclusion of immunomodulators enhances the generation of neutralizing antibodies suggesting a role in the host immune response. Taken together, this work provides novel insight into the development of vaccines against influenza carrying molecular markers and immunomodulators.

Research Grant: United States Department of Agriculture (USDA)/National Institute of Food and Agriculture (NIFA)
Student Support: Boehringer Ingelheim Veterinary Scholars Program (BIVSP)

Clinical features, imaging findings, treatment, and outcome in dogs with discospondylitis

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Discospondylitis is an infection of the intervertebral discs, adjacent cartilaginous endplates, and/or vertebral bodies. A paucity of information exists regarding canine discospondylitis which negatively impacts clinical management. The aims of our study were to describe signalment, clinical findings, treatment, and outcome in dogs with discospondylitis. Study methods included retrospective case review (01.01.10 - 12.31.21) at 4 referral institutions. A total of 172 dogs were identified. The median age of dogs was 6 years (range, 11 weeks - 15 years) and male dogs were overrepresented. Of the cases where duration of signs were known (n = 136), 92 (68.1%) were chronic in duration. Twenty-three patients (17%) presented with subacute disease and 20 (14.8%) presented acutely. The most common clinical signs were lethargy, pain, and decreased appetite. Positive bacterial cultures were noted in 46 dogs (26.7%) and fungal cultures in 4 dogs (0.23%). The most common affected site was L7-S1. Twenty-one patients had evidence of discospondylitis on advanced imaging but no evidence of disease on initial radiographs. Treatment was medical in 159 dogs and surgical in 18 dogs. Of the patients with follow-up data available (n = 58) 51 patients showed signs of clinical improvement while 3 patients had progressive disease, 3 patients initially improved but relapsed, and 1 patient never improved despite radiographic resolution of disease. Patients clinically improved at a median of 20 days from diagnosis (range 1-545 days). Twenty-six patients exhibited radiographic resolution of discospondylitis at a median of 110.5 days post treatment (range 20-604 days). Median duration of antibiotic use was 20 weeks.

Research Grant: NIH Grant 5T35OD016477-20
Student Support: NIH Grant 5T35OD016477-20

Safety of wild-caught *Musca domestica* for use as protein supplement in chicken feed

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Protein feedstuffs are a necessary, yet often expensive, additive to chicken diets and most often consist of soybean meal in the United States. To alleviate the expenses for producers as well as the carbon footprint of the soybean industry, houseflies (*Musca domestica*), can be utilized as an alternative source of protein. Previous studies show that houseflies are abundant in animal agriculture operations but also act as mechanical vectors for disease-causing pathogens. We hypothesize that wild-caught flies, treated with heat, are safe for consumption without losing nutritional value. Fly samples collected from both dairy and chicken operations were assigned to a treatment group as control (no treatment), pulverized and dried, or pulverized and heated. Treatments were then plated for microbiological identification. Bacterial DNA was extracted from live colonies following 24-hour incubation and identified. Subsets of the treatment groups were also sent to a nutrition laboratory for analysis. The results demonstrated major reductions in bacterial growth from control to dried flies or heated flies, respectively. However, nutritional analysis results indicated significant reductions in available crude protein for the heat-treated samples. Additional disinfection treatments will be conducted to determine the most effective method without damaging the nutritional contents.

Research Grant: USDA ARSX
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Exploring the role of transposable elements in female-biased embryonic lethality under genomic instability

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Maintenance of genomic integrity is essential in all aspects of organismal survival. However, how genomic instability affects embryogenesis, a period of rapid cell proliferation, is relatively understudied. Previous work from our lab has shown that genomic instability caused by persistent replication stress in mice leads to female biased embryonic lethality likely due to increased inflammation in the placenta. Interestingly, we found that placenta with high levels of DNA damage causes derepression of transposable elements. Transposable elements are implicated in triggering cell intrinsic innate immune responses via cytosolic nucleotide sensors, such as RIG1-MAVS. We hypothesized that female biased embryonic lethality is caused by transposable elements which mediate increased inflammation in the placenta. Here, we tested our hypothesis by treating pregnant females with Zidovudine (AZT), a nuclease reverse transcriptase inhibitor. Survival of female and male embryos with high genomic instability after AZT treatment is compared. In addition, we generated RIG1 knockout mice to test whether RIG1-MAVS signaling is responsible for activating innate immune response under genomic instability.

Research Grant: NIH R01 HD086609
Student Support: NIH T35 OD010941 and Cornell University College of Veterinary Medicine

Proteomic analysis of Human T-cell Leukemia Virus Type 1 reveals novel cellular proteins in viral particles

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Human T-cell leukemia virus type 1 (HTLV-1) is a human oncogenic retrovirus that causes adult T-cell leukemia/lymphoma (ATLL) and HTLV-1-associated myelopathy/tropic spastic paraparesis (HAM/TSP). It is estimated that 15-20 million people in the world are infected by this virus. In this study, we aimed to determine how HTLV-1 influences host cellular proteins to support virus replication by examining actively participating cellular host proteins incorporated into virus particles during replication. Tandem mass spectrometry was used to evaluate the proteins from purified HTLV-1 virions produced from diseased T-cells. Approximately 1700 cellular host proteins incorporated into viral particles were identified including cytoskeleton, adhesion, immune response systems and viral replication. Our ongoing experiments seek to obtain deeper insight on these cellular proteins and their respective roles in HTLV-1 replication and cell-to-cell transmission. In addition, further understanding of this virus can address gaps in the retrovirus field relating to virus-host interactions specific to HTLV-1 replication, pathogenesis, and novel therapeutic interventions.

Research Grant: None
Student Support: Supported in part by USDA Multicultural Scholars Program grant #2019-38413-29022

Does the intensity of perioperative analgesia alter the metastatic propensity of extremity osteosarcoma?

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Osteosarcoma (OS) is an aggressive bone cancer in dogs and children. Recent data suggest that the types of pain medications used during and after canine OS surgery (limb amputation) can impact time to metastasis. Here, we aimed to establish methods for modeling this phenomenon in mice. Female SCID-Beige mice underwent intratibial injections of 143B human OS cells. Ten days later, affected limbs were amputated. Mice were randomly assigned to receive low intensity analgesia (one dose of meloxicam and then buprenorphine for three days), or high intensity analgesia (meloxicam and buprenorphine for three days, plus liposomal bupivacaine at the surgical site). Amputated legs were processed for histopathology. To evaluate surgical site sensitivity, the von Frey assay was performed. Lung tumor burden was quantified using bioluminescent imaging and computed tomography. Survival was quantified. One day post-surgery, mice in the high-intensity analgesia group had lower surgical site sensitivity than mice that received low-intensity analgesia. IVIS imaging was performed 10 days postoperatively and showed no difference in lung-tumor burden between the treatment groups. Based on Kaplan-Meier survival curve analysis, there was also no detectable difference in overall survival. Leg histopathology did show variability in the amount of intra-osseous tumor. Despite significant differences in surgical site sensitivity, the magnitude of difference was small. The tumor induction technique also led to inconsistent amounts of tumor within the medullary cavity. Future investigations should: (1) further exaggerate the differences in analgesic intensity, and (2) aim to use more consistent and more biologically relevant tumor modeling techniques.

Research Grant: George H. Hitchings New Investigator Award in Health Research, a fund of Triangle Community Foundation, Boehringer Ingelheim Veterinary Scholars Program, and philanthropic donations to the Nolan lab
Student Support: NC State University Fluoroscience Endowment

Retrospective Analysis of Antimicrobial Susceptibility of *Staphylococcus* spp. in dogs, from Ontario, Canada

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Antimicrobial-resistance (AMR) is becoming increasingly common in bacteria isolated in both veterinary and human patients. Infections with drug-resistant bacteria, particularly *Staphylococcus* spp. infections are quite challenging to treat with recommended antimicrobials. The aim of this study is to investigate the AMR status of *Staphylococcus* spp. bacteria isolated from canine patients from two veterinary hospitals in the Niagara Region in Ontario, Canada. The reports were retrieved through AVImark, a veterinary practice management software from January 2015 to December 2021.The retrospective data included 1370 bacterial culture reports received from IDEXX laboratories, out of which 306 specimens (22.3%) were positive for *Staphylococcus* spp. Microsoft Excel was used to organize and analyze the data. The results showed that the most prevalent strain of *Staphylococcus* isolated is *S. pseudintermedius* (48%), followed by methicillin resistant *S. pseudintermedius* (MRSP) (28%), *S. schleiferi* (10%), *S. aureus* and other *Staphylococcus* species both at 7% respectively. The antibiotic susceptibility test results revealed a high AMR pattern for beta-lactam antibiotics (31-80%), followed by tetracycline antibiotics (20-47%), macrolides (35-40%), cephalosporins (36-37%) and fluoroquinolones (28-29%). Amikacin showed the least resistance at 1%. The study also documented evidence of multi-drug resistance (MDR) (resistance to 3 or more than 3 antimicrobial drugs) in 55% of the stains isolated. The results of the study indicate not only the emergence of AMR with recommended antimicrobials but also a high prevalence of MDR strains. Therefore, judicious use of antimicrobials is highly mandated.

Research Grant: Boehringer Ingelheim Island Veterinary Scholars Program
Student Support: St. George's University School of Veterinary Medicine, Grenada, West Indies

Optimization of a novel technique for focal ablation of TRPV1-expressing neurons in the murine tongue

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The TRPV1 protein is expressed by sensory neurons and is the molecular sensor for both capsaicin and heat. Resiniferatoxin (RTX) is a potent capsaicin analog. When given subcutaneously to mice, RTX causes systemic ablation of TRPV1-expressing neurons, resulting in desensitization to TRPV1 agonists. Techniques that localize ablation would expand the utility of RTX as a research tool. Experiments were designed to test the hypothesis that intralingual RTX injection causes desensitization of the trigeminal pathway without affecting sensitivity to TRPV1 agonists at distant sites. C57Bl/6J mice were given either intralingual RTX or control treatment. Trigeminal sensitivity to TRPV1 agonists was tested weekly by assessing responses to orally and ophthalmically-administered capsaicin. Sensitivity at distant sites was assessed using the hot-plate assay, and responses to footpad injections of capsaicin. Later, intralingual capsaicin will be administered to test local ablation. Neuronal expression of TRPV1 will be assessed in tongue, trigeminal ganglia and lung. Ongoing tests show that the positive control group (subcutaneous RTX) has significantly decreased sensitivity across all behavior assays as expected with systemic ablation of TRPV1-expressing neurons. Intralingual injections resulted in decreased responses to oral capsaicin but normal results for other assays. These early results are promising. Localizing the effects of RTX allows for targeting studies of nociceptive transmission in afferent fibers. Future research using local ablation can look towards dosage optimization, duration of action, reversibility, differences between body sites, and long-term effects of targeting specific nerve terminals.

Research Grant: Resiniferatoxin was provided as a donation from Sorrento Therapeutics; additional research funding was made possible by philanthropic support of the NC State Radiation Oncology Research Fund
Student Support: NC State University Fluoroscience Endowment

Efficacy of dilute epinephrine for use as a preventative treatment of feline scrotal hematoma

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Scrotal hematomas are the most common post-surgical complication for male cats in veterinary general practice. The authors previously investigated the use of epinephrine as a hemostatic agent at a 1:400,000 dilution but found no significant reduction in hematomas. This concentration may have been too low to induce clinically relevant vasoconstriction, and thus we hypothesize that higher concentrations of epinephrine will significantly reduce the incidence of hematomas. Male free-roaming cats trapped for Midwestern University's Trap-Neuter-Return program were enrolled into this randomized control trial. Patients were assigned to one of three treatment groups via block randomization: normal saline (control), 1:120,000 epinephrine, or 1:40,000 epinephrine. Each patient received 0.1 mL of treatment as a topical wash placed within each scrotal sac for a total of 0.2 mL. As the study is ongoing, treatments are currently labeled A, B, and C to avoid bias. Veterinarians who assess the presence and severity of hematoma at completion of surgery, at return to trap, and immediately before discharge are blinded to patients' treatment groups. The authors performing data analysis are also blinded until full enrollment is achieved. Of a calculated sample size of 500 cats, 85 cats were enrolled into groups A (n = 27), B (n = 31), and C (n = 27). The number of hematomas occurring in each group were 3 (1.1%, 95% CI [0.009, 0.25]), 6 (19%, 95% CI [0.07, 0.37]), and 2 (7.4%, 95% CI [0.009, 0.26]), respectively. A Fisher's exact test was used to compare these rates (P = 0.434). Continued enrollment to 500 cats (and consequent unblinding) will be required to determine if there are any statistically significant differences between treatments.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program and Federal Work Study

An interactive, equine neurology case simulator to improve lesion localization and problem-solving skills

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Analyzing neurologic symptoms and localizing lesions in the equine patient can be a daunting task for 4th year veterinary students and new graduates. Veterinary students entering the equine field often experience high levels of stress and concerns about limited clinical experience. The development and use of an interactive online virtual clinic in which students and new veterinarians can practice clinical equine neurology cases would provide a low stakes environment for users to revisit their knowledge of neuroanatomy and refine their problem-solving skills. The objectives of this project are to develop a variety of equine neurology cases and, in collaboration with the Veterinary Information Network (VIN)'s Virtual Clinic team, incorporate them into the existing online VIN Virtual Clinic. The simulator will utilize a branching scenario format, allowing the user to make their own choices in regard to gathering history, performing physical exams, ordering tests and diagnostics, determining a final diagnosis, and ultimately formulating a treatment plan. We plan to include interactive 3D models to simulate various components of the neurologic exam (e.g. pupillary light reflex) and neuroanatomy concepts (e.g. pathways of cranial nerves). Once this simulator is developed, we aim to conduct a study to determine the effect of engaging with this simulator on veterinary students' knowledge of neuroanatomy and confidence level as they enter their clinical rotations. We hope that the development of this equine neurology simulator will be effective in improving the competence and confidence of veterinary students and new veterinarians entering the equine field.

Research Grant: None
Student Support: Atlantic Veterinary College VetSRA, Boehringer Ingelheim Veterinary Scholar Award

Agreement between evaluators assessing bull sperm morphology by conventional methods versus video capture

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The objective of this study was to determine the agreement between skilled evaluators assessing bull sperm morphology by conventional methods versus video capture. The significance behind this study was that though intra-evaluator and inter-evaluator experiments have been conducted no studies have been reported comparing reading of stained morphology slides via microscope vs evaluation of video captured by microscopic evaluation at the same power. For this experiment semen was collected from six bulls and five eosin-nigrosin stained slides were made per bull. One slide was utilized for video capture while the other four slides were coded and sent to independent board-certified Theriogenologists with significant expertise in evaluation of bovine sperm morphology. This process was repeated for each of the six bulls. The evaluators then used individual methods to review the stained slides and assess 100 cells. For the video 100 cells were counted and labeled; the evaluators then assessed those 100 cells morphology in numerical order allowing for comparison between evaluators and methods. The results of this experiment are expected to show a greater agreement amongst trained evaluators that view the exact same 100 cells on a morphology slide.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Research Scholars Program

Diagnostic assay development of Tick Borne Pathogens in dogs

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Ticks pose as principal vectors for both animal and human bloodborne pathogens. These pathogens are responsible for a variety of diseases, such as: babesiosis, anaplasmosis, Lyme disease, and Rocky Mountain spotted fever. These tick-borne diseases are seeing greater emergence due to the geographical expansion of ticks worldwide. This growing health concern has illustrated a lack of modern diagnostic approaches to tick-borne diseases; particularly in diagnostic options provided in veterinary medicine. In human medicine, the current standard is a nucleic acid-based multiplex diagnostic assay tool. This tool can screen 384 patient samples simultaneously and can screen each individual sample for a variety of tick-borne pathogens. The tool can then provide diagnostic reports on each sample in a few hours. Development of a similar multiplex diagnostic assay in veterinary medicine will aid in earlier detection and control of infectious tick-borne pathogens in animals. Thus, our lab worked to create the foundation for a nucleic acid-based multiplex tool for use in veterinary medicine. We started with 2 major tick-borne pathogens: *Anaplasma phagocytophilum* and *Borrelia burgdorferi* species. We then used a nucleic acid-based approach to identify each pathogen using gene-specific primers and probes created in the lab. To test the specificity of the primers and probes, we screened blood from canine patients for *A. phagocytophilum* and *B. burgdorferi*. These patients did not have symptoms of tick-borne diseases nor were they diagnosed with infection from *A. phagocytophilum* or *B. burgdorferi*. Our laboratory team hopes to use these results to further develop the duplex test for *A. phagocytophilum* and *B. burgdorferi* into a multiplex test.

Research Grant: Western University of Health Sciences College of Veterinary Medicine Office for Research
Student Support: CVM Veterinary Summer Research Program

High-dose carprofen as a sole therapy in female, CD1 mice is an inadequate postoperative analgesia

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Relieving pain in laboratory mice is scientifically needed since untreated pain can influence experimental results. Non-steroidal anti-inflammatory drugs, like carprofen, are commonly given as a postoperative analgesic, but the current 5 mg/kg dosing of carprofen may not provide sufficient analgesia. We hypothesized that a higher dose of carprofen in postoperative mice will decrease pain-associated behaviors which could lead to improved analgesia compared to the current standard. Mice were anesthetized with isoflurane and received either an ovariectomy (S) or an anesthesia-only sham surgery (A) (n = 30 per group). Mice were then given either high- (10 mg/kg BID, HC) or low-dose (5 mg/kg BID, LC) carprofen or saline (NC) (n = 10 per treatment). Mouse behaviors indicative of pain: orbital tightening, arched posture, wound licking, rearing, and grooming were observed -24, 4, 8, 12, 24, and 48 hours postoperatively. All surgery groups had significantly increased scores in orbital tightening, arched posture, and wound licking compared to the anesthesia-only groups at 4, 8, 12, and 24 hours postoperatively. The SHC and SLC groups had significantly lower arched posture scores than the SNC group at 8 hours postoperatively. There was no difference in rearing or grooming behaviors between groups at any of the measured timepoints. Other than the arched posture scores at 8 hours postoperatively, there were no behaviors that suggested an analgesic effect in any of the high- or low-dose carprofen groups compared to the saline group. These results indicate that high- and low-dose carprofen is clinically insufficient as a sole analgesic therapy.

Research Grant: Office of the Vice President for Research, Colorado State University
Student Support: American Society of Laboratory Animal Practitioners Foundation

Analyses of Virulence Genes in Avian *Escherichia coli*

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Avian pathogenic *Escherichia coli* (APEC) is the causative agent of avian colibacillosis, which results in different syndroms in chickens, leading to high economic losses in the poultry industry each year. The purpose of this project is to develop a better understanding of APEC's virulence factors and its association with disease in poultry. Using PCR, 218 isolates collected from the Georgia Poultry Diagnostic Lab were analyzed for *E. coli* 16S rRNA, nine APEC virulence genes, and 28 O-serogroups. The results showed that 215 isolates were confirmed as *E. coli*, and most isolates were classified as serogroups O8 (10.23%), O78 (9.30%) and O2 (9.77%). 49.77% of the samples were non-typable. Serogroups O2 and O78 were more prevalent in diseased birds (20.7% and 27.6%, respectively) than control chicks (18% and 8%), whereas O8 was more prevalent in the control chicks (32%) than diseased birds (10.34%). Among serogroups O2, O8, and O78, the genes *ireA* and *papC* were most prevalent in disease group (O2: 41.66%, 41.66%; O8: 33.33%, 16.66%; O78: 75%, 31.25%) than the control group (O2: 11.11%, 0%; O8: 6.25%, 0%; O78: 0%, 0%). However, there was no significant difference in the prevalence of *iroN, ompT, hlyF, iss,* and *aerJ* between the control and the disease groups. The data shows that *ireA* and *papC* may be more important in causing disease compared to *iroN, ompT, hlyF, iss,* and *aerJ*. Also, O2 and O78 seem to be the most important serogroups for causing avian colibacilliosis in Georgia due to their high prevalence in diseased birds. This work gives us a better understanding of APEC virulence factors and may be useful for future vaccines targeting *ireA* and *papC* gene products.

Research Grant: USDA NIFA
Student Support: Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Ex vivo* analysis of extracellular vesicle interactions with immune cells in whole blood of *Macaca nemestrina

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Extracellular vesicles (EVs) are nano-sized, lipid bilayer particles produced naturally by cells in multicellular organisms. EVs can be loaded with therapeutic cargo and may be engineered for retention by specific body sites; therefore, they have great potential as novel carriers for delivery of drugs and biomolecules to treat diseases. Recent work in the Witwer lab demonstrated that Expi293F-derived EVs labeled with a GFP-nanoluciferase dual reporter could be detected in peripheral blood mononuclear cells (PMBCs) of *Macaca nemestrina* (pig-tailed macaques) after intravenous administration of EVs. To better understand how EVs interact with PBMCs, we developed an *ex vivo* model using whole blood collected from pig-tailed macaques to mimic the environment encountered by EVs in hosts without relying on animal models. After treating whole blood with different doses of EVs at varying time points, nanoluciferase assays and flow cytometry were conducted to detect the presence of EVs in association with different immune cell subtypes. Our data shows that EVs come in contact with both PBMCs and red blood cells (RBCs) after 5 minutes of incubation and remained detectable up to 24 hours. Importantly, EVs were found to associate with B cells at significantly higher levels than with any other PBMC subtype tested-providing further support to the Witwer's lab previous novel findings that EVs are likely taken up by B cells. Future experiments using confocal microscopy will be performed to determine the specific nature of the interactions between EVs and B cells. Overall, the findings will contribute to the growing body of knowledge around EV biodistribution in non-human primates and how EVs interact with immune cells in the blood.

Research Grant: This research project is supported by the US National Institute of Health through 5R01AI144997-03 (to Kenneth W. Witwer)
Student Support: Charles River Labs Summer Fellowship Program in Laboratory Animal Medicine

Surveillance for highly pathogenic avian influenza in common and roseate terns in Buzzards Bay, Massachusetts

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Infectious Disease and Global Health (Westervelt, Nutter, Puryear), Cummings School of Veterinary Medicine, Tufts University, Grafton, MA; Virology, Disease Ecology, and Global Health (Hill), University of Massachusetts Boston, Boston, MA; Massachusetts Division of Fisheries and Wildlife (Mostello), Westborough, MA

The common tern *Sterna hirundo* is a migratory seabird with a range that spans North America, Europe, and Asia. Tern colonies can be found along the Massachusetts coast, with the largest in Buzzards Bay. The federally endangered roseate tern *Sterna dougallii* also nests in Buzzards Bay. Avian influenza is a zoonotic virus with a natural reservoir in birds. In early 2022, the USDA announced an outbreak of highly pathogenic avian influenza (HPAI) caused by the H5N1 subtype, identified in both wild and domestic bird species across the United States. In March 2022, HPAI was found in several wild and domestic birds in Massachusetts, with shorebirds and waterfowl some of the most at-risk species, but it had not yet been documented in common or roseate terns in Massachusetts. HPAI of the strain H5N3 was first isolated and described from common terns in South Africa (Rowan 1962), establishing terns as a natural reservoir for HPAI. The purpose of this study was to monitor common tern adults and chicks as well as roseate tern chicks on Bird, Ram, and Penikese Islands in Buzzards Bay for evidence of HPAI infection. Oral and cloacal swabs were obtained from common tern adults and chicks as well as roseate tern chicks; RNA extraction and RT-qPCR were performed on these samples to determine infection status. Blood samples were taken from the cutaneous ulnar vein of common tern adults and chicks. These blood samples will be analyzed using ELISA for antibodies against H5N1 and other strains of avian influenza to look for evidence of previous infection or for protection against the virus. The infection status of common and roseate terns will provide important information regarding the distribution of HPAI in Massachusetts.

Research Grant: Edward Gorey Charitable Trust
Student Support: USDA, Tufts Summer Research Program

***In vitro* bacterial viability of commercially available veterinary fecal microbiota transplantation products**

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Fecal microbiota transplantation (FMT) for gastrointestinal (GI) and non-GI disease is increasing in veterinary medicine. Preparation of donor feces intended for FMT is labor-intensive, expensive, and requires specialized equipment. Thus, commercially available formulations are ideal in a private practice setting. The precise mechanism in which FMT confers a health benefit is unknown but is likely linked to donor feces microbial viability. Bacterial viability in canine or feline feces intended for FMT remains unknown. Our primary objective is to evaluate *in vitro* bacterial viability of veterinary commercial FMT formulations compared to lyophilized and fresh donor feces. Our working hypotheses are that all FMT preparations will exhibit some degree of viability; specifically, freshly processed FMT will yield more colony forming units than lyophilized FMT, and FMT from raw-fed donors will yield higher proportions of Gram-negative bacteria than those fed a commercial diet. Aerobic and anaerobic bacterial viability is being assessed with culture-dependent techniques using selective media; specifically, Columbia naladixic acid agar (CNA) for Gram-positive bacteria and MacConkey media for Gram-negative bacteria. AnimalBiome lyophilized FMT products (Feline Gut Restore, Canine Gut Restore, and Canine Gut Restore from Naturally Reared (raw-fed) dogs) are being compared to screened canine/feline donor fresh and lyophilized feces. Many fecal bacteria are unculturable, so this approach only represents a fraction of bacteria that may be revivified *in vivo*. However, this ongoing study will be the first to provide comparative *in vitro* bacterial viability data for FMT formulations intended for clinical use in companion animals.

Research Grant: IHS Foundation GR1225882
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Biomechanical evaluation of orthopedic cable compared to standard cerclage wire in a canine fracture model

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Cerclage wire is frequently used in simple long oblique fracture repairs adjunct to other modalities. Loosening or breakage of the cerclage wire, and displacement of fragments are complications that may be observed as healing progresses due to excessive micromotion and loading of the bone during ambulation. This necessitates the identification of a repair modality for veterinary orthopedic traumas that maintains its integrity throughout postoperative recovery periods. As there is limited veterinary literature assessing orthopedic cable systems, the aim of this study is to evaluate the DePuy Synthes 316L stainless steel orthopedic cable system compared to standard cerclage wire in a twist knot configuration under monotonic tension and 4-point bending until failure. An ex vivo fracture model using paired canine cadaver tibiae was used to evaluate orthopedic cable compared to cerclage wire using identical sized wires and cable. Mechanical testing was performed cyclically under 4-point bending until implant failure. Focusing our monotonic results, we observed that orthopedic cable can withstand 1.76 times more force than cerclage, 1139N and 644.3N respectively. At cerclage wire's maximum load to failure, it's displacement measures 4.6mm whereas at the same force, orthopedic cable's average displacement is 3.7mm. On average, orthopedic cable can withstand forces from 4-point bending 41 times longer than cerclage before failing. Finally, orthopedic cable requires 4934Nmm of work to fail compared to 1755Nmm for cerclage. Our findings suggest the use of orthopedic cable is more advantageous as an adjunct repair modality for oblique long-bone fractures when compared to standard cerclage in a twist knot configuration.

Research Grant: DePuy Synthes
Student Support: Office of Research & Graduate Studies, CVM, Mississippi State University

Development of assays to measure anthelmintic resistance at single animal resolution in *Haemonchus contortus*

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Widespread anthelmintic resistance threatens the sustainability of ruminant parasite control. *Haemonchus contortus* has developed resistance to all major drug classes, often within ten years of each drug class being introduced. While there has been progress in mapping genetic determinants of resistance, convenient *in vitro* assays have not been developed to associate phenotypic resistance with known genetic markers at the single-parasite or single-host level. I hypothesize that high-content imaging assays can be used to accurately measure anti-parasitic drug resistance in important and ubiquitous ruminant strongylid parasites at single animal resolution. To test this, I investigated whether benzimidazole responses in *H. contortus* will predict the frequency of resistance-associated β-tubulin alleles. My first aim was to develop high-content phenotypic assays to measure drug resistance at single parasite resolution. My second aim was to enable the direct association of anthelmintic responses and the frequency of known resistant markers. I used the Fecal Egg Count Reduction Test (FECRT) to confirm that animals housed at the UW Sheep Unit were infected with drug-resistant trichostrongyles. I then successfully optimized a microplate larval development assay and image processing endpoints to quantify parasite growth in the presence of drug. In parallel, I worked towards genotyping single parasites using PCR and sequencing approaches. We plan to combine these elements into a microplate assay that enables the direct association of anthelmintic responses and their genetic basis. Success in this goal would deliver an *in vitro* phenotypic and genotypic assay to elucidate within-host parasite dynamics relevant to anthelmintic efficacy.

Research Grant: Foundation of Food and Agriculture Research Vet Fellow
Student Support: None

Effect of clodronate on gene expression in the horse

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There are currently two FDA approved bisphosphonate products, Osphos and Tildren, for use in horses to treat navicular syndrome. It is hypothesized that bisphosphonates can produce analgesic effects and prevent proper healing of microcracks in bone, which are common with high-intensity exercise. Due to these effects, bisphosphonate use may lead to catastrophic injury in racehorses. However, bisphosphonates have a very short detection window in the blood before sequestering in the bone, so the reliability of current drug tests is questionable. We hypothesize that there are one or more differentially expressed genes detectable in peripheral blood from horses following administration of the bisphosphonate, clodronate. Eleven healthy exercising Thoroughbred horses were used in this study. Seven were administered clodronate (1.8 mg/kg IM) and four were administered saline as controls. RNA was isolated from peripheral blood mononuclear cells collected immediately before a single dose of clodronate or saline (day 0) and then on days 1, 6, 28, 56, and 182 post-dose. RNA was sequenced and analyzed for differentially expressed transcripts using a mixed linear model, taking both treatment and time into account. While no single transcripts were differentially expressed with a corrected $P < 0.05$, pathway analysis revealed that p38 MAPK ($P = 0.04$) and Ras ($P = 0.04$) pathways were upregulated, and cadherin signaling ($P = 0.02$) was downregulated on day 1. While our results do not support the hypothesis that there are detectable differentially expressed genes in peripheral blood of horses after clodronate administration, we have identified dysregulated pathways with acute clodronate use.

Research Grant: Grayson Jockey Club Research Foundation and The Viola Foundation
Student Support: NIH T35 Training Grant OD010956-22

Can we use electrical pulse stimulation in immortalized human skeletal muscle cells for *in vitro* exercise?

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Exercise plays a vital role in a healthy lifestyle and leads to adaptive responses in skeletal muscle. Previous studies have shown that *in vitro* electrical pulse stimulation (EPS) of primary human skeletal muscle cells can simulate some of the effects of exercise on skeletal muscle *in vivo* including changes in contractility, metabolism, gene expression, and protein markers. The use of primary cultures is limited by their slow growth, and limited ability to undergo cell divisions. To overcome these problems, an immortalized human skeletal muscle cell line (HMCL-7304) has been established. In this study, we determined if *in vitro* EPS may be used to study the effects of exercise on HMCL-7304 cells. HMCL-7304 myotubes were stimulated with chronic, low-frequency EPS (single, bipolar pulses of 2ms, with 11.5V and 1Hz) for up to 24 hours. Cell contractility was visually observed by microscopy. Glucose uptake was studied using a radio-labeled [3H] 2-Deoxy-D-Glucose assay. Glycogen content was measured using a bioluminescent detection assay. After 5 days of differentiation, most of the HMCL-7304 cells fused and formed multinucleated myotubes. After a few hours of EPS, a subset of myotubes showed noticeable contractions. Preliminary data show a trend towards increased glucose uptake after 24 hours of EPS, compared to unstimulated control cells, while the insulin effect was unaffected by EPS. We did not find changes in glycogen content with EPS compared to unstimulated control cells. Our preliminary results suggest the feasibility of using EPS in HMCL-7304 cells. Additional studies including analysis of transcriptomic and protein changes are needed.

Research Grant: Novo Nordisk Foundation and the Stanford Bio-X program (NNF17OC0025806)
Student Support: T35 Summer Research Fellowship (T35OD010989)

Effects of dietary omega-3 and -6 polyunsaturated fatty acids on muscle mitochondrial function in old mares

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Sarcopenia or age-related muscle wasting is common in geriatric horses and results in weakness and decreased muscle mass. Equine aging is associated with decreased oxidative capacity of skeletal muscle, which results in negative effects on mitochondrial function. In sedentary, older people, dietary supplementation with omega-3 (N3) polyunsaturated fatty acids (PUFA) improved muscle mass and grip strength and stimulated muscle protein synthesis. Lipid supplementation is frequently used in older horses to increase bodyweight; however, effects on muscle function have not been adequately studied, and lipid type is usually not considered. We hypothesized that dietary supplementation of lipids will increase mitochondrial function in skeletal muscle of older mares and that N3 PUFA will have more positive effects than omega-6 (N6) PUFA. Mares will receive either N3 or N6 PUFA: N3 (n = 6, mean age of 22.5 ± 2.9 years, 120 ml flaxseed oil daily) and N6 (n = 6, mean age of 23.1 ± 2.8 years, 120 ml corn oil daily). Muscle biopsies will be collected from the trapezius before and after 6 weeks of supplementation, allowing mares to serve as their own controls. Muscle samples will be permeabilized and evaluated for maximum oxygen consumption rate (OCR) and reactive oxygen species (ROS) production using the Oroboros high-resolution respirometer (O2K). Data will be analyzed by two-way ANOVA. We expect that N3 and N6 will increase muscle OCR in the old mares; however, we anticipate that N3 will be more effective than N6 at increasing OCR with less of a concomitant increase in ROS. Our findings could support dietary supplementation for the treatment and prevention of sarcopenia in older mares and have translational implications for aging humans.

Research Grant: The Cecil and Irene Hylton Foundation and The Clyde and Mary Lou Porter Foundation
Student Support: Bovine Foundation Funds

Dysbiosis and inflammation in the intestinal-specific Cftr knockout mouse

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Increasing evidence supports the association between intestinal microbial dysbiosis and inflammation in individuals affected by inflammatory bowel diseases (IBD). The global knockout of *Cftr* in mice as a model of cystic fibrosis shows intestinal dysbiosis and inflammation. Since leukocytes express CFTR1, we asked whether the intestinal-specific *Cftr* KO [B6.Cg-Tg(Vil1-cre)-Cftrf10/f10] (i*Cftr* KO) mice also show dysbiosis and bowel inflammation. Previously, we found that i*Cftr* KO mice have decreased fecal microbial diversity and population with potential pathobionts. As before, adult i*Cftr* KO and their sex-matched wild-type littermates (WT) were transitioned between two impaction preventative diets, i.e., osmotic polyethylene glycol (PEG) laxative in drinking water to a nutritionally complete liquid diet. Inflammation was assessed by an ELISA assay specific for fecal calprotectin, a protein predominantly secreted by neutrophils and used as a marker in IBD. Fecal calprotectin levels in i*Cftr* KO mice maintained on PEG were significantly higher than WT mice, indicating intestinal inflammation (20.35 μg/g vs. 2.76 μg/g; P = < 0.001, n = 10 pairs). Our next step is to perform the same calprotectin analysis in mice fed a complete liquid diet for comparison with the PEG results. We conclude that i*Cftr* mice demonstrate dysbiosis and low levels of inflammation that are independent of CFTR loss in other tissues. These results support therapeutics targeting the intestinal manifestations of cystic fibrosis.

Research Grant: Cystic Fibrosis Foundation
Student Support: Stipend for Estela Williams is supported by an endowment established by IDEXX-BioAnalytics.

A retrospective study of myxomatous mitral valve disease in Labrador Retrievers and Golden Retrievers

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Myxomatous mitral valve disease (MMVD) is the most common acquired cardiac disease in dogs, often affecting small breeds. Because small breeds are overrepresented, they have been the focus of most MMVD research in dogs to date, with minimal data describing MMVD in large breeds. The aim of this study was to describe characteristics of MMVD in Labrador Retrievers (LR) and Golden Retrievers (GR). Medical records were retrospectively reviewed, and stored echocardiograms were remeasured. Fifty-two LR (21 female, 31 male) and 20 GR (7 female, 13 male) with MMVD were identified. Median age was 10.1 years (range: 5.1-14.8) in LR and 9.2 years (range: 5.9-15.2) in GR. Forty-six LR were classified as stage B1 (88.5%), 3 as B2 (5.8%), and 3 as C (5.8%). Fourteen GR were classified as stage B1 (70.0%), 2 as B2 (10.0%), and 4 as C (20.0%). Labrador Retrievers had atrial fibrillation (AF) at the time of diagnosis less often (2/52, 3.8%) than GR (3/20, 15.0%). All five dogs with AF were in stage C, with congestive heart failure (CHF) manifesting as right-sided (N = 1 LR), left-sided (N = 2 GR) or biventricular (N = 1 LR, N = 1 GR). Ventricular arrhythmias were appreciated at the time of diagnosis in 10/52 (19.2%) LR and 3/20 (15.0%) GR. Systolic dysfunction was less common in LR (4/52, 7.7%) than GR (3/20, 15.0%), while mitral valve prolapse was more common in LR (22/52, 42.3%) than GR (5/20, 25.0%). In conclusion, these results provide descriptive information about MMVD characteristics in LR and GR. Across both breeds, MMVD was late onset, predominantly mild, and males were affected more often than females. Atrial fibrillation was common in the few LR and GR with stage C MMVD and was associated with left and/or right-sided CHF.

Research Grant: None
Student Support: NIH T35OD010991-17, Texas A&M School of Veterinary Medicine & Biomedical Sciences

Prevalence of *Dipylidium caninum* in *Ctenocephalides felis* collected from cats, dogs, and homes in Tampa, FL

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The cat flea, *Ctenocephalides felis*, is a common external parasite with the ability to infest both cats and dogs. Cat fleas can transmit a variety of pathogens, including *Dipylidium caninum*, a zoonotic tapeworm that infects the small intestine of cats and dogs. Recently, two distinct genotypes of *D. caninum*, one specific to cats and another specific to dogs, have been described. For this study, fleas were collected from cats, dogs, and the home environment of residential homes in the Tampa, FL area. A total of 1422 fleas were collected during the enrollment of this study, 281 fleas from 47 cats, 99 fleas from 9 dogs, and 1042 fleas from 63 homes. Fleas were pooled into groups of 3, whole genomic DNA was extracted, and a PCR targeting 28s ribosomal subunit of *D. caninum* was run. A total of 33 pools of fleas from cats, 8 pools from dogs, and 39 pools from the environment were tested. Of the pools tested, 2/77 (2.6%) total pools were positive for *D. caninum* (1/33 cat pools (3.0%); 0/8 dog pools (0.0%); 1/39 home pools (2.6%)). There was no significant difference in the prevalence of *D. caninum* between the three pool groups, indicating that the tapeworm is found at a low level throughout all flea populations. The prevalence of *D. caninum* in *C. felis* collected from homes, cats, and dogs in the US had not been described until now. However, the percent of positive flea pools in this study is similar to reports of *D. caninum* prevalence in cat fleas in Europe. The presence of *D. caninum* represents a significant health concern for any pets and households experiencing flea infestations. These findings provide further evidence of the importance for the use of consistent and effective flea control for cats and dogs.

Research Grant: Evaluation of an oral lotilaner product Control Flea Populations on Naturally Infested Cats in Private Residences in Tampa FL 2022 - Post-Marketing Agreement. Elanco Animal Health
Student Support: Elanco Animal Health

EHV-1 Specific Immune Response to EHV Vaccines in Horses

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EHV1 infection is ubiquitous in horse populations causing disease and economic loss with outbreaks of respiratory disease, abortion, neonatal death and myeloencephalopathy. Prevention requires an effective mucosal(IgA), systemic humoral antibody(IgG), and cell-mediated immune responses. Commercially available vaccines produce serum viral neutralizing(SVN) antibodies, but data is lacking on SVN antibody titers in horses administered EHV1 vaccines. The purpose of the study was to determine EHV1 SVN antibody titers to 3 vaccines: Vetera2XP, Calvenza EIV/EHV and Rhinomune. Hypotheses include: EHV1 vaccinated horses will have higher EHV1 antibody titers, with Rhinomune(MLV) producing the highest titers. Whole blood was drawn and serum collected on day -14 for baseline antibody titers from 52 LSU EHSP horses. Horses were randomized into 4 groups and housed on pasture (16/pasture) with 4 Group 4 (control) horses with each of the EHV1 vaccinated groups [Group 1: VETERAGOLD+VETERA2XP day 0,21,42; Group 2: VETERA EWT+WNV days 0, 21; CALVENZA EIV/EHV days 0,21,42; Group 3: VETERA 4XP+WNV,RHINOMUNE Days 0, 21; Group 4: VETERA EWT+WNV days 0,21] EHV1 serum IgG antibody titers were measured in each of the horses on days 0,21,42,63,84 prior to vaccination. Preliminary results showed significant increases in antibody titers for EHV1 vaccinated horses from day 0-21, and a significant increase for horses receiving Calvenza and Rhinomune, with the greatest increase with Calvenza. Antibody titers did not increase in the controls, suggesting a lack of exposure to EHV1 in the pastures. Although ongoing, results are expected to show an increase in titers in horses vaccinated 3 times (Group1&2) compared to horses vaccinated twice (Group3).

Research Grant: Funded by the Boehringer Ingelheim
Student Support: Funded by the Boehringer Ingelheim

Investigation of retroviral-induced lymphoid proliferation in wild turkeys using RNAscope

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Recent population declines in wild turkeys (*Meleagris gallopavo*) in the eastern and midwestern US have prompted investigations into potential underlying causes, including infectious disease, that may be contributing to these declines. Since 2009, lymphoproliferative disease virus (LPDV) has been detected in hundreds of wild turkeys across large portions of the US and Canada, with disease manifestation ranging from subclinical infection to lymphoma. Concurrently, reticuloendotheliosis virus (REV), a similar retrovirus that can cause immunosuppression and neoplasia in numerous avian species, has been detected in wild turkeys, often concurrently with LPDV. To understand the contributions of each virus to disease development in wild turkeys, we utilized RNAscope *in-situ* hybridization of formalin-fixed paraffin-embedded tissues to visualize LPDV and REV RNA in areas of notable lymphocyte infiltration. Seven wild turkeys submitted for postmortem examination that tested positive for both LPDV and REV and exhibited lymphocytic infiltration in multiple tissues were evaluated. Lymphocytic proliferation was most commonly observed in the skin, heart, liver and lung. Intravascular lymphocytes exhibiting REV and/or LPDV RNA staining were present in multiple tissues without concurrent lesions. Up to 57% of lymphocytes exhibited staining for REV RNA, manifesting as intracytoplasmic and/or intranuclear, punctate to diffuse staining. LPDV RNA was present in up to 36% of lymphocytes, as shown by intracytoplasmic, punctate to diffuse staining. Rare co-infection of individual lymphocytes was observed in multiple turkeys. This study helps further elucidate how these viruses contribute to lymphoid proliferation and disease.

Research Grant: National Wild Turkey Federation, SCWDS member states and federal partners (Federal Aid to Wildlife Restoration Act [50 Stat. 917]), and UGA Graduate School
Student Support: Boehringer Ingelheim, Veterinary Medical Experiment Station, UGA College of Veterinary Medicine

Characterization of tumor cell-intrinsic PD-1 receptor in canine urothelial carcinoma cells

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Immune checkpoint inhibition has become a promising treatment option in a number of canine and human cancers, such as invasive urothelial carcinoma (InvUC). As one of these checkpoint molecules, the programmed cell death 1 receptor (PD-1) is primarily expressed on mature cytotoxic T lymphocytes (CTLs), with ligands PD-L1 and PD-L2 expressed on tumor cells and antigen-presenting cells. The interaction between PD-1 and its ligands on tumor cells leads to CTL inactivation and immune tolerance of the tumor, making the PD-1/PD-L1 axis a key immunotherapeutic target for both veterinary and human oncology. PD-1 has also been documented to be present on the surface of a number of tumor cell types, though its function in this context is unclear. To investigate the influence of this tumor cell-intrinsic PD-1 and PD-L1 interaction on cancer cell growth, a canine InvUC cell line that overexpresses canine PD-1 (K9TCC-PU-Nk-cPD1) was developed via lentiviral transduction. Surface expression of canine PD-1 (cPD-1) in this line was confirmed via flow cytometry. Using CellTiter-Glo, soft agar, and Western blot assays, we assessed the proliferation, colony formation, and downstream signaling in the MAPK/ERK and PI3K-PKB/Akt pathways of this engineered cell line in the presence of varying amounts of cPD-L1-Fc protein. We further characterized the influence of cPD-1 on immune evasion via T-cell killing assay using activated canine PBMCs. This work in understanding the role of tumor-cell intrinsic PD-1 has significant implications for prognosis and treatment recommendations for PD-1 expressing cancers of all species.

Research Grant: Bladder Cancer Research fund
Student Support: Morris Animal Foundation

Optimization of PRO-IP-seq to reveal when key proteins interact with RNA Pol II during transcription

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Transcription of DNA into mRNA is a fundamental process in all animals. In higher eukaryotes, RNA Polymerase II (Pol II), the enzyme responsible for transcribing all mRNA, transcribes 20-60 bases before factors Negative Elongation Factor (NELF) and DRB Sensitivity Inducing Factor (DSIF) bind, pausing the complex. Pol II's release into elongation is regulated by transcription factors that recruit Positive Transcription Elongation Factor b (P-TEFb), which phosphorylates pausing factors and Pol II's C-terminal domain and promotes dissociation of NELF. Transcription has been studied for decades with primarily *in vitro* laboratory assays. However, to translate this work in basic science to medical and veterinary problems, it is important to understand how transcription functions mechanistically in living models. Assays such as precision run-on sequencing (PRO-seq) and chromatin immunoprecipitation sequencing (ChIP-seq) provide high resolution snapshots of transcription in live cells. Although it is known that proteins such as NELF, DSIF, and P-TEFb undergo rapid binding and dissociation transitions to Pol II early in transcription, current methods are insufficient to determine exactly when this happens. Thus, the goal of this study is to use a novel method, PRO-IP-seq. Preliminary IP experiments allowed for identification of ideal antibodies for future study of factors of interest, but further optimization of the method is required to achieve its full potential resolution and sensitivity. Once finalized, PRO-IP-seq should allow for the measurement of precise transitions in factor binding and dissociation during transcription and gain a better mechanistic understanding of this fundamental and highly regulated process *in vivo*.

Research Grant: NIH RMI GM139738
Student Support: NIH T35 OD010941 and the Cornell University College of Veterinary Medicine

Comparison of diet history for cats with hypocobalaminemia versus normocobalaminemia

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Low serum cobalamin (vitamin B12) concentration, or hypocobalaminemia, is often caused by gastrointestinal malabsorptive diseases in cats. Supplemental B12 alone would be unlikely to rectify this deficiency, if an absorption abnormality is the cause, but veterinarians often report a good response to therapeutic cobalamin supplementation in cats without additional therapies for malabsorptive diseases. There are no published studies discussing relationships between food and serum B12 in cats. Our objective was to describe and compare diets and food storage practices between cats with hypocobalaminemia and normocobalaminemia. Cats over one year of age with normal gastrointestinal (GI) panels (TLI, PLI, and folate values within reference) aside from cobalamin performed at the Texas A&M University (TAMU) GI Laboratory were included. Cats that received B12 therapy prior to serum B12 analysis or with a diagnosed comorbidity of GI origin were excluded. Cats were sorted into two cohorts: hypocobalaminemic (cobalamin concentrations < 350 ng/L) (*n = 6*) and normocobalaminemic cats (cobalamin concentrations > 500 ng/L) (*n = 8*). Owners were surveyed for diet history of their cat for the year preceding the GI panel. Signalment of cats, clinical signs, brand of food, form, and storage practices were compared. Cats in both groups displayed similar signalment, body condition, brand of diet, and storage practices. Hypocobalaminemic cats presented with inappetence more often. The impact of diet type or owner storage practices as a cause of hypocobalaminemia in cats was not identified in this pilot study. More research is needed to determine if reduced food intake is a cause or effect of hypocobalaminemia.

Research Grant: None
Student Support: NIH T35OD010991-17, Texas A&M School of Veterinary Medicine & Biomedical Sciences

Accumulation and feasibility of the use of bovine lactoferrin as an antibiotic alternative in chickens

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Clostridium perfringens (CP) type A/G induced avian necrotic enteritis (NE) results in $6 billion in losses to the global poultry industry annually. The surge of antibiotic resistant bacteria, and consumer demand has driven governments to restrict in-feed antibiotic use, leading to the need for antibiotic alternatives that yield good clinical outcomes. The glycoprotein bovine lactoferrin (bLF) is reportedly bacteriostatic and its digested derivative lactoferricin B (LFcin-B) is bactericidal. In preliminary work, both bLF and LFcin-B inhibited the growth of a local isolate of CP and its expression of the necrotic enteritis B-like (NetB) toxin gene *in vitro*. The hypothesis of this study was that bLF would be absorbed intact throughout the small intestine, in crop-intubated and intramuscularly (IM)-treated chickens. Additionally, detectable concentrations will accumulate within the submucosa of the jejunum and ileum 9 days post-treatment (day 14 samples), verifying bLF as an effective alternative to traditional antibiotics. Immunohistochemistry (IHC) was used to localize and confirm the presence of bLF in 5-week-old specific-pathogen-free White Leghorn chicks following supplementation for 5 consecutive days, euthanized, and sampled on days 0 (prior to treatment initiation), 3, 7, and 14. Bovine LF was detected in the submucosa, subserosa, and villous epithelium of the jejunum and ileum of treated chickens. The reported findings support that bLF could be a feasible alternative to traditional antibiotic use in chickens. Semi-quantitative assessment of staining intensity will be determined using the Image J software. Future studies should investigate the effectiveness of bLF as a prophylactic and/or therapeutic for NE.

Research Grant: Foundation for Food and Agriculture Research & the American Association of Veterinary Medical Colleges, Western University of Health Science College of Veterinary Medicine Office of Research
Student Support: Foundation for Food & Agriculture Research & American Association of Veterinary Medical Colleges

Role of the triacylglycerol hydrolase enzyme carboxylesterase 1d in lung inflammation

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Lung injury can be initiated by LPS leading to a buildup of lipid droplets in macrophages that contain polyunsaturated fatty acids (PUFAs). Murine carboxylesterase 1d (Ces1d) is associated with ER membranes in cells and it is the most abundant serine hydrolase in lung. Ces1d has a role in the catabolism of triacylglycerols (TAGs), but there is a knowledge gap regarding its role in tissue injury. We hypothesized that LPS-induced inflammation will increase oxidized (ox)TAGs in lung, and if Ces1d is inactivated the levels of oxPUFAs released from oxTAGs will decrease, thus mitigating inflammation. The objectives were to (1) determine the levels of inflammatory molecules following in vivo exposure to LPS when Ces1d is active and inactive, and (2) examine Ces1d's ability to release PUFAs and oxylipins from synthetic oxTAG in vitro. Intranasal LPS or saline was administered to male wildtype (WT) and Ces1d knockout (KO) mice (n = 5/group). BALF and lungs were collected at 6 h and lipid mediators and Il-1β levels determined. PGE_2 levels were unchanged in WT BALF following LPS treatment, whereas they increased 10-fold in KO BALF. This difference also correlated with increases in lung Il1-β in KO lung. When synthetic oxTAG 18:2 (containing 9- and 13-HODE) was incubated with lung membrane fractions from WT and KO mice, the KO lungs exhibited slower rates of HODE release than WT lungs. However, levels of HETEs and HODEs (both oxPUFAs) were unaltered by LPS in lungs of either genotype, indicating that the absence of Ces1d did not reduce oxPUFAs. Indeed, Ces1d deficiency appeared to make these mice more sensitive to LPS. Thus, we need to revisit our hypothesis to consider other possible TAG hydrolases that might alleviate inflammation.

Research Grant: NIH R15HL157818-01A1
Student Support: NIH T35 OD 010432-21

Understanding the genetic basis of Pelger-Huet anomaly in Australian shepherd dogs

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Pelger-Huet anomaly (PHA) is an inherited disorder that has been reported in humans, dogs, horses, and mice. Affected humans and animals exhibit hyposegmentation and altered chromatin structure in granulocytes, especially neutrophils. These morphologic changes do not cause functional abnormalities in heterozygotes, with affected individuals being healthy, but can mimic a left shift on cytology. To date, PHA has been reported in both mixed breed and purebred dogs, including Australian shepherds. Veterinary literature suggests a higher incidence of PHA in Australian shepherds, with one study finding 9.8% of Australian shepherds in California affected. The causative gene mutation for PHA has never been identified in dogs, though the lamin B receptor gene (*LBR*) has been identified as the causative gene in humans and mice, with 11 distinct mutations reported to date. Based upon this, we hypothesized that a heterozygous mutation in *LBR* causes PHA in Australian shepherd dogs. Sanger sequencing was used to compare the coding regions of *LBR* in an affected Australian shepherd to three unaffected Australian shepherds. All exons were sequenced and showed no variants unique to the affected dog. We also performed whole genome sequencing (WGS) on the same affected dog and compared its genome to a private database of 671 dogs. Analysis of the WGS data confirmed our Sanger sequencing findings, however we noted that a large segment of the 5' UTR and promoter region was not covered by WGS reads due to high G-C content. Sanger sequencing is underway to sequence this region upstream of the *LBR* coding sequence. Concurrently, several other candidate genes for PHA are being evaluated using WGS data for the affected animal.

Research Grant: Research Grant: Departmental Funds
Student Support: Student Support: NIH, Office of the Director, Award Number T35OD011118

Osteoinductive hydrogels for treatment of vertebral compression fractures: an osteopenic leporine model

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Vertebral compression fractures (VCFs) affect approximately 1.5 million people in the US each year, resulting in significant pain and morbidity. Approximately 25% of post-menopausal women will have a VCF in their lifetime, with osteoporosis being the major risk factor. Currently, the gold standard surgical treatment to address VCFs is percutaneous vertebroplasty, in which a polymethylmethacrylate (PMMA) bone cement is injected into the vertebral body to stabilize the fracture. However, PMMA is not an ideal material for vertebroplasty due to being much stiffer than the surrounding tissue as it possesses an ultimate compressive strength one to two orders of magnitude greater than native bone. This mechanical mismatch leads to stress shielding into adjacent vertebral bodies causing them to fracture and require further surgical intervention. Previous *in vivo* leporine studies have shown calcium phosphate-loaded, chitosan-based osteoinductive hydrogels to be effective in vertebral fusion procedures, leading to intervertebral stabilization and new bone growth. Our objective is to demonstrate the feasibility of utilizing these bioactive hydrogels for the treatment of VCFs. Hydrogel compressive strength will be measured by mechanical testing as well as short and long-term biocompatibility determined *in vitro* using cell culture experiments. *In vivo* assessment of the biomaterial will be conducted in an osteopenic leporine model. Following percutaneous vertebroplasty with our osteoinductive hydrogel, computed tomography, biomechanical testing, and histology will be used to measure the quantity and quality of new bone growth in the injected vertebrae.

Research Grant: University of Missouri Coulter Program
Student Support: IDEXX BioAnalytics

Establishing the prevalence of ESBL and carbapenemase-producing Enterobacterales in sheltered animals

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Antibiotic resistance is a rising global crisis and critically threatens the basis of modern medicine. Beta-lactam antibiotics are among the most commonly used antibiotics and the most important drugs in both human and veterinary medicine. In veterinary patients, extended-spectrum beta lactamases (ESBL) and carbapenemases present a huge concern for the treatment of Gram-negative bacterial infections. Although infections caused by these bacteria are traditionally viewed as of nosocomially acquired, increasing reports of ESBL-producing Enterobacterales (EPE) and carbapenemase-producing Enterobacterales (CPE) isolated from companion animals suggest the need for surveillance among a variety of animal populations to establish baselines and identify potential sources. Therefore, in this study, we will collect fecal samples from sheltered animals within 1 week of admission to 3 shelter facilities in the Philadelphia region. For each specimen, selective culture with phenotypic and PCR confirmation will be performed to identify EPE and CPE. As we have little knowledge on the prevalence of CPE and ESBL carriage among animals, the proposed research will lay a foundation for future surveillance projects such that we can develop proper control strategies to be implemented in veterinary settings to protect the health of animals and the people who care for them.

Research Grant: None
Student Support: NIH/BI Student Scholars Program

Potential biological implications of *Ascaris suum* infection on porcine microbiome

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Ascaris, a large intestinal-dwelling nematode, is a parasite of public health importance in the family Ascarididae. Different species of this helminth infect all classes of vertebrates (including humans), with *Ascaris suum* infections causing important economic losses in swine - mostly due to reduced feed conversion and impaired liver metabolism - as well as risks of zoonotic infection. As intestinal helminths have previously been found to markedly change gut microbiome composition (and related aspects of host metabolism), it is important to further understand such gut microbial responses as part of future explorations in swine (and human) management/medicine. The aim of this study was to better understand how parasitic infections alter gut microenvironments, and disrupt the structure and function of the gut microbiota in their host. The composition of the gut microbiota of five pigs challenged (infected with *A. suum*), and five age-matched naive controls, was compared using 16S rRNA gene based Illumina sequencing and bioinformatics tools. The results demonstrated that *A. suum* infection was associated with a significant change in relative abundances of various taxa. Among infected pigs, significant increases were seen in the abundance of taxa such as Proteobacteria, several types of Clostridiales, and Spirochaetes; notable decreases were seen in others, e.g. Veillonellaceae, Bacilli, and Coprococcus, suggesting that roundworms may be capable of altering host immunity to increase local colonization of pathogenic bacteria. These findings improved our understanding of the pathophysiological consequences of intestinal nematode infections, and may provide new foci for future microbial medicine and ecology engineering.

Research Grant: USDA (research grant) 8042-32000-116-000D
Student Support: USDA-BIVSP Stipend, USDA ARS NBAF AGREEMENT NO: ARS 59-3022-1-003

Ex vivo biomechanical and microscopic comparison of two cortical screw sizes in fetlock joint arthrodesis

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In horses, osteoarthritis (OA) is especially prevalent in the metacarpophalangeal (MCP) joint. The current treatment for refractory OA of the MCP joint is surgical arthrodesis using locking compression plates in combination with a palmar tension band. One method of palmar tension band application is lag screw fixation of the proximal sesamoid bones (PSBs) to the third metacarpal bone condyles. The aim of this study was to compare the biomechanical properties and microscopic damage of two cortex screws (4.5-mm and 5.5-mm diameter) inserted into the PSB after loading. We hypothesize that increased screw diameter decreases construct failure load and that microscopic damage will be greater in medial lag screws due to horses bearing greater weight in the medial side of the limb. Screws were inserted in five pairs of cadaver forelimbs. After single cycle to failure axial loading, screws were removed and evaluated by low power stereomicroscopy and high resolution scanning electron microscopy. All constructs failed by transverse fracture of the PSB through the screw holes. There was no significant difference in construct stiffness or mean failure load between the 4.5-mm and 5.5-mm screw groups. These results reveal how screw size is not a critical determinant for tension band strength. Surgeons can be confident that either screw size will provide proper strength to the construct. This allows them to prioritize other factors for screw selection such as patient size, screw availability, or price.

Research Grant: None
Student Support: Boehringer Ingelheim and Purdue University's College of Veterinary Medicine

Distribution of tick vectors and risk for tick-borne infectious disease across land use gradients in Oregon

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Ticks are responsible for many vector-borne infections in humans and domestic animals. While incidence of tick-borne disease transmission has increased in the western United States, there is still limited research on disease distribution. Driving forces involved in the emergence of tick-borne diseases are complex and include changes in climate, host and vector abundance, human behavior, and land use. Specifically, patterns of pathogen transmission risk across various land use types are not fully understood. We take a first step in determining the presence and abundance of ticks and their life stages across land use gradients of minimally disturbed, agricultural, and urban environments at two climatically different study sites in Oregon. We are sampling the environment for ticks along transects paralleling the Mary's River in Willamette Valley and the White River, Tygh Creek, and Three Mile Creek in Tygh Valley. Known tick species in the area include *Ixodes pacificus, Dermacentor variabilis, Dermacentor occidentalis* and *Rhipicephalus sanguineus*. Collected ticks will be identified and quantified based on sex and life stage (adult, nymph, larva). Based on anecdotal reports of ticks noted on the local human and pet population, we expect tick abundance to differ between land use types. In a future step, tick DNA will be extracted and amplified via PCR to screen for pathogen presence. Understanding the abundance and distribution of tick vectors at our study sites establishes the foundation for determining the prevalence of infectious pathogens harbored by the respective tick populations and assessing the risk of disease transmission.

Research Grant: Western University of Health SciencesU Intramural Team Grant to Hanselmann and Steinauer; Oregon State University Department of Biomedical Sciences Intramural support to Beechler
Student Support: WesternU Research Fellowship; CVM Scholar Program; Intramural Grant: Hanselmann and Steinauer

Characterization of glucose transport in skeletal muscle of the New Zealand White rabbit

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Skeletal muscle is typically the largest metabolic sink found in animals and is responsible for absorbing significant amounts of glucose and amino acids. The composition of skeletal muscle includes multiple fiber types and is classically divided into Type I (oxidative) and Type II (glycolytic) fibers. Metabolic diseases have risen in prevalence within the animal kingdom and specifically, alterations to glucose handling (ie. Diabetes mellitus) has become a focus of the One Health paradigm. However, some species are known to be highly resistant to diabetes, but the underlying composition of skeletal muscle and overall metabolic pathways that control glucose trafficking remain unexplored. The purpose of this study was to characterize skeletal muscle fiber composition in the New Zealand White rabbits (*Oryctolagus cuniculus*) and identify relevant glucose transporters. Ten, fresh cadaver, male rabbits were utilized, with non-fasting glucose and HbA1c's being recorded. Samples of biceps femoris and apex cardiac muscle being obtained. A novel dot blot assay was used to characterize the composition of the fibers and qRT-PCR was used to determine the expression of 7 glucose transporters (Glut 1-4,8,10,12). Data collection and analysis is ongoing. The results of this study are: 1) To validate a novel dot blot assay for characterization of fiber type using minimal sample and 2) To determine expression of relevant glucose transporters in skeletal and cardiac muscle in a highly diabetes-resistant species. Taken together, this study has the potential to inform diagnosis and treatment plans for metabolic diseases in both human and animal health.

Research Grant: Oklahoma State University College of Veterinary Medicine, Debbie and Wayne Bell Professorship in Veterinary Clinical Sciences
Student Support: Oklahoma State University Summer Research Training Program for Veterinary Students

MAGResp — a database of respiratory metagenome-assembled genomes (MAGs) for cattle and swine

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Respiratory health and disease has a major financial impact on commercial swine and cattle production systems. Shotgun metagenomic sequencing can help improve our understanding of the microbial composition of the respiratory tract, and the creation of metagenome assembled genomes (MAGs) may allow for more specific taxonomic classification. MAGResp will be a database of swine and cattle respiratory MAGs generated from open-source shotgun metagenomic datasets. The goals of this project were to identify candidate datasets for MAG assembly and to generate MAGs for at least one of those datasets. Datasets were identified using PubMed with the key search phrases "swine/pig respiratory shotgun metagenomic" and "cattle respiratory metagenomic/shotgun metagenomic". Results that did not study swine or cattle, utilize shotgun metagenomic data, sample a portion of the respiratory tract, or make their sequencing data open source were excluded from candidacy. Raw datasets were downloaded from their respective repositories, filtered for removal of low-quality and host reads, and MAG assembly was performed using methods described in the PIGC pipeline. The above search terms yielded 32 unique studies, and application of exclusion criteria identified four promising candidate datasets. One of these datasets has been downloaded and is undergoing metagenomic binning. While the literature search is by no-means comprehensive, it highlights the current lack of swine and cattle respiratory studies that utilize shotgun metagenomic data to assess microbial composition. Upon completion of this database, users will be able to curate results based on metadata filters to produce custom reference databases for their respiratory metagenomic studies.

Research Grant: None
Student Support: 2022 FFAR Veterinary Student Fellowship; NIH, Office of the Director, Award Number T35OD011118

Effects of extracellular vesicles from triple-negative breast cancer cells on the tumour microenvironment

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Extracellular vesicles (EVs) are non-classical secretory vesicles that have been shown to play key roles in intercellular communication, particularly in the context of promoting cancer progression. The two major classes of EVs produced by cancer cells; plasma membrane-derived microvesicles (MVs) which range from 200 to 1000nm in diameter and endosomal multivesicular body-derived exosomes which are 30 to 150nm in diameter contain proteins, lipids, nucleic acids and metabolites that exert pro-tumourigenic effects. We have discovered that the MVs and exosomes produced by the triple-negative breast cancer cell line, MDA-MB-231 cells, are highly enriched in Survivin and KRAS, two proteins that have been heavily implicated in driving tumourigenesis. As such, this study investigated the effects MDA-MB-231 derived EVs had on MCF10A (normal mammary epithelial) cells as well as the impact they have on the survival of doxorubicin-induced senescent human dermal fibroblast cells. Collectively, our findings highlight how the EVs generated by highly aggressive forms of cancer cells have the ability to impact the function of non-cancerous cell types within the tumour microenvironment to drive changes that promote cancer progression. Findings from this study, relating to the composition of EVs and their downstream effects on tumour development, provide significant clinical benefit by enabling more efficient cancer diagnosis.

Research Grant: NIH R01 CA259195-01A1
Student Support: Cornell Veterinary Leadership Program

Cortisol levels, hematology, and biochemistry of spawning male Atlantic sturgeon

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This project investigates the health and physiology of endangered Atlantic sturgeon (*Acipenser oxyrhynchus oxyrhynchus*) during spawning in the Hudson River, New York. Throughout their ranges, Atlantic sturgeon are threatened by overfishing, habitat loss, and pollution. Because sturgeon move between saltwater and freshwater at different life stages, their survival and reproductive success depends on the health of both systems. Sturgeon arrive from marine and estuarine feeding areas in good condition and fast during the 4-6 week spawn. Their body condition visibly declines, and related physiologic changes have not been studied. Hematologic and biochemistry reference intervals have also not been reported. This project compares blood levels of glucocorticoid stress hormones between early (late May) and late spawning season (end of June), and develops hematologic and biochemical reference intervals. In collaboration with New York State Department of Environmental Conservation and Delaware State University, samples were collected from 163 spawning male sturgeon in the Hudson River from late May through June. Complete blood counts were performed using Natt and Herrick's stain and a hemocytometer; differential cell counts will be performed on Diff-Quik stained slides. Biochemistry analysis will be performed using automated methods. Cortisol levels will be analyzed using Arbor Assays' Cortisol ELISA Kit. Establishing baseline health information for sturgeon in good condition will help us understand changes due to normal events (spawning) and facilitate future comparisons between populations and across habitats of different quality. Better information on sturgeon life cycle will inform future conservation measures.

Research Grant: Environmental Fellowship, Tufts University Institute of the Environment
Student Support: Unknown

Serum TNF-α and IL-6 concentrations as indicators of early placentitis in pregnant mares

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Infectious placentitis is an important cause of abortion in mares, with a herd prevalence of 9.8-38.1%. Currently, diagnosis is made based on abnormal transrectal and transabdominal uterine ultrasonography, presence of vaginal discharge, early udder development, prenatal lactation, and abortion or foal prematurity. Early diagnosis of placentitis is critical for prompt treatment. Serum inflammatory cytokine concentrations have been shown to increase in mares with advanced experimental placentitis but have not been studied in early natural placentitis. The hypothesis of our study was that increased serum TNFα and IL-6 concentrations are associated with the early onset and severity of placentitis. Pregnant Thoroughbred mares were evaluated for placental health or inflammation starting at 180 days of gestation, and blood samples were taken monthly. Two studies measured serum TNFα and IL-6 concentrations: 1) Pre-onset and early-onset of placentitis: mares with placentitis (n = 12) and with gestational-age matching control healthy pregnancies (n = 12) were tested; early-onset was defined as the time point of the first clinical evidence of placentitis diagnosed by ultrasonography, and the pre-onset was the month before the early onset of placentitis; and 2) Severity of placentitis: severity was based on a scoring system, and the following groups were determined: control (n = 49), pre-onset (n = 11), mild (n = 15), and moderate to severe (n = 23) placentitis. Serum TNFα and IL-6 concentrations were determine using ELISAs. The data are being analyzed statistically to compare cytokine concentrations between groups (placentitis and control), time-points (pre-onset and early onset), and severity (control, mild, moderate and severe).

Research Grant: Harry M. Zweig Memorial Fund for Equine Research
Student Support: Boehringer Ingelheim and Cornell University College of Veterinary Medicine

Developing a behavioral phenotype for Trichotillomania in the rhesus macaque (*Macaca Mulatta*)

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Trichotillomania (TTM) or hair-pulling is an understudied psychiatric disorder in humans primarily affecting females. Barbering in mice has been established as an animal model for TTM to understand the underlying physiological mechanisms. Our goal was to develop a model of TTM in a species that has been reported to naturally exhibit hair-pulling behavior and that is more closely related to humans, the rhesus macaque. Previous studies in nonhuman primates have identified hair-pulling in two ways: by reviewing veterinary health records for reports of hair-pulling, or as a single behavioral category as part of general behavioral assessments. The goal was to further refine the description of hair-pulling by isolating different typologies of the behavior. Identifying and describing the range and types of hair-pulling is foundational for both identifying potential environmental triggers for the behavior and measuring physiological changes in affected individuals. We developed a coding scheme that identified 6 types of hair-pulling behavior and differentiated whether the behavior was self or cagemate-directed. Using this coding scheme to identify hair-pullers will lay a foundation for both identifying potential environmental triggers and measuring underlying physiological mechanisms. Previous research has found improvement in TTM expression with antioxidant supplements, and we aim to treat hair-pulling in nonhuman primates with supplements in addition to enrichment in the future. This project will take us one step closer to discovering the mechanism behind hair-pulling and allow us to develop interventions and treatments to benefit the animals' outcomes, as well as provide potential targets for treating the human condition.

Research Grant: NIH P51OD011106
Student Support: The School of Veterinary Medicine Dean's Office

Effect of day of additional PGF2α during timed-AI on ovarian dynamics and fertility of Holstein heifers

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All recommended timed-artificial insemination (AI) programs for dairy heifers use intravaginal progesterone (P4) implants; however, P4 implants are costly and onerous. Results from our laboratory indicated that using prostaglandin F2α (PG) 2 d before the initiation of these programs allows the removal of the P4 implants from the protocol without compromising the proportion of heifers synchronized (PG-2d-GnRH-6d-PG-1d-PG-1d-GnRH + AI). Our previous results also showed that an additional PG at the end of the protocol was necessary to maximize luteal regression and optimize pregnancies per artificial insemination (P/AI). Our objective was to determine if delaying the additional PG treatment until the day of the last GnRH and AI would affect P/AI in Holstein heifers receiving a modified timed-AI program. Combining PG and GnRH treatments and AI on the same day would reduce animal handling requirements, diminishing labor, cost, and animal stress. We hypothesized that delaying the additional PG treatment would not affect P/AI after timed-AI. To test this hypothesis, Holstein heifers (n = 244) from 3 commercial dairy farms were randomly assigned to either control (CON) or treatment (Tx). Both groups received PG (0.5 mg of cloprostenol i.m.) on d -8, GnRH (86 mcg i.m.) on d -6, and PG on d 0. Heifers in CON received the extra PG on d 1 and GnRH and timed-AI on d 2, while heifers in Tx received the extra PG simultaneously with GnRH and timed-AI on d 2. Farm veterinarians performed pregnancy diagnosis using ultrasound from 33 to 35 d after AI. Our preliminary results from a subset of heifers (n = 123) indicate that delaying the additional PG to the same day of GnRH and timed-AI seems to reduce P/AI (CON = 54% vs. Tx = 42%).

Research Grant: Boehringer Ingelheim
Student Support: Foundation for Food & Agriculture Research 2022 Veterinary Student Research Fellowship Grant