Posting From the Event?

Use #ResearchAtPennVet

Cover Photo:

Matrix-degrading enzyme activity (green) clears a pathway for invading breast cancer cells (red) at the tumor-stromal border. Photo credit: Becky Brisson, Volk Laboratory
8:30 a.m.  
**REGISTRATION & CONTINENTAL BREAKFAST**

8:55 a.m.  
**OPENING REMARKS**
Phillip Scott, PhD  
Monserrat C. Anguera, PhD  
*Vice Dean for Research & Academic Resources  Chair, Research Retreat Organizing Committee*

9:00–10:05 a.m.  
**BIOMEDICAL SCIENCES – Introduction by Michael J. May, PhD**

9:05–9:25 a.m.  
Unusual cell lineage plasticity caused by knock-out of transcription factor YY1  
Michael L. Atchison, PhD

9:25–9:45 a.m.  
Global mechanisms of protein regulation  
Anna Kashina, PhD

9:45–10:05 a.m.  
Rewired regulatory pathways involving retrotransposons impact reproduction and early development  
Andrew J. Modzelewski, PhD

10:05–11:10 a.m.  
**CLINICAL SCIENCES AND ADVANCED MEDICINE – Introduction by Anna M. Massie, DVM**

10:10–10:30 a.m.  
When the scalpel doesn’t cut it...A veterinary surgeon-scientist’s approach to regenerative medicine and comparative oncology  
Susan W. Volk, VMD, PhD, DACVS

10:30–10:50 a.m.  
Targeting regulatory T-cells in canine glioma—Clinical implications with translational potential  
Wojciech K. Panek, DVM

10:50–11:10 a.m.  
Minimally invasive thoracic duct embolization (TDE) for chylothorax  
Dana L. Clarke, VMD

11:10–11:45 a.m.  
**POSTER SESSION – Introduction by Montserrat C. Anguera, PhD**

11:45–12:45 p.m.  
**LUNCH**

12:50–1:45 p.m.  
**MARSHAK LECTURE – Introduction by Montserrat C. Anguera, PhD**

Is cognition the secret to dogs’ success?  
Brian Hare, PhD

1:45–2:50 p.m.  
**PATHOBIOLOGY – Introduction by Louise H. Moncla, PhD**

1:50–2:10 p.m.  
Canine neurologic diseases as natural models for human disease  
Molly E. Church, MS, VMD, PhD, DACVP

2:10–2:30 p.m.  
Wildlife Futures research  
Erick Gagne, PhD

2:30–2:50 p.m.  
Parasite sex and why it’s important to understand  
Boris Striepen, PhD

2:50–3:10 p.m.  
**BREAK**

3:15–4:20 p.m.  
**CLINICAL STUDIES—NEW Bolton CENTER – Introduction by Mary A. Robinson, VMD, PhD**

3:20–3:40 p.m.  
Flow-controlled ventilation—A new way to improve pulmonary mechanics  
Klaus Hopster, DVM, PhD

3:40–4:00 p.m.  
Microbiome medicine: Towards sustainable livestock systems  
Dipti Pitta, BVSc, MVSc, PhD

4:00–4:20 p.m.  
Classification of equine ambulatory events using machine learning analysis of inertial measurement data  
Darko Stefanovski, PhD

4:20–4:30 p.m.  
**ZOEITIS PRIZE – Awarded by Christopher J. Lengner, PhD**

4:30–5:30 p.m.  
**PRIZES, POSTER SESSION, AND RECEPTION**
Acknowledgments

Faculty Organizing Committee:

Montserrat C. Anguera, PhD, Chair
Matthew J. Atherton, BVSc, PhD
Charles W. Bradley, VMD
Anna M. Massie, DVM
Michael J. May, PhD
Andrew J. Modzelewski, PhD
Louise H. Moncla, PhD
Mary A. Robinson, PhD, VMD
Phillip Scott, PhD

The University of Pennsylvania School of Veterinary Medicine is grateful to the organizations, sponsors, donors, and families who have made our research possible.

Zoetis: Our thanks to Zoetis for the 2023 Zoetis Award for Veterinary Research Excellence to be presented to a member of the Penn Vet faculty. The award aims to foster innovative research by recognizing outstanding research effort and productivity in the veterinary profession.

Robert R. Marshak Lectureship: We are grateful to the memory of Dean Emeritus Robert R. Marshak, who was the dean of the School of Veterinary Medicine from 1973 to 1987. He was a visionary leader of the profession and a pioneer of bovine practice.

We thank our generous and helpful friends and colleagues, including John Donges; Ashley Hinton; Colin Redick; Stephen Hawkins; Mary Berger; Dr. Thomas Parsons; and Melissa Sage, Inn at Swarthmore.

Photos (clockwise from top):
1.) Mouse embryonic fibroblasts expressing GFP-tagged actin isoforms. (Anna Kashina)
2.) Mouse preimplantation embryo that resembles a mouse, for fun. (Andrew Modzelewski)
3.) Wild turkey sampling as a part of the turkey health project. (Erick Gagne)
Brian Hare, PhD
Professor of Evolutionary Anthropology
Duke University

Is cognition the secret to dogs’ success?

Dogs have more jobs than ever, but the demand for the best trained dogs far exceeds the supply. The challenge is identifying dogs that are most likely to succeed with working dog training. Here I will present data on individual differences in dog psychology that demonstrate that dogs have different types of cognition and these abilities vary independently. I will then explain how the existence of cognitive profiles in dogs has the potential to enhance the selection, breeding and rearing of working dogs. To tell the story I will share what we have learned so far about puppies, service dogs, pets, and even some wolves. The ending we are working toward is one where 1) we can train dogs for jobs they are most likely to succeed in and 2) more people in need can benefit from the help a dog can provide. On the way we will continue translating what we learn so it is relevant to the well-being of all dogs – including our family dogs. I will conclude by sharing how we are currently working toward these goals.

Dr. Brian Hare is a core member of the Center of Cognitive Neuroscience, a professor in evolutionary anthropology, and psychology and neuroscience at Duke University. He received his PhD from Harvard University in 2004, and in 2005, following his work at the Max Planck Institute in Leipzig, was awarded the Sofia Kovalevskaja Award—Germany’s most prestigious award for scientists under 40. In 2009, after arriving at Duke University, he established the Duke Canine Cognition Center. Hare has published over 100 scientific papers including in Science, Nature, and PNAS. He has received external support from NIH, NSF, ONR, and a number of private foundations. He co-authored The Genius of Dogs, a New York Times Bestseller, and Survival of the Friendliest, an international bestseller with his wife Vanessa Woods. Their third book together, The Puppy Kindergarten, will be out in summer 2024 from Random House.
Michael L. Atchison, PhD
Dr. Michael Atchison obtained his BS degree in biology from the State University of New York at Albany and obtained his PhD in cell and molecular biology from New York University School of Medicine. He moved to Philadelphia to perform a postdoc with Robert Perry at Fox Chase Cancer Center and joined the Penn School of Veterinary Medicine as an assistant professor in 1988. Currently, he is a professor of biochemistry in the Department of Biomedical Sciences. He has directed the VMD-PhD program at Penn Vet since 2001 and co-directed the NIH/BI Summer Scholars Program since 1990. His laboratory studies transcriptional regulation and epigenetic mechanisms of lineage development within the hematopoietic system, particularly the B cell lineage.

Molly E. Church, MS, VMD, PhD, DACVP
Dr. Molly Church earned her VMD from Penn Vet in 2009 and joined the faculty in 2016 as an assistant professor of anatomic pathology in the Department of Pathobiology. She received her bachelor's and master's degrees at the University of California (UC) Santa Cruz. After earning her VMD, she completed her residency training in anatomic pathology, as well as a PhD in comparative pathology, at UC Davis. Since returning to Penn Vet as a faculty member, she has had the opportunity to hone her diagnostic abilities in its busy biopsy and autopsy services and to work with a diverse group of clinicians and researchers, resulting in numerous exciting collaborative research projects. As a CE faculty member, her research and clinical interests are intertwined and reflect her passion for neuropathology. Her interests are focused not only on the diagnosis of neuroinflammatory disease and neoplasms of the central and peripheral nervous system, but also on detailed molecular examination of these diseases in an effort to improve diagnostics and gain a better understanding of the underlying pathogenesis.

Dana L. Clarke, VMD
Dr. Dana Clarke graduated from the University of Pennsylvania in 2006. After graduation, she completed a one-year rotating internship at Michigan State University, followed by a residency in emergency/critical care at the University of Pennsylvania. Upon completion of her residency in 2010, she spent one year observing in the interventional radiology service at the Hospital of the University of Pennsylvania. She then became the director of the interventional radiology program at Penn Vet and has a dual appointment in the sections of surgery and critical care. In 2015, she was appointed to the first faculty position in interventional radiology in veterinary medicine. Her research and clinical interests include developing a better understanding of the progression and physiology of tracheal collapse, improving tracheal stent design and sizing, vascular malformations and obstructions, and all forms of respiratory disease within the ICU.
Erick Gagne, PhD
Originally from Philadelphia, Dr. Roderick “Erick” Gagne obtained his master’s degree and PhD from Tulane University in ecology and evolutionary biology. There he studied the disease ecology of an invasive parasite infecting Hawaiian stream fishes. Subsequently, he was a postdoc at Colorado State University where he used genomic data of the host and pathogen to assess how landscape features and demographic factors influence the spread of disease. He is currently an assistant professor of wildlife ecology at the University of Pennsylvania School of Veterinary Medicine and a part of the Wildlife Futures Program. His research integrates genetic and ecological approaches to evaluate infectious diseases. His focus is on understanding the transmission of wildlife disease on landscapes as well as the ecology and evolution of pathogen spillover.

Klaus Hopster, DVM, PhD
Dr. Klaus Hopster is the Marilyn M. Simpson Associate Professor of Large Animal Anesthesia in the Department of Clinical Studies—New Bolton Center. Dr. Hopster received his DVM degree from the Hannover School of Veterinary Medicine in Germany in 2006. He then performed a rotating internship at the Equine Hospital while being enrolled in a doctorate program that he completed in 2007 with a thesis entitled “Open-Lung-Concept ventilation during general anesthesia of the horse and its influence on the early post-operative period.” From 2007 to 2011, Dr. Hopster was enrolled in an ECVAA-approved residency program in veterinary anesthesia and analgesia, and after the successful conclusion of this program, he obtained the ECVCAA diplomate status. Upon finishing his training program, Klaus accepted the position of senior lecturer in anesthesia, pain management, and critical care at the Hannover School of Veterinary Medicine's Equine Hospital, which he held until his departure to the University of Pennsylvania in 2016. Over the past 15 years, his research activities have focused on intraoperative lung function and tissue oxygenation in the horse. He has published numerous other original scientific and review articles, book chapters, abstracts, and proceedings in the field of veterinary anesthesiology and is a frequent speaker at national and international conferences and seminars. Dr. Hopster is a member of the Association of Veterinary Anaesthetists (AVA), the European College of Veterinary Anaesthesia and Analgesia (ECVAA), and the Anesthesia, Intensive & Emergency Care, and Pain Management (VAINS) section of the German Veterinary Association (DVG).

Anna Kashina, PhD
Dr. Anna Kashina's research focuses on protein regulation, including posttranslational modifications, protein isoform diversity, and protein misfolding in disease. She has been a faculty member at Penn Vet since 2004. Her research pioneered the studies of protein arginylation, a posttranslational modification that acts as a global biological regulator and plays a major role in embryogenesis and prevention of heart disease and neurodegeneration. She also discovered that nucleotide coding sequence and silent substitutions can regulate biological functions of closely related protein isoforms. Her recent interests include prion diseases and novel approaches to diagnostics of chronic wasting disease in deer.
Andrew J. Modzelewski, PhD
Dr. Andrew J. Modzelewski (Dr. Modz) is an assistant professor in the Department of Biomedical Sciences who was recruited to Penn Vet last year. Dr. Modz received his BS from Penn State University with a major in Biochemistry and Molecular Biology. Dr. Modz then went to Cornell University for his PhD in genetics, genomics and development with Dr. Paula Cohen where he developed an interest in reproduction and development with a special interest in non-coding RNAs. He did his postdoc at the University of California at Berkeley with Dr. Lin He, an expert on miRNAs and cancer, but shifted to early embryos and ancient viral elements (retrotransposons). Dr. Modz modified and developed various tools to study the phenomenon of retrotransposon reactivation that occurs in all mammalian preimplantation embryos. One of these tools is an electroporation-based CRISPR/Cas9 delivery system called “CRISPR RNP Electroporation of Zygotes” (CRISPR-EZ). Despite being called “Junk DNA,” Dr. Modz published evidence of the first essential retrotransposon in mammalian preimplantation development, suggesting instead a “symbiotic” instead of parasitic relationship. At Penn, Dr. Modz plans to further study the developmental roles of retrotransposon reactivation in the early embryo and reproduction and extend this to instances of epigenetic breakdown that occur in aging, disease, and cancer, where retrotransposons frequently re-emerge and potentially contribute to malignancies.

Wojciech K. Panek, DVM
Dr. Wojciech Panek serves as an assistant professor of neurology and neurosurgery at Penn Vet. He graduated from Wroclaw University of Environmental and Life Sciences, Poland. Following rotating and surgical internships, he completed a 2-year neuro-oncology fellowship at NU, Feinberg School of Medicine in the Department of Neurology/Neurosurgery, Chicago, IL. He then went on to complete a 2-year fellowship focusing on neuro-aging in companion dogs at NC State University, followed by a 3-year neurology and neurosurgery residency program at UC Davis. His research includes translational neuro-oncology and neuro-aging and primarily focuses on developing a comprehensive understanding of the regulatory signals that govern the immune system mobilization and/or exhaustion in patients suffering from CNS tumors and canine cognitive dysfunction.

Dipti Pitta, BVSc, MVSc, PhD
Dr. Dipti Pitta completed her Bachelor of Veterinary Science and Master of Veterinary Science programs in India. She then received her specialty training in ruminant nutrition and microbiology as a part of a PhD degree program from Massey University, New Zealand. Her career took her to AgResearch, New Zealand as a junior scientist, then to Texas A&M University before joining as a faculty member at the University of Pennsylvania School of Veterinary Medicine. She has worked on several projects involving forages, environmental issues, nutritional aspects, microbial ecology, biometrics, and food safety. In her Agricultural Systems and Microbial Genomics (ASMG) Laboratory at Penn Vet, she has several ongoing projects using dairy cattle both at Penn Vet’s dairy herd and commercial herds. The current work predominantly focuses on two major areas: (i) understanding the mechanistic basis of methanogenesis to develop novel mitigation strategies for enteric methane abatement, and (ii) investigating the lasting effects of early life microbial interventions on health, well-being, productivity, and methane emissions in ruminants. Dr. Pitta is also involved in several collaborative projects to investigate the role of gut microbiome in health and disease in equine, swine, and honeybees. She has secured over $4 million in extramural support, serves as an advisory board member for multiple organizations, and mentors graduate students from diverse disciplines.
**Darko Stefanovski, PhD**

Dr. Darko Stefanovski’s independent research program is in the area of biostatistical and biomathematical modeling and, most recently, the integration of these two fields under the umbrella of machine learning. Furthermore, he oversees the maintenance and development of the mathematical modeling software WinSAAM. For many years, his research has been focused on glucose metabolism and associated pathologies such as sleep disorders, obesity, diabetes, cardiovascular disease, and cancer.

**Boris Striepen, PhD**

Dr. Boris Striepen studied biology at the universities of Bonn and Marburg, and conducted undergrad research on liver flukes in Bonn, and trypanosomes in Bobo Dioulasso, Burkina Faso. He earned a PhD for work on parasite biochemistry with Ralph Schwarz, was a postdoc with David Roos, studying parasite cell biology, and started his own laboratory at the University of Georgia in 2000. In 2017, he moved to the University of Pennsylvania. Dr. Striepen studies the cell and molecular biology of apicomplexan parasites. His current research focus is the parasite *Cryptosporidium*, a leading global cause of severe diarrhea and mortality in young children. His lab pioneered molecular genetics and mouse models for this important infection and leads a range of interdisciplinary efforts to understand fundamental parasite biology and advance translation toward drugs and vaccines.

**Susan W. Volk, VMD, PhD, DACVS**

Dr. Susan Volk is the Corinne R. and Henry Bower Professor of Small Animal Surgery at the University of Pennsylvania School of Veterinary Medicine. Dr. Volk completed the Veterinary Medical Scientist Training Program (VMD-PhD), as well as a small animal rotating internship and surgical residency at the University of Pennsylvania, before joining the faculty of Penn Vet in 2007. Her laboratory is focused on defining mechanisms by which the extracellular matrix, particularly collagens, regulate cell activities and fate in both regenerative and tumor microenvironments. This NIH, private foundation, and industry-sponsored research has basic and translational components, including clinical trials in veterinary patients. As a veterinary surgeon-scientist, she is passionate about applying this basic science knowledge to develop innovative regenerative and oncologic therapies to close clinical gaps for both veterinary and human patients. In addition to her translational research and clinical work, she also serves in national leadership positions in the field of tissue repair and regeneration, including currently serving on the board of directors for the North American Veterinary Regenerative Medicine Association and as vice president of the Wound Healing Society.
Abstract Numbers

The abstract numbers below correspond to the poster board number. Abstracts are ordered alphabetically by the presenting author's last name.

1. Jennifer I. Alexander
2. Tatyana Appelbaum
3. Nasreen Bano
4. Madeline Boyes
5. Madeline Boyes
6. Rachel Butler
7. Michelle D. Cully
8. Jessica F. DiStefano
9. Valerie L. Dufour
10. Thomas Ede
11. Kristen Esannason
12. Lidia Flor Cuenca
13. Lidia Flor Cuenca
14. Jeffrey S. Frankel
15. Ronald N. Harty
16. Ronald N. Harty
17. David Holt
18. Sarah Ibach
19. Amy Johnson
20. Jennifer C. Kwok
21. Alessandro P. Lamacchia
22. Elizabeth M. Lennon
23. Ning Li
24. Amritha Mallikarjun
25. Marcella Massimini
26. Leif K. McGoldrick
27. Shelby Monnin
28. Kapil S. Narayan
29. Imani Nicolis
30. Mana Okudaira
31. Marisol Parada Sarmiento
32. Brianna E. Parsons
33. Olivia A. Pilling
34. Meghan T. Ramos
35. Antonia Rotolo
36. Leandro Sabei
37. Sulagna Sanyal
38. Carlo Siracusa
39. Georgia Skelton
40. Kristofer C. Smith
41. Daniel Sorobetea
42. Jack Swain
43. Kei Takahashi
44. Eoin Christopher Whelan
45. Clara Wilson
46. Abigale Zoltick
1. **Hedgehog Signaling Converges with HAP40 to Control Intestinal Aging and Disease.**

Jennifer I. Alexander¹,², Alana M. O’Reilly¹, and Christopher J. Lengner².

¹Cancer Signaling and Microenvironment, Fox Chase Cancer Center, Philadelphia, PA; ²Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Alarmingly, Colorectal Cancer (CRC) incidence is disproportionately rising in young patients (EAO-CRC) without known cause. However, the underlying mechanisms at the intersection of aging biology and EAO-CRC pathogenesis remain elusive. By leveraging the powerful molecular genetics of the fly-to-human intestinal organoid pipeline, our goal is to delineate the mechanisms of aging that drive EAO-CRC. We discovered a novel Hedgehog (Hh)-dependent mechanism that balances autophagy-based cellular repair and proliferative regeneration to sustain healthy tissue aging. Specifically, we identified the Hh effector Patched (Ptc) as the critical switch for regulating both processes. We also found that the autophagy-associated transcript, HAP40, was reduced in Ptc hypomorphs. We then determined the impact of gain or loss of HAP40 in intestinal cells and observed evidence of increased cellular aging coupled with decreased survival and proliferation. We then queried human-derived transcriptomic data to assess HAP40 status in Hh-dependent cancers and found that 7% of patients displayed either gene amplification or deep deletion. We next compared the diagnosis age of patients, finding that patients bearing mutations in the HAP40 gene were diagnosed younger than those without mutations. Moreover, survival probability of patients with normal HAP40 and Hh pathway signaling was significantly better than patients with mutant HAP40 or Hh effectors. The phenotypic similarities and dependency of HAP40 expression on Ptc suggests a new link between Hh and HAP40 signaling that is critical for intestinal tissue maintenance and aging regulation in flies. Taken together, this suggests a model in which Hh pathway components intersect with an understudied gene as a dual signaling cassette that impacts patient outcomes. Our next goal is to generate isogenic patient-derived CRC organoids to validate our experimental and transcriptomic data. Delineation of the sequential activation steps of this novel pathway will establish a new paradigm for the biological impact of Hh signaling driving EAO-CRC.

**Research Grant:** GMAP Region 4 Pilot Funding in Cancer and Cancer Health Disparities Award

2. **The Landscape of the RPGR Exon ORF15–Haplotype Diversity in Human Populations.**


Department of Clinical Sciences & Advanced Medicine, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Genetic variants in the RPGR exon ORF15 cause X-linked retinitis pigmentosa (XLRP) characterized by severe visual impairment early in life. The underlying basis for extensive clinical heterogeneity in RPGR-XLRP remains to be fully elucidated. We performed in-depth studies of exon ORF15 sequence variation across human populations to provide more insights on the genetic architectures of this gene region. Specifically, phased X chromosome.vcf files were downloaded from the 1000 Genomes website (whole-genome sequencing data from 2504 normal individuals). The data covers 26 populations encompassing 5 major ancestral groups (Africans [AFR], admixed Americans [AMR], East Asians [EAS], Europeans [EUR], and South Asians [SAS]). Sequencing datasets were processed extracting the phased information, calculating allele frequency and counting the haplotypes. We localized a total of 24 sequence variants (20 SNPs and 4 indels) within the ORF15 region. Additionally, haplotypes of the interval (n=34) were detected. Analysis of haplotype distribution and linkage disequilibrium patterns in ancestral groups identified the prevalence of 5 major haplotypes with frequencies exceeding 5% in at least 3 out of total 5 ancestral groups. The major haplotype corresponding to RPGR reference sequence NM_001034853 with no polymorphisms has the highest frequency, and varied between 32% (EAS) to
64% (EUR). The frequencies of 4 other major haplotypes have population specific pattern ranging from 37.6% to 2.5% between groups. From the remaining 29 haplotypes, two were exclusive for AMR group (2% and 8.3%, respectively), 4 haplotypes were present with frequency between 2.1% to 3.2% in at least one out of the 5 ancestral groups, and 23 haplotypes were not exceeding 1% frequency. The prevalence of the haplotype with no polymorphisms in major ancestral groups provides clear evidence for selective constraint in this RPGR gene region. We also found evidences of recombination among ORF15 haplotypes potentially creating the diversity of low-frequency ORF15 haplotypes.

Support: EY-06855, -17549, the Foundation Fighting Blindness, the Alcon Research Foundation

3. YY1’s Role in B Lineage Commitment.

Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Stem cells produce various lineages during hematopoietic development, but after a specific progenitor stage, each lineage follows a predetermined path. In bone marrow, lymphoid-primed progenitors differentiate into B, T, and NK cells. In transitioning from the pre-pro-B cell to the pro-B cell stage, cells exclusively commit to the B-cell lineage. We have investigated the impact of conditional knockout (KO) of the transcription factor (TF) YY1 in pro-B cells. We previously showed that when grown on DL-4 feeder cells which provide Notch signaling, YY1KO pro-B cells, but not wild-type pro-B cells, developed into T lineage cells. We found that YY1KO pro-B cells that had developed into T-lineage-like cells showed RNA-seq patterns that closely resembled the T-lineage, while simultaneously losing their B-lineage expression profile. We evaluated chromatin and RNA expression properties of YY1KO vs wild-type pro-B cells to determine the mechanism of this unusual lineage plasticity. DNA binding sites for TF important for B-lineage development (Pax5, EBF1, E2A) were in less accessible chromatin in YY1KO compared to wild-type pro-B cells as seen in the ATAC-Seq data. Simultaneously, DNA binding sites for TFs important for alternative hematopoietic lineages (Elf4, Runx1, IRF3) were in more accessible chromatin in YY1KO pro-B cells. Gene Ontology analyses showed genes in pathways involved in development of alternative lineages were also in more accessible chromatin in YY1KO pro-B cells. The ATAC-seq peaks were reduced for some B-lineage genes (Cd79a and Rag2), whereas increased for alternative lineage genes (Gata3 and Ccr2). Finally, scRNA-seq data from YY1KO pro-B cells showed a multiplicity of genes expressed representative of diverse hematopoietic lineages. These data indicate that YY1 is necessary for maintenance of B lineage commitment, and for repression of alternative lineages.


Madeline Boyes⁵, Axel C. Moore⁶, Julie Engiles⁵, Benjamin Sinder⁷, Klaus Hopster⁵, Jason Anari⁷, Srima Balasubramanian⁴, Edward Vresilovic⁵, Dawn M. Elliott⁶, Thomas P. Schaer⁶, Brian D. Snyder⁵, and Patrick J. Cahill⁵.

¹School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA; ²University of Delaware, Newark, DE; ³Children’s Hospital of Philadelphia, Philadelphia, PA; ⁴Drexel University, Philadelphia, PA; ⁵Boston Children’s Hospital, Boston, MA.

IVD distortion contributes to early AIS, with subsequent vertebral wedging as deformity progresses. Spine anatomy can be modulated by manipulating the mechanical milieu during growth (Heuter-Volkmann). Using a growing pig model where a posterolateral tether produced a lateral bending moment to induce a progressive scoliosis, we investigated hierarchical tissue remodeling.

Rapidly-growing 12-wk old Yorkshire pigs (n=3) were instrumented with a subcutaneous CoCr tether spanning the thoracolumbar(TL) and lumbar(L) spine to create a lateral bending moment that incited a progressive scoliosis. Changes to vertebral body(VB) and IVD anatomy over time and space were measured by serial CT and MRI (T1-FLASH, T2-CPMG). After sacrifice @ 22 wks post-op, functional spine units were isolated at the apex of deformity for μCT and histology.

Acute scoliosis (17°) was mediated by IVD wedging. From 6-12 wks deformity was shared between TL(45-38%) and L(41-47%) regions, with IVD(40-46%) and VB(60-54%) wedging contributing to overall scoliosis. By 19 wks, deformity was
mainly imparted by VB wedging. Asymmetric loading modulated tissue structure and function. MRI and histology demonstrate translation of the nucleus pulposus (NP) from concavity (compression) towards convexity (tension). μCT and histology suggest that compression inhibits physeal growth manifest by $\downarrow$epiphyseal height @ concavity and reactive changes in bone morphology: $\uparrow$6.4% endplate thickness; $\uparrow$2.4% trabecular number. Cartilaginous endplate(CEP) thickening may reduce tissue diffusivity, leading to IVD degradation as evidenced by degeneration of annulus fibrosis(AF) by chondroid metaplasia and fibrillation of inner AF rings. Degenerative changes within NP include multifocal loss of notochordal cells(NC) and extracellular matrix with NC necrosis.

These multi-level, hierarchical data indicate that mechanically induced asymmetric spine growth provokes IVD distortion and degenerative processes; initiated by CEP thickening that may reduce endplate diffusivity, followed by compression mediated physeal growth inhibition resulting in progressive vertebral wedging.

5. **In Vivo Measurement of Tether Tension in a Pig Model of Spinal Deformity.**

Madeline Boyes¹, Axel C. Moore², Klaus Hopster¹, Benjamin Sinder³, Jason Anari³, Sriram Balasubramanian⁴, Edward Vresilovic⁵, Dawn M. Elliott², Thomas P. Schaer¹, Brian D. Snyder³, and Patrick J. Cahill⁶.

¹School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA; ²University of Delaware, Newark, DE; ³Children’s Hospital of Philadelphia, Philadelphia, PA; ⁴Drexel University, Philadelphia, PA; ⁵Boston Children's Hospital, Boston, MA.

Surgical interventions such as Vertebral Body Tethering (VBT) attempt to correct scoliosis, while preserving spinal motion. A static tether tension is set during surgery, however *in vivo* the loading environment is quite different: rather than a constant applied load/bending moment, there is variation in applied stresses/strains in time and space that result in stochastic loading. The purpose of this study was to determine the *in vivo* variation in tether tension in a porcine model of spinal deformity.

Under IACUC approval, in a 6-week-old, 20 kg, female Yorkshire pig, a unilateral tether was placed in the thoracic to lumbar spine using a subcutaneous laterally offset stainless-steel cable spanning two posterior pedicle screw clusters at T9-10 and L4-5 respectively. The applied cable force was measured in real time using an in-line spring assembly, attached to a submersible load cell. Cable force was recorded.

During surgery ~40N of tension was applied to the cable connecting the thoracic and lumbar vertebral anchor points. There was a decline in cable force to ~15-20N over the ensuing 24hrs reflecting the viscoelastic stress relaxation of the soft tissues. Videos synchronized with force data revealed variation in cable force associated with movement. Cable force measured 7 days following surgery revealed that ambulation produced forces ranging from 0 to 40N. CT imaging at day 7 with the pig positioned sternal recumbency demonstrated a cable force of 7 N. Bending obtained under general anesthesia demonstrated that a lateral bend of 8° away from the tether produced a tension of 24N while a 35° lateral bend towards the tether resulted in a force of 10N.

Imposed forces were dynamic, exhibiting a wide range of amplitudes and frequencies. Future work will focus on longer-term monitoring of the dynamic forces generated by the tether during controlled treadmill walking combined with real-time motion capture imaging to determine the kinematics of spinal motion.

**Support:** Funded by Wyss Campbell Center for Thoracic Insufficiency Syndrome (Children’s Hospital of Philadelphia).

6. **Pre-Clinical Large Animal Model of Thoracic Insufficiency Using Yucatan Mini Pig.**

Rachel Butler⁷, Klaus Hopster³, Madeline Boyes¹, Benjamin Sinder³, Patrick Cahill³, Brian Snyder³, and Thomas Schaer³.

⁷School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA; ³Children’s Hospital of Philadelphia, Philadelphia PA; ³Boston Children's Hospital, Boston MA.

Thoracic Insufficiency Syndrome (TIS) represents a novel form of postnatal pulmonary hypoplasia and restrictive respiratory disease that occurs in children (<10 yrs.) with congenital or acquired anomalies of the spine and thorax.
Developing a pre-clinical large animal model for TIS as a testing platform to evaluate respiratory development, the pathoanatomy associated with thoracic insufficiency, and to parametrically evaluate the efficacy of different treatment strategies is fundamental to reducing the morbidity and mortality of TIS. The purpose of this study was to develop a pre-clinical mini-pig model of TIS, induced by tethering the rib cage. Under IACUC approval two ♀ 8-week-old Yucatan mini-pigs weighing 8 kg were used (n=1 tethered animal, TA; n=1 age-matched control, AMC). Ribs were surgically divided into three groups and tied together. Pigs were anesthetized for serial CT scans at various timepoints pre/postoperatively (0-68 weeks) to document progressive thoracic and scoliotic deformity, and corresponding lung hypoplasia. The extent of scoliosis, lung morphology (LM), and mean lung volume (MLV) were evaluated from reconstructed CT images. Pulmonary function was evaluated by calculating dynamic lung compliance (Cdyn). The LM and MLV of TA initially revealed differences compared to AMC, however by 68 weeks they were similar. Cdyn was most affected. Intermittent positive pressure ventilation (IPPV) increased Cdyn by more than 65% for AMC @ 68 weeks, while IPPV increased Cdyn for TA by only 15% @ 68 weeks. TA reached a maximum Cdyn by 6 weeks (32 mL/cmH\textsubscript{2}O) where AMC reached maximum Cdyn at 20 weeks (48 mL/cmH\textsubscript{2}O). In contrast, flow-controlled expiration (FLEX) increased Cdyn for AMC by 120% and TA by 100% at 68 weeks. Like children with TIS, despite normalization of overall lung volume and morphology, the result of compensatory hypertrophy from constricting the chest wall by rib tethering decreased respiratory compliance in the Yucatan mini-pig model.

**Research Grant:** The Wyss/Campbell Center for Thoracic Insufficiency (Children’s Hospital of Philadelphia)

### 7. Loss of IKKα in Lymphatic Endothelial Cells Disrupts Lymphoid Organ Formation and Protects Mice from Infection with Influenza Virus.

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Immune surveillance and protection of tissues from infection relies on strategically located secondary lymphoid organs (SLOs), including lymph nodes (LNs), and the lymphatic vasculature which connects them. LNs provide an environment for efficient antigen sampling from the lymph and support robust lymphocyte responses. The non-canonical NF-κB pathway is required for SLO development and recent evidence suggests that activation of this pathway in lymphatic endothelial cells (LECs) promotes lymphoid organogenesis. To study this, we generated mice lacking LEC-intrinsic IKKα, a central kinase in the non-canonical NF-κB pathway. These mice fail to develop LNs and strikingly, we found aggregates of immune cells in their lungs in the absence of inflammation. Further assessment of these immune cell accumulations revealed them to be tertiary lymphoid organs (TLOs). TLOs are ectopic lymphocyte follicles that arise in a range of inflammatory diseases, but the mechanisms that control their formation and function remain incompletely understood. As formation of TLOs in response to infection has been shown to provide protection against respiratory pathogens, we hypothesized that the pre-existing TLOs would provide a more robust local immune response in the lung to influenza infection. Surprisingly, both the B cell response and the rate of viral clearance were similar to littermate controls. Consequently, while our findings reveal that targeting IKKα in LECs disrupts lymphoid organ formation, the precise mechanisms underlying the protection from influenza infection remain to be established. Our ongoing work is focused on determining if protection is due to the presence of TLOs or if this is a function of a separate role for LECs.


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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. 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, Boehringer-Ingelheim

9. Stargardt Disease: Phenotypic Characterization and Natural Disease History of ABCA4 Mutation in a Canine Model.

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Purpose: A 4176insC in ABCA4 exon 28 results in a frameshift and premature stop, and causes a recessively inherited retinal disease in dogs. A research colony has been established, and dogs from the service dog population have been recruited for studies to characterize the phenotype and natural disease history.

Approach: Eleven affected dogs (5M/6F) and 9 heterozygous controls (4M/5F) were phenotyped non-invasively between 9 weeks and 10.8 years of age by fundus photography, cSLO/sdOCT, ERG and visual function assessment.

Results: Functional and structural abnormalities were limited to the ABCA4-affected dogs, and heterozygotes were normal under all study conditions. Earliest fundus abnormalities consisted of increased reflectivity with occasional darkening in the fovea-like region at the center of the area centralis; this advanced to a thin band-shaped streak of retinal thinning along the visual streak with a variable rate of progression, but no generalized degeneration. cSLO analysis in blue autofluorescence mode showed a focus of increased autofluorescence in the fovea-like region which extended medially and laterally albeit with reduced intensity. At this time, sdOCT of the fovea-like region generally showed no abnormalities, but within several months the fovea-like region began to thin, and, with time, atrophic changes in this region developed. The most striking early abnormality, however, was in the cone ERG. By 6 months, most dogs had reduced cone amplitudes, either with single flash or flicker stimuli under photopic conditions, and these changes were progressive with almost non-recordable cone responses under some stimulus conditions at older ages. On the other hand, rod ERG components remained normal to slightly reduced in amplitude even in the older dogs. The older dogs in the study served as guide dogs for the blind. While the graduates who used these dogs for daily activities did not indicate any vision problems, assessment by an experienced trainer identified vision problems, almost exclusively in the dark in all of these dogs, among which included: severely compromised vision at night even when lights are on, running into fences and doorways in subdued light, difficulty in adjusting from light to darkness, but not going from into bright sunlight. These vision abnormalities under dim or relative dark conditions were present at the time when the rod ERG was normal or slightly reduced in amplitude, but cone ERGs were markedly reduced or absent.
Conclusions: Stargardt disease in the canine model is a cone-rod dystrophy with pathology that begins in the fovea-like region with increased short wavelength autofluorescence and selective thinning of this region. While the generalized cone ERG functional abnormalities suggest a generalized cone defect, the presence of functional photopic vision indicates that cones are present although their function is impaired. This suggests that successful therapeutic intervention, e.g. through gene therapy, could reverse the functional deficits and arrest disease progression. These studies are ongoing.

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Surgical castration of piglets is a routine procedure, commonly done without pain control. While acutely painful, little is known about the impact of castration in the hours following the procedure on the animal’s affective state. A conditioned place aversion paradigm was implemented to test piglets’ memory of the procedure. A testing apparatus was created with 3 equally sized arenas. The two outer ‘treatment’ pens each contained unique cues while the center pen remained neutral. Piglets (n=22) were subjected to surgical castration following topical local anesthesia in one outer pen or a sham procedure in the other followed by a 2h recovery period. Treatments were balanced and given 48h apart. Twenty-four hours after the last treatment, piglets were returned to the center chamber with free access to all pens, to investigate how they divide their time between the treatment pens. Piglets displayed no aversion to the castration pen. A second trial was conducted with 22 additional piglets where a presumed positive experience was evaluated following the same paradigm. The two treatments were a sham procedure identical to the first trial vs. an enriched arena including straw, toys and sucrose solution. We expected piglets to develop a preference for the enriched arena, but piglets did not show a difference in time spent. We hypothesize the lack of place aversion or preference can be explained by an apparent indifference of piglets between treatments (either sham vs. castration or sham vs. enrichment). Alternatively, the paradigm might not be sensitive enough to detect differences experienced by the animals, and linked to multiple methodological factors. These findings also could result from the inability of neonatal piglets to learn the differences between treatments or their inability to focus on the environment rather than their current affective state. Further studies are ongoing to differentiate these possible interpretations.

11. High Mortality in Juvenile Rainbow Trout.

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Five hundred juvenile rainbow trout fish were ordered from a reputable source slowly began to experience a mortality of ~3% within the first 2 hours after their arrival to our aquatics facility. This was presumed to be a typical occurrence due to shipping stress and acclimating to a new tank system. Modifications were made to the tanks to decrease the speed of the water flow rate to prevent the fry from fatiguing while swimming and could ultimately lead to their mortality. Routine water tests were performed to ensure that the water temperature, nitrate, and nitrite levels were appropriate. As the week progressed, the mortality increased over the next few days from 10% to 40% to eventually 80%. Fish were noted to have a corkscrew swim pattern, some of the fish had increased pigmentation, and fecal casts extending from their vent. Due to the likelihood that this was an infectious disease outbreak, we elected to euthanize the remainder of the fry in the tank and the tank was disinfected. Fry were submitted for viral culture, PCR, and histopathology. The results of our diagnostic testing were conclusive for an infectious pancreatic necrosis outbreak. We moved forward with contacting the vendor to warn them of the potentiality of disease in their colony.

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Swine are a clinically relevant animal model for the development of novel cellular immunotherapies for tolerance induction to solid organ transplants and prevention of graft-versus-host disease after bone marrow transplantation. The similarity of swine to human in anatomy, immune function and metabolism enhances its translational value in preclinical studies. Based on the promise of this clinically relevant model, we have generated strategies to manufacture Chimeric Antigen Receptor (CAR) T cells for transplant tolerance and cancer-related applications. Previously, we have developed novel protocols to activate, expand and transduce swine CAR T cells \textit{in vitro} using artificial antigen presenting cells (aAPCs) for stimulation and a lentivirus-based system for CAR transduction. Hence, we optimized our protocols to expand Tregs in order to manufacture CAR Tregs. Tregs are relevant due to main role in the regulation and homeostasis role in the immune system. In pig, Tregs are defined as a CD25\textsuperscript{high} CD4\textsuperscript{+} T lymphocyte expressing the FoxP3 (Forkhead Box P3) as transcription factor. Tregs (CD25\textsuperscript{high} CD4\textsuperscript{+}) were efficiently sorted and lentivirally transduced with an anti HLA-A2 CAR molecule. CAR Tregs expanded up to twenty-fold over 11 days. These Tregs maintained CAR and FoxP3 expression throughout extended culture and became stimulated and expanded specifically by the engagement of the CAR binding to antigen positive target cells. In summary, we previously developed effector CAR Tcells and now have generated robust protocols to develop swine CAR Tregs as a therapy for future translational studies in transplantation tolerance and cancer immunotherapies.

Research Grant: ITMAT


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Animal models of human disease have always played a central role in biomedical research, but the predictive value gap between humans and rodent models have hindered the applicability of these discoveries. Swine are ideally positioned as a cancer model as their anatomy, physiology, and immunology closely resemble that of humans. However, the current lack of transplantable hematologic cancers limits the use of swine as a model for cancer studies. The Oncopig model is a transgenic swine model that recapitulates human cancer through development of site and cell specific tumors following Cre recombinase induced expression of heterozygous KRAS\textsuperscript{G12D} and TP53\textsuperscript{R167H}, mutations that have been demonstrated to be key drivers of human cancer. We have previously determined lymphoid leukemias can be generated \textit{in vivo} by injection of Adenovirus Cre-recombinase into secondary lymphoid organs surgically in three Oncopigs. We found that this procedure resulted in lymphadenopathy, suggestive of lymphoma. Flow cytometry data from tissue samples showed a massive predominance of CD8\textsuperscript{+} T cells. We are now transforming isolated Oncopig PBMCs and CD3\textsuperscript{+} T cells \textit{in vitro} with the goal of generating T cell specific leukemias to later be transplanted back into the host swine. To date, we have isolated CD3\textsuperscript{+} T cells from Oncopigs and transformed them by Cre-mediated oncogene activation. Preliminary flow cytometry data from these samples have also shown a predominance for CD8\textsuperscript{+} T cells to expand, corresponding with the results seen \textit{in vivo}. The overall goal is to establish a reliable and reproducible swine cancer model and increase the predictive value of pre-clinical cancer studies.

Research Grant: ITMAT, Internal funds

Student Support: NIH Grant T35 OD 010919-25
14. Hindlimb Monoparesis in a Cynomolgus Macaque (*Macaca fascicularis*).

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A 2.5-year-old, 2.64-kg, singly-housed female cynomolgus macaque was reported for intermittently holding her right hindlimb in full flexion or extension while ambulating. At the time, she was able to bear full weight, but muscle atrophy of the limb was noted cageside. She received meloxicam (0.2 mg/kg SC once, followed by 0.1 mg/kg SC SID for 3 days). Five days after presentation, she was sedated for diagnostic work-up. At the right hindlimb, there was moderate muscle atrophy and crepitus of the coxofemoral joint. X-rays showed marked hypoplasia of the right femoral head. Bloodwork showed mild dehydration, inflammation, and osteolysis. She became intermittently-non-weight-bearing on the affected limb 3 days later; carprofen treatment (4.4 mg/kg PO SID) began. She continued to lose weight, and femoral head ostectomy (FHO) was offered to the lab; this was declined in favor of transferring her to an acute-use experiment and continuing daily carprofen. On repeat examination and diagnostics, she had a body condition score of 1.5/5, with severe crepitus of the right coxofemoral joint and marked muscle wasting of the entire limb. Abdominal ultrasound was within normal limits. X-rays showed rapid disease progression: severe degeneration of the right femoral head and marked acetabular lysis. Bloodwork showed evidence of mild dehydration, inflammation, and osteolysis. Carprofen ended, and she received dexamethasone SP (1 mg/kg IM) and famotidine (0.5 mg/kg IM) in preparation for CSF tap, MRI, and brain injection with AAV vector per protocol. Acupuncture was also performed at this time. Buprenorphine (0.01 mg/kg SC) was administered, and she was maintained on a 7-day dexamethasone taper per protocol. Once the steroids ended, carprofen was restarted (2.2 mg/kg PO SID) until endpoint 1 week later. Grossly, the right femoral head was small (5 mm diameter), rough, and irregular with an absence of articular cartilage. The joint capsule was thickened. Radiographic and pathologic findings were consistent with avascular necrosis of the femoral head. This has only been reported twice in nonhuman primates, though it is a well-characterized condition in young small-breed dogs for which conservative (analgesics, cage rest) or surgical (FHO) treatment options are available.

15. Chaperone Assisted Selective Autophagy (CASA) Targets Filovirus VP40 as a Client and Restricts Egress of Virus Particles.

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The filovirus VP40 protein directs virion egress, which is regulated either positively or negatively by select VP40-host interactions. We demonstrate that host BAG3 and HSP70 recognize VP40 as a client and inhibit egress of VP40 VLPs by promoting degradation of VP40 via Chaperone Assisted Selective Autophagy (CASA). Pharmacological inhibition of either the early stage formation of the VP40/BAG3/HSP70 tripartite complex, or late stage formation of autolysosomes, rescued VP40 VLP egress back to WT levels. The mechanistic target of rapamycin complex 1 (mTORC1) is a master regulator of autophagy, and we found that surface expression of EBOV GP on either VLPs or an infectious VSV recombinant virus, activated mTORC1. Notably, pharmacological suppression of mTORC1 signaling by rapamycin activated CASA in a BAG3-dependent manner to restrict egress of both VLPs and infectious EBOV in Huh7 cells. In sum, our novel findings highlight the involvement of the mTORC1/CASA axis in regulating filovirus egress.
16. **Contrasting Effects of Filamin A and B Proteins in Modulating Filovirus Entry.**

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Ebola (EBOV) and Marburg viruses (MARV) cause severe hemorrhagic fever associated with high mortality rates in humans. A better understanding of filovirus-host interactions that regulate the EBOV and MARV lifecycles can provide biological and mechanistic insight critical for therapeutic development. EBOV glycoprotein (eGP) and MARV glycoprotein (mGP) mediate entry into host cells primarily by actin-dependent macropinocytosis. Here, we identified actin-binding cytoskeletal crosslinking proteins filamin A (FLNa) and B (FLNb) as important regulators of both EBOV and MARV entry. We found that entry of pseudotype psVSV-RFP-eGP, infectious recombinant rVSV-eGP-mCherry, and live authentic EBOV and MARV was inhibited in filamin A knockdown (FLNaKD) cells, but was surprisingly enhanced in filamin B knockdown (FLNbKD) cells. Mechanistically, our findings suggest that differential regulation of macropinocytosis by FLNa and FLNb likely contributes to their specific effects on EBOV and MARV entry. This study is the first to identify the filamin family of proteins as regulators of EBOV and MARV entry. These findings may provide insight into the development of new countermeasures to prevent EBOV and MARV infections.

17. **A Near-Infrared Choline Kinase Fluorophore for Detection of Lung Tumor Margins.**

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Introduction: The most important prognostic indicator following lung cancer surgery is complete tumor resection. Methods of intraoperative margin assessment during lung cancer excision are lacking. The aim of this study was to evaluate intraoperative, near-infrared (NIR) imaging of canine lung tumors to detect tumor margins using JAS 239, a fluorophore targeting choline kinase. Choline kinase is an oncogene that is overexpressed in many cancers during tumorgenesis.

Materials and Methods: Dogs with primary lung tumors were enrolled in the study with informed owner consent. Dogs underwent open thoracotomy and tumor excision following intravenous injection of JAS 239. Lungs were imaged using a NIR imaging system both in vivo and ex vivo. Fluorescent margins were marked and compared to histopathological margins. The wound bed was re-imaged for residual fluorescence suspicious for positive tumor margins. Tumor signal-to-background ratio (SBR) was calculated using Image J.

Results: NIR imaging identified canine lung tumors in 13/13 cases. Mean tumor SBR was 4 (range =1.4-7). Tumor margins identified by NIR fluorescent imaging correlated well with histopathological margins. Areas of minimal fluorescence within tumors correlated with necrosis in 4 cases.

Conclusions: NIR imaging using JAS 239 provided reliable intraoperative assessment of canine primary lung tumor margins. Canine primary lung tumors are a spontaneous large animal model of human non-small cell lung cancer. This study suggests that intraoperative NIR imaging using JAS 239 may be of benefit in human non-small cell lung cancer surgery patients.

**Research Grant:** NIH R01CA226412-01

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Qualitative Behavior Assessment (QBA) is a welfare evaluation tool that uses a holistic approach to capturing an animal’s emotional state. Due to its holistic nature, assessors should be trained in the nuances of species-specific behavior and how to properly use QBA. The objective of this study is to probe the efficacy of a training program designed to familiarize students with no prior experience on sow welfare assessment using QBA. Fourteen veterinary students were recruited to partake in a four-hour long training session on sow behavior and how to use QBA. Students then completed QBA on a video library of post-weaned sows selected to capture the breadth of behavior. Training efficacy was assessed by comparing the QBA scores of students to those of five pig experts. QBA scores from experts and students were analyzed separately using principal component analysis, and the first two PCs with Eigenvalues greater than 1 were selected. Expert PC1 ranged from Tense/Agitated to Enjoying/Happy and accounted for 36% of variance while PC2 ranged from Indifferent/Calm to Active/Lively and accounted for 22% of variance. Student PC1 ranged from Tense/Distressed to Playful/Enjoying and accounted for 41% of variance while student PC2 ranged from Listless/Indifferent to Active/Agitated and accounted for 20% of variance. Experts displayed almost perfect agreement on PC1 (W=0.91), and moderate agreement on PC2 (W=0.66) while students displayed moderate agreement on both PC1 (W=0.63) and PC2 (W=0.72). However, a significant correlation was found between student and expert PC1 (r=0.927, p<0.001) and PC2 (r=0.958, p<0.001). These results indicate that when trained, student assessors may assess welfare similar to experts, however, limitations in experimental design make it difficult to assess whether similarities are due to the robust nature of QBA or the sufficiency of the training program. Future research will work to further elucidate these results.

Research Grants: Pennsylvania Department of Agriculture, Center for Poultry and Livestock Excellence

19. Association of Vitamin E-Responsive Myopathy (VEM) And Equine Neuroaxonal Dystrophy / Equine Degenerative Myeloencephalopathy (eNAD/EDM).

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Equine neuroaxonal dystrophy/equine degenerative myeloencephalopathy (eNAD/EDM) is a common postmortem diagnosis in neurologic horses. Though temporal vitamin E deficiency is etiologically implicated, eNAD/EDM remains a diagnosis of exclusion in the living horse. Vitamin E-responsive myopathy (VEM) is diagnosed by mitochondrial alterations in highly oxidative muscle fibers within the sacrocaudalis dorsalis medialis (SCDM). These alterations might be a proxy for central nervous system oxidative damage. The objective was to determine the percentage of horses with concurrent eNAD/EDM and VEM. Percentages of horses with eNAD/EDM exhibiting low serum [vitamin E] and increased serum and CSF [phosphorylated neurofilament heavy subunit (pNF-H)] were also compared. In this descriptive case series, SCDM biopsies were obtained from 68 client-owned horses presented to Penn Vet's New Bolton Center between 2021-2023 with postmortem lesions compatible with eNAD/EDM. Biopsies were histopathologically evaluated for mitochondrial alterations. Serum [vitamin E] was determined with HPLC and [pNF-H] with sandwich ELISA. 28/68 (41%) horses with eNAD/EDM exhibited comorbid VEM. 3/36 (8%) exhibited elevated serum [pNF-H], and 8/36 (22%) exhibited increased CSF [pNF-H]. 11/59 (19%) had low serum [vitamin E]. In conclusion, SCDM mitochondrial alterations were identified in >40% of horses with eNAD/EDM and can increase antemortem suspicion of neurodegenerative disease. Investigation of VEM frequency in normal horses and those with other neurologic conditions will enable estimation of specificity.

Research Grant: This work was funded by generous donations to the Neurologic Research Fund at New Bolton Center from clients and veterinarians.
20. Retinal Phenotype and Genetic Basis of cord1-PRA in English Springer Spaniels Affected With the RPGRIP1 Variant.


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A commonly found form of canine cone-rod dystrophy (cord1) is caused by an insertion in the retinitis pigmentosa GTPase regulator-interacting protein 1 (RPGRIP1) gene, which encodes for a photoreceptor connecting cilia. In humans, mutations in RPGRIP1 lead to Leber congenital amaurosis (Dryja et al, 2001).

RPGRIP1 canine cone-rod dystrophy as a cause of PRA was first identified in the miniature longhaired dachshund (Mellersh, 2006), but was later found in many dog breeds. In the English Springer Spaniel (ESS) dog, this disease appears to have a later onset and slower progression. In some cases, affected dogs may never show behavioral signs of vision loss, despite there being electoretinographic changes. Nevertheless, the possibility of developing debilitating comorbidities such as cataracts, lens luxation and glaucoma, make RPGRIP1-PRA a noteworthy disease in the breed.

In this study, we examined over 494 ESS dogs to characterize disease phenotype and identify disease-modifying factors. An online questionnaire was used to collect data from additional ESS dogs. Dogs that were examined received ophthalmic examinations alongside morphological and functional assessments, with some (n=76) dogs receiving followed-up exams throughout the 4-year span of this study. These follow-ups showed varied rates of decline in electoretinographic response, corresponding to varied rates of ONL thinning on OCT. With most affected dogs, owners report no clinical signs of vision loss. Other dogs however show nyctalopia when they are at mid to advanced stages of retinal degeneration.

RPGRIP1 mutations have a huge prevalence in the ESS population, although the subtle clinical signs and slow progression may have allowed affected cases to remain unnoticed. One or more disease-modifying genes may be at play in phenotype variability. It is advisable for now to breed affected dogs only to homozygous unaffected dogs. For affected dogs, repeat ERG testing is recommended to predict the rate of disease progression.

21. Type 2 Diabetes Mellitus in Tupaia belangeri (Northern Tree Shrew).

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Three older adult, singly housed, northern tree shrews presented for a range of clinical signs within a 1-year period, including lethargy, hind limb lameness, decreased visual acuity when performing tasks, polyuria, and polydipsia. Diagnostics included point of care blood glucose and ketone measurements, as well as urine dipstick readouts. These three shrews were found to have varying degrees of hyperglycemia, glucosuria, and ketonuria. The combination of these clinical signs and diagnostics led to a previously undescribed, presumptive diagnosis of diabetes mellitus type 2. Management of this disease process included modification of baseline diet to a feline diabetic management chow, as well as transitioning from their typical frugivorous diet to vegetable and protein sources such as peas, carrots, mealworms, and eggs. Additional medical intervention included metformin dosed at 10 mg/kg based on human and non-human primate literature. Bi-weekly blood glucose readings and urine dipstick measurements were used to track diabetic management and potential relapse. Using these techniques, animals were able to be clinically managed for 3-8 months from initial diagnosis, with demonstratable reduction in blood glucose values. Diagnosis of type 2 diabetes mellitus was confirmed through terminal blood collection measuring plasma insulin levels, as well as HbA1c measurements, CBC, and chemistry analysis. Post-mortem and histopathological examinations were performed, and main findings included pancreatic islet cell lipidosis, glycogen vacuolation of the pancreatic, biliary, and renal ductules or tubules, and glomerulosclerosis. Streptozotocin-induced type 1 diabetes has been previously described in tree shrews, which provided the literature characterizing their normal versus diabetic hematologic values; however, this is the first report of spontaneously occurring type 2 diabetes in this species and may present opportunities for future translational model development.
22. Mast Cells Limit Intestinal Inflammation by Restraining IgA Production.

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Ulcerative colitis (UC) is a debilitating and poorly understood disease that is characterized by neutrophilic infiltration of the colon. Recent evidence from human and mouse studies show how non-secretory IgA retained in the lamina propria complexes with translocated antigens, activating neutrophils and inciting a self-amplifying loop of neutrophilic inflammation and IgA production that contribute to UC pathology. IgA class-switching can be modulated by mast cells in the colonic lamina propria, but how this impacts intestinal inflammation is unknown. Our previous work in both a chronic, spontaneous colitis model and chemical colitis model demonstrate that mast cell-deficient mice have more severe colitis than wildtype cagemates, and that mast cell reconstitution ameliorates colitis, demonstrating that mast cells suppress colonic inflammation. Since excessive IgA levels exacerbate local intestinal inflammation, we hypothesized that mast cells in the colonic lamina propria limit colitis by regulating IgA production. To test our hypothesis, we applied the oxazolone colitis model and discovered that mast cell-deficient mice have increased IgA+ plasma and memory B cells in both the colonic lamina propria and draining lymph node as well as elevated total serum IgA protein compared to wildtype controls. These data suggest that mast cells act upstream of a previously defined pathogenic IgA-neutrophil loop, and that further studies are required to establish whether runaway IgA production causes the enhanced colitis observed in mast cell deficiency as well as identify candidate mast cell signals. This project aims to determine if and how mast cells regulate IgA production in a mouse model of ulcerative colitis, indicating a new line of inquiry into the pathogenesis of this disease with the potential to discover a new therapeutic target.

23. APC and P53 Mutations Synergize to Create a Therapeutic Vulnerability to NOTUM Inhibition in Advanced Colorectal Cancer.

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Colorectal cancer (CRC) represents a major unmet medical need and cause of cancer-related deaths globally. APC inactivation results in the constitutive transcriptional activation of the canonical WNT signal transduction pathway effector β-Catenin. However, APC loss also activates feedback inhibitors, including the extracellular palmitoleoyl-protein carboxylesterase NOTUM. Here, we show NOTUM retains cell-autonomous tumor suppressive activity in APC-null adenomatous lesions but switch into an obligate oncogene in APC/P53 mutant advanced adenocarcinomas. We found that NOTUM tumor suppressive activity in APC-null adenomas results from cleaving of Glypicans1 from the cell surface to antagonize mTORC1 activity, and its oncogenic activity in advanced adenocarcinomas results from its cleavage of Glypicans4 from the cell surface to antagonize tumor suppressive TGFB signaling. Ultimately, preclinical mouse models of CRC and human tumoroid cultures demonstrated that pharmacological inhibition of NOTUM is highly effective in arresting primary adenocarcinoma growth and inhibiting metastatic colonization of distal organs. Our results demonstrate the role of the NOTUM in mammalian intestinal biology and colon cancer. The finding that this single agent is effective in treating highly aggressive cancers for which there exist few viable therapeutic options has major implications for the treatment of late-stage colorectal adenocarcinomas.
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Narcotics detection dogs are at increased risk for exposure to powerful opioids like fentanyl and carfentanil due to high levels of drug trafficking and illegal manufacturing of these opioids. When these dogs are exposed to opioids and demonstrate signs of intoxication, an opioid reversal agent is indicated, although the type of opioid antagonist has been subject to debate. We previously demonstrated that intranasal and intramuscular naloxone were equivalent in reversing fentanyl sedation in working dogs (Essler et al., 2019). This study examines the efficacy of three reversal agents (naloxone, nalmefene, and naltrexone). While naloxone is most readily available, some studies have suggested that nalmefene and naltrexone may be more efficacious for reversal of long-acting opioids and prevention of re-narcotization (Wilhelm et al., 1995). Additionally, we examined the effect of methadone administration and subsequent reversal agent administration on dogs' ability to detect a trained target odor 4 hours, 24 hours, and 48 hours after administration. All reversal agents were found to be effective and there was no evidence of adverse effects or re-narcotization six hours post-exposure. We also found that none of the reversal agents impacted dogs' odor detection performance at any timepoint. Given the drug market is moving towards more potent synthetic opioids, further experimentation will be needed to assess the effectiveness of different reversal agents for ultra-potent or longer-acting opioids, and the potential impacts on dogs' working performance.

Research Support: Our first study was funded by the Department of Homeland Security HSHQDC-17-P-00112. Our second study was completed through a cooperative agreement from the U.S. Army Research Office (ARO Proposal #: 77711-ST) with funding provided by the Defense Health Agency.

25. Leveraging Multi-Specific CAR-iNKTs to Tackle Solid Tumor Heterogeneity.

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Introduction: Solid cancers proved resistant to Chimeric Antigen Receptor T (CAR-T) cells. Unlike CAR-T, CAR-invariant NKT (CAR-iNKT) cells kill CD1d-positive macrophages within the tumor microenvironment, known to inhibit CAR-Ts. Additionally, CAR-iNKTs kill malignant cells more effectively than CAR-Ts through simultaneous recognition of multiple targets, which is desirable to prevent immune escape in solid tumors. We developed CAR-iNKTs targeting human and canine solid tumor antigens B7H3 and IL13Rα2 and investigated feasibility of these CAR-iNKTs for the treatment of osteosarcoma (OS), a paradigm of uncurable solid tumor.

Methods: We employed real-time-qPCR and flow cytometry to determine CD1d, B7H3 and IL13Rα2 expression in six human (134B, HOS, MG63, U-2OS, G-292, Saos2) and four canine (MC, SK, BW, CS-KOSA) OS lines. Using multiplex
immunofluorescence and single-cell RNA-sequencing, we investigated CD1d, B7H3 and IL13Rα2 expression in patient primary and metastatic tumors. To elucidate the underpinning regulatory mechanisms, we leveraged post hoc ChiP-seq and TF motif analyses and in vitro pharmacological assays using epidrugs.

Results: qPCR assays showed higher expression of B7H3 mRNA in all human and canine lines compared to IL13RA2 and CD1d. Flow cytometry confirmed similar protein expression patterns. CAR-iNKTs killed OS cells via CAR-B7H3 more effectively than CAR-IL13Rα2, suggesting that low IL13Rα2 levels can limit CAR-iNKT efficacy. To put our findings in clinical context, we evaluated patient samples, revealing B7H3, IL13Rα2 and CD1d profiles consistent with OS lines. Further IL13RA2 promoter interrogation suggested that reduced histone acetyl marks may contribute to low IL13RA2 expression in OS. Accordingly, when treating OS cells with the histone deacetylase inhibitor SAHA, IL13Rα2 was effectively upregulated.

Conclusions: We developed B7H3- and IL13Rα2-targeting CAR-iNKTs for human and canine solid tumors. Epidrugs may enhance CAR-iNKT multi-targeting capabilities, reducing immune escape of antigen-negative malignant cells. Future investigation in canine OS patients will determine feasibility of this approach and potential for translation into the human clinics.

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Drug testing of human and equine athletes is critical for maintaining the integrity of sport. Identifying the presence of banned and controlled substances enables regulatory bodies to deter use of these substances, thus protecting the athletes’ health, while also providing a fair environment for those who are competing. A method utilizing GC-MS/MS was developed to accurately, quickly, and repeatably screen for and analyze equine plasma samples for 31 compounds from three different classes-NSAIDs, Steroids, and Cannabinoids.

The samples were prepared utilizing a modified acidic liquid-liquid extraction that was published prior for the extraction of NSAIDs from equine plasma, and derivatization with BSTFA+TMCS. The instrument was a Thermo Scientific Trace 1310 GC and TSQ9000 Triple Quadrupole MS. The run time was 4.7 minutes, allowing for the method to be used in a high-throughput environment. 1 µL sample was injected into the instrument and a TraceGold-SQC 15m column was used. Due to the intricacy of the processing method, results were automatically generated and analyzed utilizing multiple factors: retention time, ion ratios between both confirming ions and quantitation ion, ion coelution, and added tolerances for these values to allow for day-to-day instrument fluctuations and possible slight changes at lower concentrations.

This method was able to correctly and repeatably identify 31 compounds including 23 NSAIDs, 5 steroids, and 3 cannabinoids. Extraction recovery was examined with an overall average value of 75%, with the cannabinoids having an average of 43%, NSAIDs at 77% and steroids at 85%. Matrix effect was also examined with the matrix causing ion suppression to some extent for 21 of the 31 compounds and ion enhancement for the rest.

Research Funding: Pennsylvania Department of Agriculture

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The state of Pennsylvania is home to lush forests and mountains that provide an ideal habitat to support large populations of white-tailed deer, *Odocoileus virginianus*. Cervids frequently harbor multiple tick species and their associated pathogens, which can pose a significant health risk to deer and humans alike. This study sought to identify known zoonotic pathogens in ticks and matched deer blood samples collected from across the state of Pennsylvania. 184 ticks and host blood samples were collected from 62 deer. Ticks were identified by sex, genus, species, age, and extent of engorgement, and total nucleic acids were extracted using the Qiagen DNA/RNA AllPrep kit. Nearly all of the ticks we identified were either *Ixodes scapularis* or *Haemaphysalis longicornis*, the latter being an introduced species in the state of Pennsylvania for which little is known about distribution and contribution to pathogen transmission. All ticks and matched deer blood samples were tested by QPCR using primer-probe assays for pan-*Ehrlichia*, pan-*Rickettsia*, pan-*Apicomplexa*, pan-*Borrelia*, and Powassan virus lineage II. Our results identified a prevalence of 40% (73/184) for *Apicomplexa* spp., 25% (47/184) for *Ehrlichia* spp., 38% (27/74 non-*Ixodes* ticks) for *Rickettsia* spp., 10% (18/184) for *Borrelia* spp., and 0% for Powassan virus. QPCR analysis of deer blood samples is ongoing. Finally, we selected 48 of the 184 ticks for 'shotgun' metagenomic sequencing to determine sensitivity for detecting known tickborne pathogens, as well as discovering potential novel tickborne pathogens. Continued research on these samples include RT-QuIC for prions among ticks from Chronic Wasting Disease suspect cases. This study on the prevalence of tickborne diseases gives researchers an evolving look into the landscape of zoonotic and infectious pathogens present in ticks and their deer host in verdant Pennsylvania.

**Research Grant:** Richard King Mellon Foundation


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Enteric methane (CH4) emissions from dairy cows contribute to 30% of total methane emissions and are considered a net energy loss to the animal. In the rumen, methanogens (hydrogenotrophic, methylotrophic, and methylaminotrophic) reduce carbon dioxide (CO2), methanol, and methylamines, respectively to generate CH4 via different mechanisms. Although several methanogenic inhibitors have been investigated to reduce CH4 production, their effect on individual methanogens is unknown. Thus, this study investigated the effect of either 3-nitrooxypropanol (3-NOP) or *Asparagopsis taxiformis* (AT) on individual methanogens in rumen samples of dairy cows from two different experiments. First, we identified 13 ruminal methanogens which represented the three major substrates for methanogenesis along with the commonly present 16S rRNA archaeal gene. The copy number of individual methanogens was quantified using sequence specific primers using quantitative PCR (qPCR). These data were then compared to the 16S rRNA gene-based archaeal diversity retrieved via Illumina Mi-seq platform. The copy number of the 16S rRNA did not differ with either 3-NOP or AT, however, the individual methanogens showed differences amongst the two inhibitors. The copy numbers of hydrogenotrophic methanogens such as *M. ruminantium*, *M. smithii*, *M. millerae*, *M. olleyae*, and *M. sp. YE315* were reduced (P<0.05) with 3-NOP and AT, whereas *M. sp. AbM4* and *M. formicicum* were reduced (P<0.05) only with AT supplementation. Among methylotrophic methanogens, *M. intistilis* increased (P<0.05) with 3-NOP whereas *M. archean* ISO4*H*5 was reduced (P<0.05) with AT. Among methanol utilizing methanogens, *M. stadtmanae* were completely reduced (P<0.05) with AT and *M. mazei* increased (P<0.05) with 3-NOP. The 16S rRNA analysis revealed that 3-NOP is more inhibitory on *Methanobrevibacter* and AT is more specific to inhibit *Methanosphaera*. This comparative study revealed novel information on the effects of 3-NOP and AT on individual methanogens. Further studies are required to understand the action mechanisms of these inhibitors on individual methanogens.
29. Group 2 Innate Lymphoid Cells Are Required for Protective Immunity in Helminth Infected Mice.

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Experimental studies employing the murine hookworm *Nippostrongylus brasiliensis* are widely used to elucidate the pathogenesis and immunology of helminth infection, which is a major cause of morbidity in human and animal health. Hookworms parasitize the host through skin penetration by third stage infectious larvae (iL3), migration through lung tissue and entry into the gastrointestinal (GI) tract for egg production by adult stage worms. Type 2 immune responses, characterized by Interleukins (IL’s) 4, 5, 9, 13, 25, and 33, drive host protection through worm clearance and tissue repair, while Interferon gamma (INF-g) and IL-17A responses can drive susceptibility and disease exacerbation. The relative contributions of CD4+ T helper (Th2) cells versus group 2 Innate lymphoid cells (ILC2s) to host immunity and tissue repair remain unclear, largely due to the lack of genetic systems that selectively eliminate only one of these populations. The recent generation of mice deficient in Locus Control Region 1 (LCR$$^{-/-}$$), which allows a selective loss of ILC2 with an intact Th2 compartment, provides a critical tool for addressing this long-standing controversy. In this study, LCR1$$^{-/-}$$ mice or wild-type controls (C57BL/6, n = 15/group) were subcutaneously infected with *N. brasiliensis* iL3 and evaluated for parasitological impact and extent of lung injury. Data show 144- and 49-fold higher fecal egg loads and intestinal worm numbers and significantly more red blood cells in the lungs of LCR1$$^{-/-}$$ mice vs. controls, indicating greater host susceptibility and lung damage in mice lacking ILC2s. LCR1$$^{-/-}$$ produced less IL-4 but higher IL-17A levels than WT controls. As expected, LCR1$$^{-/-}$$ mice had significantly fewer GATA3+ST2+ILC2s, as well as fewer eosinophils, but Th2 cells were equivalent between groups. This study sheds new light on the mechanisms of resistance against hookworms and supports the idea that ILC2s are essential for both host protection and tissue repair independently of Th2 cells.


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Despite pathologic changes commonly observed in equine proximal sesamoid bones (PSBs), the development and maturation of these bones is still poorly understood. The objective of this study was to describe the process and pattern of endochondral ossification in PSBs obtained from fetuses and young horses using micro-CT and histology. Proximal sesamoid bones from 12 horses ranging in age from a 105-day old fetus to a 540-day old yearling were collected. Mid-sagittal histologic sections of PSBs were stained with hematoxylin and eosin and safranin O/fast green and examined to describe the growth cartilage, ossification center, spherical growth plate activity, articular cartilage, and entheses formation. For samples with adequate mineralization, micro-CT analysis was performed to characterize tissue volume (TV), bone volume fraction (BV/TV), height, width, depth, trabecular thickness (Tb.Th), and anisotropy. The study demonstrated that equine PSBs mineralize by endochondral ossification during the late gestation to early post-natal period. The spherical growth plate activity was variable across apical, flexor, basilar, and articular ossification fronts. Structural organization of the articular cartilage and fibrocartilaginous entheses occurred after cessation of growth activity of the spherical growth plate. The fibrocartilaginous entheses of the flexor cortex of the PSB was not mature at 540-days post-gestation. The delayed maturation of the fibrocartilaginous entheses and articular cartilage may play a role in the pathophysiology of equine PSB fracture and warrants further investigation.

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Pigs in commercial settings face several challenges that compromise their welfare. Common housing and husbandry practices inhibit the development and expression of species-specific behavior and compromise their homeostasis. Individual stalls or crates are the most common way that sows are housed. These crates drastically restrict sow social, explorative, and maternal behavior, and likely induce high levels of stress. Here, we studied the effect of crates on the circadian rhythm of cortisol in gilts as a complementary indicator of welfare.

Twelve gilts (TN70-Topigs) were housed in group pens for five weeks (W1 to W5), then housed in individual crates for four weeks (W6 to W9). Saliva was collected at 7:00a.m. and 6:00p.m. during three consecutive days in each of the nine weeks. Salivary cortisol concentrations were determined using a Cortisol Enzyme Immunoassay Kit (Salimetrics®). Only results with a coefficient of variance <15% were considered. A ratio between the a.m. and p.m. period was calculated and used as a dependent variable in a linear mixed model. The week and the animal identification were used as fixed and random effects, respectively. An additional model was performed focusing on only the weeks when the gilts were crated (W6 to W9) and keeping the same random effect. The results indicated a weak effect on the cortisol ratio (p=0.01). The cortisol ratios of weeks W6, W7, W8, and W9 were lesser than W1 (p ≤0.01). When using only the weeks when the gilts were crated, the ratios of W8 and W9 were greater than W6 (p≤0.02).

An organized relationship between morning and afternoon cortisol could be considered a welfare indicator together with behavioral measures. However, on W6, the cortisol ratio gets the closest to 1 (\( \bar{X} = 1.26 \)), indicating that moving the animals from group housing to an individual-restrictive one was enough to disorganize the cortisol circadian rhythm.

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32. Using Veterinary Expertise to Counter Climate Change by Merging Social Entrepreneurship with Sustainable Agriculture.

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Problem Statement: Agriculture is the backbone of society, feeding our growing world, and both contributing to and offering opportunity to counter societal challenges like climate change and global inequality. Merging social entrepreneurship with sustainable agriculture provides a strategy for communities and ‘experts’ to co-create equitable agricultural initiatives and envision healthful food systems by identifying interlinkages, gaps, and opportunities between human, animal, and environmental health, while considering operations realities of current sociopolitical economic systems.

Methodology: To enhance community-based collaborations, we have developed a new framework for applying social entrepreneurial principles to sustainable agriculture and food systems. We collaborated in two countries in Sub-Saharan Africa – Gambia and Botswana – to conduct feasibility studies and refine this novel methodology. Social entrepreneurship is a tool to de-risk initiatives, integrate systems-thinking, and quickly develop solutions to societal challenges.
Results: Gambia Goat Dairy was built through this methodology and scaled in collaboration with Penn Vet to become FAIR Farms, the Foundation for Agro-Farming Innovation and Resilience. A theory-of-change logic model shows this research and demonstration farm model can achieve impact across 13 of the 17 United Nations Sustainable Development Goals, with the hypothesis that equitable agricultural development promotes inclusive economic development with secondary benefits of improved health and nutrition, environmental stewardship, and community empowerment. We further tested this methodology through an in-country feasibility study in Botswana in 2023, using human centered design to interview over 70 stakeholders. All preliminary impact assessments of operations in Gambia show progress, while ongoing operations inform development of new initiatives like that in Botswana.

Conclusions: Future research to define and publicize this methodology could benefit stakeholders across the globe working to achieve the U.N. Sustainable Development Goals. Moreover, as a solution-building methodology, social entrepreneurship leverages relevant veterinary expertise in collaboration with community partners and interdisciplinary teams, to develop climate crisis solutions.

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33. Selective Whole-Genome Amplification Reveals Population Genetics of *Leishmania braziliensis* From Primary Patient Samples.


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In Brazil, *Leishmania braziliensis* is the main causative agent of the neglected tropical disease, cutaneous leishmaniasis (CL). CL can present on a spectrum of disease severity and often is refractory to treatment, yet the parasite factors that may contribute to disease presentation and patient treatment outcome are not well understood, in part because successfully isolating and culturing parasites from patient lesions remains a major challenge, and because adaption to culture has been shown to induce widespread genetic changes in *Leishmania*. Here we describe the development of selective whole genome amplification (SWGA) for *Leishmania* and show that this method enables culture-independent analysis of whole parasite genomes obtained directly from primary patient skin samples, all while avoiding artifacts associated with adaption to culture. We show that SWGA can be applied to multiple *Leishmania* species residing in different host species, suggesting that this method can be broadly useful in both experimental infection models and clinical studies. Finally, we show that parasite genomes generated by SWGA of skin biopsies collected from patients in Corte de Pedra, Bahia, Brazil exhibit substantial genetic diversity and can be integrated with published whole genome data from parasites isolates to expand our understanding of *Leishmania* population genetics in Brazil.


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Working dogs are at a substantial risk of canine non-pyrogenic hyperthermia, a life-threatening condition that can occur due to physical exertion or environmental factors that inhibit dogs’ ability to cool themselves. Two often recommended cooling methods to reduce body temperature are water immersion and application of isopropyl alcohol to paw pads. This crossover study compared the relative efficacy of these methods in 12 working-dogs-in-training post exertional heat stress. Each study day, dogs had a physical exam and performed recalls sprints in which dogs ran approximately 25 meters
between two designated handlers until duration reached 10 minutes or they showed multiple signs of heat stress, or their core temperature reached 105°F (40.6°C). Dogs’ temperature and heart rate were collected after each recall. Dogs completed three study days, and each day randomly received one of three interventions: passive cooling (no intervention), partial cool water immersion, or isopropyl alcohol on the paw pads. Post-intervention the dogs were rested and monitored for 20 minutes. Partial water immersion and isopropyl alcohol both cooled dogs more than no intervention, and water immersion cooled dogs more efficiently than isopropyl alcohol. Additionally, application of isopropyl alcohol raised dogs’ heart rates more than water immersion or no intervention, suggesting that the odor of the isopropyl alcohol or the application on the paw pads is potentially stressful to the dogs. Thus, partial water immersion is preferred to treat dogs’ post-exertion hyperthermia due to its more efficient cooling and better tolerance of use.

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Student Support: American Kennel Club Canine Health Foundation

35. Allogeneic Chimeric Antigen Receptor Invariant NKT Cells for the Treatment of Canine and Human Solid Tumors.

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Introduction: Invariant Natural Killer T (iNKT) cells are CD1d-restricted lymphocytes that do not cause graft-versus-host disease, have natural tumor tropism and recognize CD1d on tumor-associated macrophages as well as malignant cells. Using a comparative approach in immunocompetent dogs, an unrivaled large-animal model for adoptive cell therapies (ACT), we investigated feasibility of allogeneic (allo-)iNKT ACT to improve clinical outcomes in solid tumor patients.

Methods: iNKTs from 10 canine and 10 human healthy donors were isolated, expanded and characterized by killing, proliferative and cytokine immunoassays alongside scRNA-seq. To determine allo-iNKT ACT feasibility and safety, and assess donor cells persistence and immune effects, unedited canine allo-iNKTs were infused into MHC-mismatched non-lymphodepleted recipients and monitored by serial flow cytometry and RT-qPCR analysis in recipients’ blood and bone marrow. Canine and human iNKTs were engineered with CARs targeting the solid tumor antigens B7H3 and IL13Ra2 and characterized by scRNA-seq and killing assays against human and canine osteosarcoma lines.

Results: Canine iNKTs recapitulated unique genomic, immunophenotypic and transcriptomic features of human iNKTs. When comparing different donors, we identified donor-specific functional and transcriptomic signatures of immunological fitness. Infusion of 4x10^8 unedited iNKTs into MHC-mismatched, non-lymphodepleted recipients was safe, and the donor cell persisted retaining immune functions for at least 118 days. Compared to unedited iNKTs, canine CAR-iNKTs exhibited greater cytotoxic and proliferative reactivity against B7H3 and IL13Ra2-expressing lines, with individual anti-tumor responses that correlated with donor-specific molecular profiles.

Conclusion: We established a powerful canine iNKT model, showing the feasibility of allo-iNKT therapy with long-lasting therapeutic potential. Canine allo-CAR-iNKTs displayed enhanced anti-tumor effect, in addition to recapitulating clinically relevant features of human CAR-iNKTs. Donor-specific signatures may identify optimal CAR-iNKTs products for maximal anti-tumor responses. Our model can be leveraged to accelerate clinical development of iNKT-based therapies and realize a potent, globally accessible ACT to improve outcomes in canine and pediatric solid cancer patients.
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36. Inheriting the Sins of Their Fathers: Boar Life Experiences Can Shape the Emotional Responses of Their Offspring.

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The welfare of breeding boars is often overlooked, resulting in limited scientific data to foster discussion of the topic. We aimed to investigate the effect of different boar housing conditions on their offspring’s emotionality. Eighteen boars were housed in three different conditions: crates (C;n=6), pens (P;n=6), or enriched pens (E;n=6). Boars were distributed by semen quality (SQ). Three semen pools were used to inseminate 13 gilts housed in outdoor paddocks. At 25 days of age, 138 suckling piglets were subjected to open field (OF), novel object (NO), and elevated plus maze (EPM) tests. Saliva was collected before and after the OF and NO tests to measure cortisol concentrations. At the end of the experiment, hair samples were collected for DNA paternity tests. Piglets were classified based on their behavioral responses using hierarchical cluster analysis of the principal components extracted from factor analysis of mixed data. The variables were reduced to seven principal components (dimensions, Dims), which explained 73% of the total variation, and were analyzed using linear mixed models. The models included each Dim as a dependent variable, paternal treatment and body weight as fixed effects, and paternal SQ as a random effect. Kruskal-Wallis and Wilcoxon rank-sum tests were used to compare the cortisol concentration ratios (before/after) between groups. There was an effect of treatment on Dim3 (EPM; activity/fear), with higher values in C piglets than E piglets (p=0.047). Although C piglets had significantly higher values than P piglets in Dim4 (EPM; anxiety; p=0.029) and Dim6 (NO; inactivity far from the object/exploration; p<0.0001), the effect of the paternal treatment×body weight interaction was significant in both dimensions (p<0.05). The cortisol ratio in E piglets was greater than that in P and C piglets (p<0.05). Our findings indicate that boar breeding environments affect the stress response and emotionality (anxiety, fear, and exploration) of their offspring.

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Note: LS is a visiting scholar in the research group of Dr. Thomas D. Parsons.

37. Exploring the Mechanisms Behind YY1-Mediated Regulation of B-Cell Lineage Commitment.

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The proper development of various immune cells depends upon transcriptional and epigenetic regulatory mechanisms that control lineage specification, development, and commitment. Epigenetic modifications and changes in 3D chromatin architecture have been proposed to be determinants in lineage development. Transcription factor Yin Yang 1 (YY1) can both activate and repress transcription, and YY1 controls long-range chromatin interactions (LRCIs) in a lineage-specific manner despite its ubiquitous expression pattern. We found that conditional knock-out of YY1 in the B cell lineage leads to the loss of B-lineage commitment, resulting in the ability of YY1-null pro-B cells to develop into T-cell lineage cells both in vitro and in vivo. To explore the mechanism behind this YY1-mediated lineage commitment regulation we are studying YY1’s control of LRCIs, changes in genomic compartments, and presence of any heterochromatic structures that maintain
lineage specificity. To identify the YY1 regulated changes in chromatin accessibility followed by alterations in genomic compartments and 3D genome structure, we are performing high-throughput Next Generation sequencing experiments including ATAC-seq and Hi-C in wild type and YY1-null pro-B cells. These data are being compared to transcriptome profiles. Initial analyses of the ATAC-seq and Hi-C data have revealed global changes in chromatin structures comparing wild type and YY1-null pro-B cells. We hypothesize that YY1 knockout ablates LRCIs that stabilized B lineage gene expression patterns while simultaneously removing YY1’s repressive impact on alternative lineage gene expression. Additional in-depth comparative analyses will enable us to determine the intricate mechanisms involved in this unique YY1-mediated regulation.

38. An Assessment of the Relationship Between Physical and Behavioral Health in Senior Cats.

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Senior cats can show signs of cognitive decline associated with disorientation, altered interactions, altered sleep-wake cycle, housesoiling, activity levels, and anxiety (DISHA). We hypothesized that health conditions would affect the severity of DISHA signs and the performance in a spatial memory test.

We conducted a multi-centric study, including clinically healthy cats over the age of 7 that attended two selected veterinary centers for routine health exams including blood work (CBC, biochemistry, metabolic function) and a cognitive decline assessment through semi-structured interviews and tests.

On a sample of 29 cats (MdAge=9 years, range=7-15 years; Females=18, all spayed), attended two veterinary centers for routine health exams and underwent a physical examination, blood tests, semi-structured interviews and questionnaires for cognitive and behavior assessment, and a spatial memory test performed at home by their owner. An overall cognitive dysfunction score (frequency x severity of signs) and DISHA domains scores were calculated. For the memory test, the cats witnessed the owner baiting 1 of 5 identical plastic containers. After a 30 seconds distraction, the cats were allowed to seek for the food. The test was repeated once per container in a pseudo-randomized order.

Results from generalized linear models indicated that increased body condition score (BCS), ALT, and creatinine were predictive of a greater frequency and severity of cognitive decline symptoms. In the spatial memory test, cats with more severe housesoiling were less likely to find food on their first attempt, and performed more mistakes or did not engage in the task, especially in the latest trial.

These findings highlight the importance of early screening for clinical and cognitive alterations and suggest the role of weight and some metabolic parameters as early markers of altered sleep-wake cycle. The results of the cognitive test evidence of a relationship between spatial memory and owner-reported behavior problems.

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Note: The project has received the approval of the Institutional Animal Care and Use Committee (IACUC protocol 807030). All authors declare no conflict of interest.


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Radiography is not considered useful for the diagnosis of acute laminitis because traditional radiographic measurements of distal phalanx (DP) displacement lack sufficient accuracy to detect the small changes that occur early in the disease process. Digital radiography has improved visualization of tissue layers in the lamellar region and recently it was shown that measurements between the inner hoof wall and outer edge of the DP (termed “lamellar lucent zone”; LLZ) correspond
well with the actual width of the combined lamellar and sub-lamellar tissue. Since stretching and/or separation of lamellae is a consistent histological feature of acute laminitis, we hypothesized that acute laminitis would be associated with measurable increases in LLZ. Our objective was to compare LLZ between healthy and acutely laminitic feet.

Forelimb radiographs from 32 healthy and 17 acutely laminitic mixed-breed horses were analyzed using Osirix software. Acute laminitis was defined by clinical signs (at least 2 of the following: acute multi-limb lameness, increased digital pulse amplitude, increased hoof heat) with a duration of ≤ 3 days and a lack of radiographic evidence of chronic laminitis (³ 3° palmar rotation; remodeling of the DP). Sixteen radiographic parameters were compared between laminitic (n=39) and control (n=64) radiographs using ANOVA. A receiver-operator characteristic curve was created for select measurements.

The mean [95% confidence interval] LLZ (mm) was increased in acute laminitis compared to control in the proximal (9.25 [8.8-9.67] vs 7.33 [7.15-7.5] ; P<0.0001), middle (9.1 [8.7-9.5] vs 6.78[6.6-6.9]; P<0.0001), and distal (9.75 [9.1-10.4] vs 7.21[7-7.42]; P<0.0001) dorsal hoof wall. ROC analysis of the middle dorsal LLZ revealed an AUC of 0.94. With a cut-off of 7.9mm, the middle dorsal LZ had a sensitivity of 82.1% and specificity of 95.3% for the diagnosis of acute laminitis.

Digital radiographic measurement of the LLZ is accurate for the diagnosis of acute laminitis.

40. **Impact of Mechanical vs. Manual Catching on Stress, Fear, and Carcass Quality in Slower Growing Broilers.**

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Before slaughter, broiler chickens must be moved from the floor of a farm into transport coops to be moved to the processing facility. There are currently two common methods of catching the birds and placing them in coops. This study evaluated manual and mechanical catching methods on slower growing broiler chickens on the day of processing. Ten catching events, five mechanical and five manual, were evaluated for animal welfare and an additional set of 241 catches, 128 manual and 113 manual, were analyzed to determine effect on carcass quality. No significant difference in serum corticosterone concentration (CORT) was found between the catching methods (p = 0.9). Pre-catching CORT (15.07 ± 2.24) was significantly lower than post-catching (25.41 ± 2.22) (p < 0.001). Manually caught broilers had four times greater odds of tonic immobility (TI) than mechanically caught birds (OR 4.0, 95% CI: 1.54 – 10.54) (p < 0.001). Birds also had 77% lower odds of TI before being caught than after, irrespective of catching method. Manually caught birds had 19% greater risk of bruised wings (p < 0.05) and 23% greater risk of bruised legs (p < 0.05). Lower odds of TI and decreased risk of injury in the machine caught birds indicate improved welfare and carcass quality compared with manual catching. Overall, machine catching was found to improve welfare and carcass quality in these slower growing broilers.

Research Grant: United States Department of Agriculture

41. **A TNF-IL-1 Circuit Controls *Yersinia* Within Granulomas.**

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Tumor necrosis factor (TNF) is a pleiotropic inflammatory cytokine that mediates antimicrobial defense and granuloma formation in response to infection by numerous pathogens. *Yersinia pseudotuberculosis* colonizes the intestinal mucosa and induces recruitment of neutrophils and inflammatory monocytes into organized immune structures termed pyogranulomas that control the bacterial infection. Inflammatory monocytes are essential for control and clearance of *Yersinia* within intestinal pyogranulomas, but how monocytes mediate *Yersinia* restriction is poorly understood. Here, we
demonstrate that TNF signaling in monocytes is required for bacterial containment following enteric *Yersinia* infection. We further show that monocyte-intrinsic TNFR1 signaling drives production of monocyte-derived interleukin-1 (IL-1), which signals through IL-1 receptor on non-hematopoietic cells to enable pyogranuloma-mediated control of *Yersinia* infection. Altogether, our work reveals a monocyte-intrinsic TNF-IL-1 collaborative circuit as a crucial driver of intestinal granuloma function, and defines the cellular target of TNF signaling that restricts intestinal *Yersinia* infection.

42. **Generation of Swine Gamma Delta CAR T Cell Lines.**

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Chimeric antigen receptor (CAR) T cell therapies use T cells engineered to target antigens expressed in cancer cells. Research has been developed to optimize this technology using animal models such as rodents. Unfortunately, rodent models have demonstrated poor clinical predictive value, limiting the translatability to man. Swine share many anatomical, physiological, and immune characteristics with humans, making them ideal for clinical translation. Gamma delta T cells (GDCs) are a subset of CD3+ cells that can be found in the peripheral blood and mucosal tissues and have strong anti-tumor properties and no MHC restriction. This makes them an attractive cell type as there is minimal risk for inducing graft-versus-host disease compared to alpha-beta T cells. We sought to take advantage of both CAR technology and GDC characteristics to generate a large animal CAR GDC. Using swine peripheral blood mononuclear cells, we isolated and expanded GDCs for transduction with a characterized CAR molecule. Optimization of expansion techniques are being studied using zoledronic acid, IL-2, and artificial antigen presenting cells (aAPCs) based on a K562 cells line expressing human CD86 and CD64 loaded with anti-swine CD3. To date, using 1uM ZA, 1000 IU/IL2 and a 10:1 aAPC:GDC ration lead to a >4-fold expansion of absolute GDCs within 8 days. Flow cytometry data suggests that the majority of these GDCs are CD4- and CD8-.

Lentiviral transduction of GDCs with an anti-HLA A2 CAR, characterized previously in human and swine T cell effectors, yielded up to a 24.8% CAR transduction making them a viable new cell line for clinical application upon further refinement of the protocol.

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**Student Support:** Internal funds

43. **Optimization of Laser Capture Microdissection Protocol for Transcriptomic Analysis from PFA-Fixed Archival Retinal Tissues.**

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RNA sequencing (RNA-seq) coupled with laser capture microdissection (LCM) can be a powerful tool for transcriptomic analysis in tissues of experimental animal models. Paraformaldehyde (PFA) fixation, followed by embedding in Optimal Cutting Temperature (OCT) medium, is a common approach for long-term storage of vertebrate ocular tissues. However, the quality of RNA derived from archival PFA-fixed, OCT-embedded samples is limited, affecting its utility as an accurate source for transcriptomic analysis. The aim of this study was to develop a methodology to obtain high quality RNA from PFA-fixed canine eyes by utilizing LCM to isolate retinal tissue. We demonstrated the utility of an optimized LCM and RNA purification protocol for transcriptomic profiling from PFA-fixed retinal samples by comparing four pairs of canine posterior ocular cups, wherein one eye was PFA-fixed before embedding in OCT medium and the contralateral eye embedded without fixation (fresh frozen, FF). Since mRNA obtained from PFA-fixed retinas were contaminated with genomic DNA, two rounds of DNase I treatment were applied to obtain usable quality RNA for RNA-seq. RNA-seq libraries were prepared using SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian and the libraries were sequenced on Illumina NextSeq 2000. Quality of sequencing reads obtained from PFA-fixed and untreated tissues were found to be comparable. Comparison of the normalized read counts of 25 genes specifically expressed in the retina showed that most of these genes were not differentially enriched between FF and PFA-fixed samples. Gene set enrichment analysis of our
RNA-seq datasets showed that transcripts coding for extracellular matrix proteins were higher in FF samples in comparison to PFA-fixed samples. Our study provides an optimized workflow for tissue isolation with LCM, RNA extraction, quality assessment on RNA and cDNA libraries, and transcriptomic profiling from PFA-fixed archival canine retina.

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44. Induced Pluripotent Stem Cells Recapitulate Human Germ Cell Development.

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Introduction: Human induced pluripotent stem cells (hiPSCs) provide a ready source of cells that can be used to generate a variety of cell lineages. We investigated generation of male germline cells from hiPSCs to understand the development of spermatogenesis and elucidate mechanisms of infertility.

Methods: hiPSC cells were differentiated via a defined culture scheme into primordial-germ-cell-like cells (PGCLCs). PGCLCs were mixed with mouse fetal somatic cells and transplanted under the kidney capsule of recipient immunocompromised female mice, leading to the formation of xenogeneic reconstituted testes (xrTestes). xrTestes were recovered 120 to 251 days after transplant, analyzed by immunohistochemistry and single-cell RNA-sequencing (scRNAseq) and compared with normal human testes samples from gestational week 6 through to post-pubertal samples.

Results: We observed reconstitution of testicular cords within xrTestes, persisting for >8 months and showed basal colonization by human-mitochondrial-antigen-positive hiPSC-derived cells. PGCLC cells matured progressively into M (TFAP2C+, DDX4 low) then T1 prospermatogonia (TFAP2C-, DDX4 high, MAGEC2+), undifferentiated spermatogonia (UTF1+, GFRA1+), differentiating spermatogonia (KIT+, KI67+) and finally preleptotene spermatocytes (gH2AX+, REC8+).

scRNAseq analysis of xrTestes revealed distinct cell states, identified through unbiased cell clustering and specific gene expression markers and profiles. RNA velocity and pseudotime analyses showed a stepwise progression of clusters that corresponded to developing germ cell types. Alignment of iPSC-derived germ cells to in vivo human spermatogenesis displayed strong overlap in clustering, with spermatogonial populations exhibiting the highest similarity, suggesting that our hiPSC-derived system represents a faithful model of early-stage human germ cells development. hiPSC-derived germ cells closely mirrored all germ cell types up to preleptotene spermatocytes. However, beyond this stage, no further developmental progress was detected.

Conclusions: Our findings demonstrate that iPSC-derived germ cells successfully differentiate within xrTestes, although they arrest at the onset of meiosis. All developmental stages, ranging from embryonic to adult germ cell stages, were represented within xrTestes. hiPSC-derived germ cells displayed remarkable morphological and transcriptomic similarity to their counterparts in normal human germ cell development, highlighting the potential of our iPSC-derived platform for accelerating our understanding of germ cell development and for the design of innovative therapeutics to combat infertility.
45. The Use of Alternative Aids for Chronic Wasting Disease Odor Learning in Detection Canines.

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Chronic Wasting Disease (CWD) is a highly infectious, fatal prion disease that affects cervid species. One promising method for CWD surveillance is the use of detection dogs trained on the volatile organic compound signature of CWD fecal matter. However, using CWD-positive fecal matter itself poses a biohazard risk; the CWD prion can bind to soil particles and remain infectious in contaminated areas for extended periods of time. One solution is to use noninfectious alternative aids that can replicate the odor of CWD-positive and CWD-negative fecal matter and are safe to use in the environment. In this study, trained CWD detection dogs’ (N = 6) sensitivity and specificity for different alternative aid materials (cotton, GetXent tubes, and PDMS polymer) incubated with CWD-positive and CWD-negative fecal matter at two different temperatures (21°C and 37°C) for three different lengths of time (6 hours, 24 hours, and 48 hours) were evaluated. Results show that cotton incubated at 21°C for 24 hours is the best aid based on the dogs’ performance (69.4% sensitivity and 75% specificity) and practical needs for alternative aids in the field. These findings inform and contribute to the practical application of CWD field detection dogs.

46. Risk Factors for Lameness in Finisher Pigs.

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Lameness is a common condition of growing pigs that compromises welfare and production performance. The objectives of this study are to 1) determine the prevalence of lameness at two different stages of a finisher pig’s growing life; 2) investigate sow and finisher farm-level risk factors for lameness; and 3) quantify antimicrobial use on finisher farms. A visual locomotion scoring system was used to evaluate pigs on a 0-2 scale ranging from no lameness to severe lameness. Finisher farm-level risk factors investigated included housing factors, nutrition, and presence of Mycoplasma hyosynoviae in oral fluid samples. Multivariable linear regression analysis was used to examine the association between farm-level factors and lameness prevalence. Preliminary findings were obtained from 6,862 pigs from 4 companies assessed on 26 different farms during the first targeted stage of growth (63-108 days). The mean (SD) percentage of lame pigs per farm was determined to be 23.8% (10) with a range of 7-41%. Of the pooled oral fluid samples, 26.9% tested positive for M. hyosynoviae and 19.2% of samples from pens with the highest combined lameness score tested positive for M. hyosynoviae. There were no statistically significant associations between lameness prevalence and any farm-level factors. The results of this study demonstrate a higher lameness prevalence than previously reported in the literature (1-20%). Prevalence of lameness and M. hyosynoviae oral fluid results do not appear to be related, at least at this age. Additional data collection from more farms and from older pigs on each farm is ongoing. Identification of risk factors for lameness may help elucidate disease pathogenesis and direct producers towards beneficial management and husbandry practices, including antimicrobial stewardship.

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