ILLUMINATING NEW FRONTIERS

The Future of Research at Penn Vet

NOVEMBER 1, 2024

The Inn at Swarthmore // Swarthmore, Pennsylvania

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PROGRAM

8:30 a.m.	**REGISTRATION & CONTINENTAL BREAKFAST**	
9:00 a.m.	**OPENING REMARKS**	Phillip Scott, PhD *Vice Dean for Research & Academic Resources*
9:10 a.m.	**PATHOBIOLOGY** Talks: 15 min with 5 min Q & A	
9:10 a.m.	The future of research in Pathobiology	Igor E. Brodsky, PhD
9:30 a.m.	The cryptic role of MHC-E-restricted T cells in flu infection	Michael J. Hogan, PhD
9:50 a.m.	Translation-dependent downregulation of Cas12a mRNA by an anti-CRISPR protein	Nicole D. Marino, PhD
10:10 a.m.	**CLINICAL SCIENCES & ADVANCED MEDICINE** Talks: 15 min with 5 min Q & A	
10:10 a.m.	The future of research in the Department of Clinical Sciences & Advanced Medicine	Mark A. Oyama, DVM, MSCE, DACVIM
10:30 a.m.	STING agonist immunotherapy in canine solid tumors	Jennifer A. Lenz, DVM, DACVIM (Oncology)
10:50 a.m.	Temporal profiling of the tumor microenvironment immune landscape in canine nasal carcinoma: Single-cell RNA sequencing	Thomas Lee, DVM, MVM, MS
11:10 a.m.	**BREAK**	
11:30 a.m.	**ROBERT R. MARSHAK LECTURE** Using the immune system as our new drug paradigm: From T cell exhaustion to Immune Health 40 min with 10 min Q & A	E. John Wherry, PhD
12:25 p.m.	**LUNCH**	
1:20 p.m.	**POSTER SESSION**	
2:00 p.m.	**BIOMEDICAL SCIENCES** Talks: 15 min with 5 min Q & A	
2:00 p.m.	Animal biology is biomedical science	Christopher J. Lengner, PhD
2:20 p.m.	Vascular regeneration and angiocrine inflammation in viral pneumonia	Andrew E. Vaughan, PhD
2:40 p.m.	Comparative oncogenomics, for dog and man	Timour Baslan, PhD

PROGRAM CONT'D.

3:00 p.m.	**BREAK**	
3:20 p.m.	**CLINICAL STUDIES—NEW BOLTON CENTER** Talks: 15 min with 5 min Q & A	
3:20 p.m.	The future of research in the Department of Clinical Studies—New Bolton Center	Katrin Hinrichs, DVM, PhD
3:40 p.m.	Equine viral hepatitis: Clinical conditions and epidemiology	Joy E. Tomlinson, DVM, PhD, DACVIM (LAIM)
4:00 p.m.	Translational animal models of subchondral bone injury offer opportunities to advance osteoarthritis research	Holly Stewart, VMD, PhD
4:20 p.m.	**ZOETIS PRIZE**	Awarded by Katrin Hinrichs, DVM, PhD
4:30 p.m.	**RAFFLE, POSTER SESSION, AND RECEPTION**	

PHOTOS ON FACING PAGE (FROM TOP TO BOTTOM):

1. Confocal microphotograph of an equine oocyte fertilized by standard *in vitro* fertilization, showing the sperm tail stained with Mitotracker Red (center) adjacent to the decondensing sperm chromatin, and the oocyte chromatin in anaphase of the second meiotic division, adjacent to the first polar body at 4:00. (Katrin Hinrichs)
2. Lineage tracing of pulmonary veins generating new lung capillaries after influenza infection. (Andrew Vaughan)
3. Equine parvovirus-hepatitis infects hepatocytes and results in hepatocellular necrosis and lymphocytic infiltrates. An infected hepatocyte is shown (arrow, EqPV-H *in situ* hybridization). (Joy Tomlinson)

ACKNOWLEDGMENTS

Organizing Committee

Phillip Scott, PhD
Katherine A. Kruger, MSW

THE UNIVERSITY OF PENNSYLVANIA SCHOOL OF VETERINARY MEDICINE IS GRATEFUL TO THE ORGANIZATIONS, SPONSORS, DONORS, AND FAMILIES WHO HAVE MADE OUR RESEARCH POSSIBLE.

Dean Emeritus Alan M. Kelly

We honor the memory of Dean Emeritus Alan M. Kelly, who created the Research Retreat in 1994 to bring together basic science and clinical faculty from the Philadelphia and New Bolton Center campuses to meet one another and learn about each other's research. The first retreat was held on June 14, 1994.

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.

Zoetis

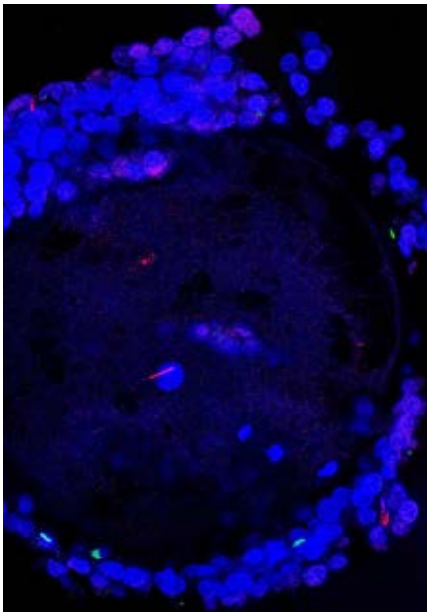
Our thanks to Zoetis for the 2024 Zoetis Award for Veterinary Research Excellence to be presented to a member of the Penn Vet faculty. The award aims to foster innovative research by recognizing outstanding research effort and productivity in the veterinary profession.

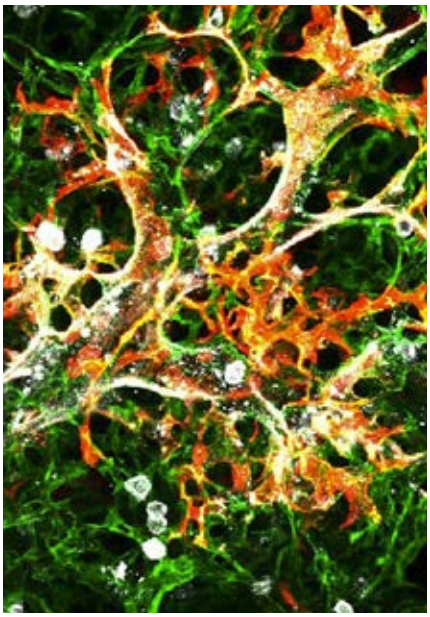
Department Chairs

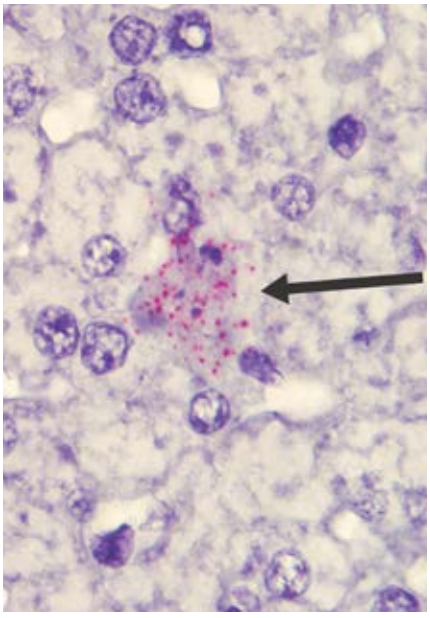
Sincerest thanks to Drs. Igor Brodsky, Katrin Hinrichs, Chris Lengner, and Mark Oyama for their assistance with the *30 Years of Penn Vet Research* graphic.

Thank You

We thank our generous and helpful friends and colleagues, including Sue Waddington-Pilder; Caitlin Ware; John Donges; Colin Redick; Stephen Hawkins; Anne Marie Kane, Imogen Design, LLC; and Melissa Sage & Carly Warner, Inn at Swarthmore.







ROBERT R. MARSHAK LECTURE

THE ROBERT R. MARSHAK LECTURESHIP WAS ESTABLISHED IN 1993 IN HONOR OF DEAN EMERITUS ROBERT MARSHAK, THE NINTH DEAN OF THE SCHOOL OF VETERINARY MEDICINE.

Using the immune system as our new drug paradigm: From T cell exhaustion to Immune Health

E. John Wherry, PhD

E. JOHN WHERRY, PhD

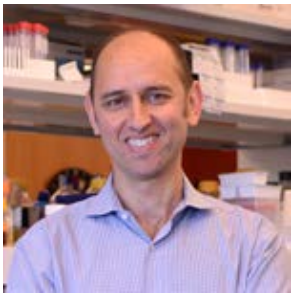
Richard and Barbara Schiffrin President's Distinguished Professor
Director, Institute for Immunology
Chair, Department of Systems Pharmacology & Translational Therapeutics
Perelman School of Medicine

Dr. E. John Wherry is a professor of immunology and the director of the Institute for Immunology at the Perelman School of Medicine. Dr. Wherry's research focuses on T cell exhaustion in chronic infections and cancer and on the mechanisms by which immunoregulatory "checkpoint" pathways, such as PD-1, control T cell exhaustion. His work has advanced the understanding of how gene expression changes affect this exhaustion, which has led to strategies to improve the effectiveness of T cell targeting immunotherapies.

FACULTY SPEAKERS

Timour Baslan, PhD

Dr. Baslan leads a multidisciplinary research group that employs computation and functional biology to advance an understanding of cancer genetics and biology with the aim of developing early detection strategies and therapeutics.



Igor E. Brodsky, PhD

The Brodsky lab is interested in host-pathogen interactions, with a focus on innate immune defense against enteric bacterial pathogens and bacterial evasion strategies. Dr. Brodsky is especially interested in how a particular arm of the innate immune system, known as 'The Inflammasome,' which triggers an inflammatory form of cell death, termed pyroptosis, detects and responds to bacterial infection. Dr. Brodsky obtained his undergraduate degree in molecular biology at Princeton University and pursued PhD studies at Stanford University in the Department of Microbiology and Immunology, studying the adaptation of Salmonella to antimicrobial peptides. His postdoctoral training in immunology was conducted at Yale University, where he focused on the innate immune detection of bacterial pathogens. Recent work in the Brodsky lab has investigated the molecular basis for inflammasome activation in response to pathogen manipulation of NF-kB signaling, has investigated the role of inflammatory monocytes in the formation of intestinal granulomas during gastrointestinal bacterial infection, and has explored the cell biology of the cytosolic response to lipopolysaccharide from Gram-negative bacteria. The Brodsky lab's long-term research goals are to continue to define the fundamental mechanisms that govern innate detection of microbial infection with the goal of improving human and animal health.

Dr. Brodsky joined the Department of Pathobiology in 2011 as an Assistant Professor, was promoted to Associate Professor in 2017, and became Department Chair in 2021. His long-term goal as Chair of Pathobiology is to continue to grow and support an exceptional community of productive, engaged, and energetic faculty who are recognized for their research, teaching, and clinical missions, as well as their leadership in research in the areas of immune responses to infectious diseases, biology of infection, and organismal pathology.

FACULTY SPEAKERS

Katrin Hinrichs, DVM, PhD

Dr. Hinrichs is the Harry Werner Endowed Professor of Equine Medicine and Chair of the Department of Clinical Studies—New Bolton Center. She obtained her DVM from the University of California, Davis, and her PhD from the University of Pennsylvania. Dr. Hinrichs' laboratory has pioneered research into equine assisted reproduction, developing the first successful program for *in vitro* production of equine blastocysts via intracytoplasmic sperm injection (ICSI) in the US. Her research has led to methods for shipment of immature oocytes and for biopsy and cryopreservation of equine embryos, now utilized worldwide, and most recently, the first efficient protocol for standard *in vitro* fertilization in the horse.

Michael J. Hogan, PhD

Dr. Hogan is a new tenure-track assistant professor based in the Department of Pathobiology, and opened his lab on January 1, 2024. The goals of his lab are to understand the mechanisms of T cell-based protection from viral infections and to translate these lessons into the design of better vaccines. Selected topics of interest include CD8 T cell control of respiratory virus infections, the mechanisms of mRNA vaccine immunogenicity, and the development of mRNA/lipid nanoparticle vaccines for animal species with veterinary and agricultural importance. Mike did his postdoctoral fellowship at the Children's Hospital of Philadelphia (CHOP) in the lab of Dr. Laurence "Ike" Eisenlohr, where he studied the T cell response to influenza virus. Before that, he did his doctoral training at the University of Pennsylvania in the labs of Drs. James A. Hoxie and Drew Weissman, where he helped to describe the nucleoside-modified mRNA vaccine platform used against COVID-19.

Thomas Lee, DVM, MVM, MS

Dr. Thomas Lee is a new faculty member of radiation oncology at the Penn Vet Cancer Center. He earned his DVM degree from the National Taiwan University (NTU) School of Veterinary Medicine. Following a rotating internship and a combined residency in medical oncology and a master's degree program, Dr. Lee served as a clinical instructor at NTU for two years. During this time, he collaborated with a human radiation oncology team, treating animal patients and developing a deep interest in radiation therapy. His growing passion for this field led him to pursue and complete a residency in veterinary radiation oncology at the Flint Animal Cancer Center at Colorado State University (CSU), where he also earned a second master's degree. Dr. Lee became a board-certified veterinary radiation oncologist in 2021 and is currently finalizing his PhD in cell and molecular biology with a specialization in cancer biology. His research interests focus on head and neck cancers, radioimmunotherapy, and the biology and application of advanced radiation technologies such as stereotactic body radiation therapy (SBRT) and FLASH radiation therapy. His professional goal is to excel as a clinician-scientist, providing compassionate patient care with cutting-edge technology, while conducting translational cancer research that benefits patients across species.

Christopher J. Lengner, PhD

Dr. Lengner is the Harriet Ellison Woodward Professor and Chair of the Department of Biomedical Sciences, where his lab uses molecular genetic and genomic tools to understand the organization of adult stem cell compartments and their oncogenic transformation. The Lengner lab currently focuses on endodermal organs and disease states, including understanding the fundamental organization of the intestinal stem cell compartment and how its dysregulation initiates tumorigenesis, how telomere biology disorders impact stem cell function and crosstalk with the microenvironment, and how new genetic tools can be harnessed to understand lineage in tissue regeneration and cancer metastases.

Prior to assuming the role of Department Chair, Dr. Lengner acted as the Co-Director, along with Dr. Jeremy Wang, of Penn Vet's Center for Animal Transgenesis from 2014-2023. Dr. Lengner also served as the Associate Director of the Institute for Regenerative Medicine at the University of Pennsylvania from 2017-2023.

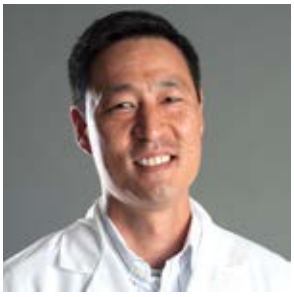
Jennifer A. Lenz, DVM, DACVIM (Oncology)

Dr. Lenz is an Assistant Professor of Medical Oncology in the Department of Clinical Sciences and Advanced Medicine. Dr. Lenz earned her DVM degree from the University of Wisconsin—Madison in 2014. She completed her residency in medical oncology at the University of Minnesota, obtained board certification through the American College of Veterinary Internal Medicine (Oncology), and joined the faculty at the University of Pennsylvania in 2018. Dr. Lenz's primary research interest is to characterize the immune landscape of the tumor microenvironment in companion animals and identify spontaneous cancer models for translational research. Dr. Lenz is a member of the Atherton Laboratory and, in collaboration with the Penn Vet Comparative Pathology Core, designed an immunohistochemical panel to characterize and quantify tumor-infiltrating lymphocytes (TIL) in canine tumor tissues. Dr. Lenz plays an active role in the Comprehensive Cancer Care service of Ryan Veterinary Hospital, which provides tertiary-level care to canine and feline cancer patients. She also serves as principal investigator for several ongoing veterinary clinical trials evaluating novel immunotherapeutics.

Nicole D. Marino, PhD

Dr. Marino is an Assistant Professor in the Department of Pathobiology. She received her Bachelor of Arts from Rice University in Houston, Texas, with dual majors in biochemistry & cell biology and classical studies. She obtained her PhD in 2018 from Stanford University, where she studied *Toxoplasma gondii* pathogenesis in Dr. John Boothroyd's laboratory. Her fascination with parasites and infectious disease led her to explore the ancient arms race between bacteria and their genetic parasites (phage) as a postdoctoral fellow in Dr. Joseph Bondy-Denomy's lab at the University of California, San Francisco. Her lab currently investigates the molecular mechanisms that bacteria use to thwart phage infection and how phage overcome these defenses. Outside of the lab, Dr. Marino enjoys tango dancing, cooking, live music, and comedy shows.

FACULTY SPEAKERS



Mark A. Oyama, DVM, MSCE, DACVIM

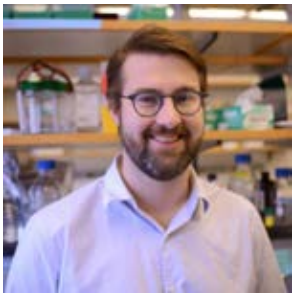
Dr. Oyama is a veterinary cardiologist and Chair of the Department of Clinical Sciences and Advanced Medicine (CSAM). He received his DVM degree from the University of Illinois, performed his internship at the Animal Medical Center in NYC, and his residency at UC-Davis. He has been actively involved in applied and clinical cardiology research for over 20 years.

Holly Stewart, VMD, PhD

Dr. Stewart received her veterinary degree from the University of Pennsylvania, followed by an internship at Pioneer Equine Hospital in Oakdale, California. Following her internship, she completed a large animal surgery residency at the University of Pennsylvania's New Bolton Center. After her residency, Holly pursued a PhD in clinical orthopedics at Colorado State University. Her doctoral research focused on the evaluation of bone edema (bone marrow lesions), including the development of translational experimental models for bone edema and optimization of volumetric imaging for the detection of fluid within bone. She stayed on at Colorado State University after her PhD and worked at the Preclinical Surgical Research Laboratory within the Translational Medicine Institute. She returned to New Bolton Center as an Assistant Professor of Large Animal Surgery in 2023, where she has continued her research focused on understanding the role of subchondral bone in joint health and disease.

Joy E. Tomlinson, DVM, PhD, DACVIM (LAIM)

Dr. Tomlinson is an Assistant Professor of Large Animal Medicine in the Department of Clinical Studies—New Bolton Center. She received her degrees in biological and environmental engineering and veterinary medicine from Cornell University. After graduation, she completed a private practice internship at Chino Valley Equine Hospital in southern California, followed by a residency in large animal internal medicine at New Bolton Center, achieving board certification in large animal internal medicine in 2014. After her residency, she worked at Cornell University as a lecturer and senior research associate before returning to the University of Pennsylvania in her current role in 2024. Her research focuses on equine viral hepatitis, including the pathogens equine parvovirus-hepatitis (EqPV-H) and equine hepacivirus (EqHV). Her seminal work in this area has defined new diseases, resulting in federal regulations to prevent disease transmission. The American Association of Equine Practitioners recognized her work as having significant impact on the diagnosis, treatment, or prevention of equine disease with the conferral of the AAEP 2021 Research Award. Dr. Tomlinson's ongoing research plans include both a big-picture approach to mapping the transmission, epidemiology, and disease associations of both viruses, as well as a finer look at the role of the immune responses in liver injury and determinants of disease severity.



Andrew E. Vaughan, PhD

Dr. Vaughan's principal research interests lie in characterizing the relevant cell types and molecular mechanisms that orchestrate regeneration of epithelial tissues in order to promote more effective repair. He is especially interested in the delicate balance that exists between appropriate tissue repair (euplasia) and maladaptive/dysplastic tissue remodeling. He was initially trained in basic molecular biology and biochemistry as an undergraduate at the University of Nebraska. Dr. Vaughan developed a broad range of experiences throughout graduate school and during his postdoctoral fellowship, including molecular virology, lung biology, and regenerative medicine. His focus has ultimately coalesced around employing a cellular and molecular framework to study lung injury, in particular, understanding how the various pulmonary tissue compartments are able to (successfully or unsuccessfully) reconstitute themselves after severe lung injury. Dr. Vaughan established a research group as an assistant professor at the University of Pennsylvania in 2017, and both as a trainee and now as a principal investigator, his lab continues to make major progress in understanding the molecular mechanisms needed for successful lung repair, especially after respiratory viral infections (e.g., H1N1 influenza, SARS-CoV-2).

30 YEARS OF PENN VET RESEARCH

30TH ANNIVERSARY RESEARCH RETREAT (1994-2024)

For 140 years, Penn Vet researchers have transformed veterinary medicine, improved animal health and welfare, and contributed to significant advances in human medicine.

Discover how the last three decades of Penn Vet's scientific innovations and breakthroughs have illuminated new frontiers.

1994

Ralph Brinster, VMD, PhD, authors a series of landmark studies describing the development of conditions enabling the stable culture of male spermatogonial stem cells and, subsequently, the transplantation of these stem cells to reconstitute spermatogenesis in recipient animals.

Phillip Scott, PhD, and collaborators show that IL-12 dramatically enhances the effectiveness of a vaccine against leishmaniasis.

1995

Under the guidance of **James D. Ferguson, VMD**, a team at New Bolton Center develops the concept of systemic breeding of dairy cows in an integrated program, leading to global changes in the dairy industry.

John H. Wolfe, VMD, PhD, and collaborators demonstrate the potential of neural progenitor cell engraftment to correct lysosomal storage disorders in a mouse model of mucopolysaccharidosis VII (Sly syndrome).

Gail Smith, VMD, PhD, receives a patent for the PennHIP method of assessing canine hip dysplasia.

1996

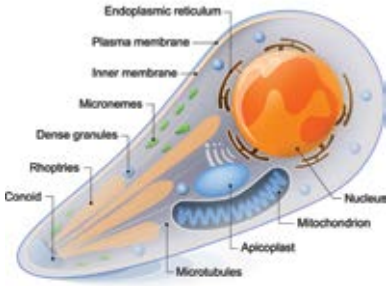
2001

Thomas D. Parsons, VMD, PhD, creates a prototype of a non-competitive electronic sow feeding (ESF) system. The system uses electronic ear tags to dispense the proper amount of feed to specific animals. Today, more than 200,000 sows across the country are being raised using similar systems.

2002

Ina Dobrinski, Dr. med. vet., MVSc, PhD, develops a novel technique for inducing and maintaining complete spermatogenesis from immature pig and goat testes by grafting them into immunocompromised mice. The findings have implications for preserving the germ lines of critically endangered species.

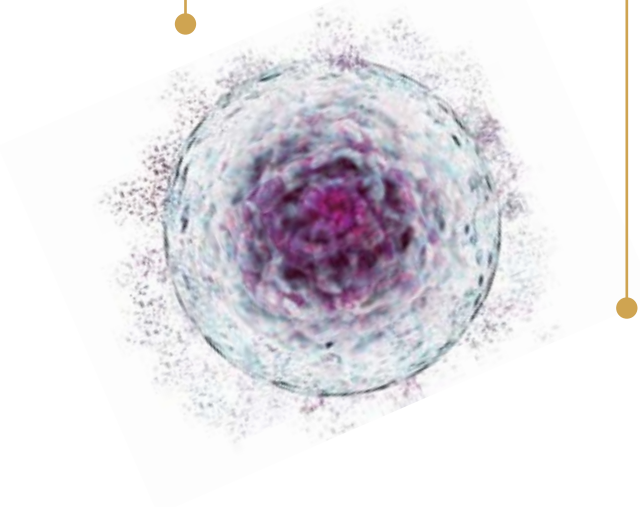
2003



Christopher A. Hunter, PhD, and collaborators discover that the IL-27R (WSX-1) is required to suppress T cell hyperactivity and is critical for survival during *Toxoplasma gondii* infection.

James A. Serpell, PhD, develops the Canine Behavioral Assessment and Research Questionnaire (C-BARQ), the first canine behavioral assessment of its kind to be extensively tested for reliability and validity on large samples of dogs of many breeds.

Hans R. Schöler, PhD, makes seminal contributions to the study of pluripotency—the ability of a stem cell to make any cell type in the body—by describing the transcription factor OCT4 as a central regulator of pluripotency. Dr. Schöler's work laid the foundation for the generation of induced pluripotent stem cells.

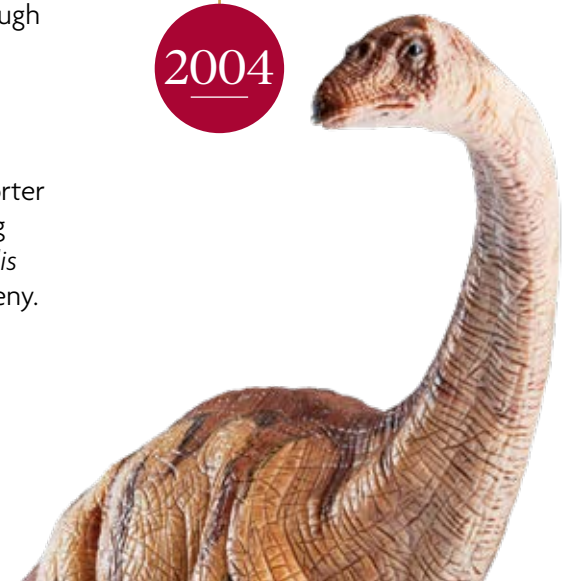


Mark Haskins, VMD, and collaborators produce the first successful application of gene therapy in preventing the clinical manifestations of a lysosomal storage disease (mucopolysaccharidosis VII) in a large animal model (dogs).

James B. Lok, PhD, through gonadal microinjection of DNA constructs, successfully introduces and achieves transient expression of two reporter transgenes in free-living *Strongyloides stercoralis* females and their progeny.

Peter Dodson, PhD, describes a new diplodocoid sauropod dinosaur species, *Suuwassea emilieae*, that was found by in 1998 by William J. Donowick, DVM, emeritus professor of surgery, while on a horseback ride in Montana.

2004



2005

Charles H. Vite, DVM, PhD, performs the first successful gene therapy treatment of a central nervous system disease (α-mannosidosis) in a large animal model (cats).

2006

J. Oriol Sunyer, PhD, and collaborators demonstrate that B cells in teleost fish and amphibians possess robust phagocytic and microbicidal abilities, suggesting a potential evolutionary link between B lymphocytes and macrophages.

2011

Ralph Brinster, VMD, PhD, receives the National Medal of Science from President Barack Obama. Dr. Brinster is the first veterinarian to win the medal.

2017

Serge Y. Fuchs, MD, PhD, and collaborators demonstrate that degradation of IFNAR1 undermines survival and activities of cytotoxic T lymphocytes and is a major driver of immunosuppression in colorectal tumors, illuminating IFNAR1 as a promising target for novel anti-cancer therapies.

2008

Lawrence R. Soma, VMD; Cornelius E. Uboh, PhD, and colleagues become first in the world to develop a method for confirming and differentiating blood-doping agents—recombinant human erythropoietin (rhEPO) and darbepoetin alfa (DPO)—in equine athletes by testing plasma through liquid chromatography coupled to tandem mass spectrometry.

2014

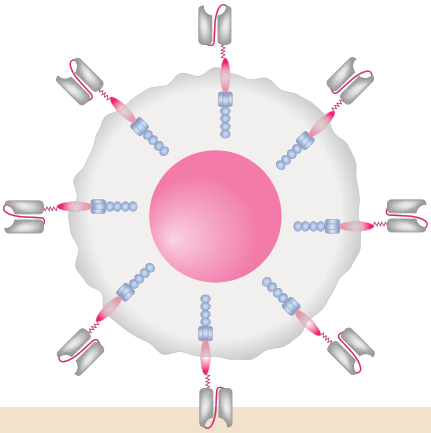
Dean W. Richardson, DVM, and a co-investigator develop methods for computed tomography-guided internal fixation of equine orthopedic fractures in clinical patients. This technique offers significant advantages in accuracy, planning, and postoperative assessment.

Ellen Puré, PhD, and collaborators describe how fibroblasts within tumors activate expression of a cell surface protein called fibroblast activation protein (FAP) that plays a crucial role in promoting tumor progression by suppressing the immune response to tumor cells.

The work of **Gustavo D. Aguirre, VMD, PhD**, and **William A. Beltran, DVM, PhD**, leads to FDA approval of the first gene therapy for biallelic RPE65 mutation-associated retinal dystrophy, an inherited condition that results in progressive vision loss and complete blindness in some patients. This is the first directly administered gene therapy approved in the US that targets a disease caused by mutations in a specific gene.

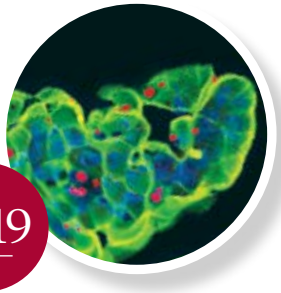
2018

Susan W. Volk, VMD, PhD, receives a patent for her approach to reengineering the tumor microenvironment, using collagen type III, to prevent metastatic spread following surgical removal of tumors.



2019

Boris Striepen, PhD, and collaborators demonstrate that sexual reproduction is not only necessary for the transmission of the parasite *Cryptosporidium* from one host to the next but also to sustain continued infection, suggesting blocking parasite sex as a novel target to cure or prevent this infection.



2020

Nicola J. Mason, BVetMed, PhD, and collaborators establish a model system for evaluating CAR T cell therapy using dogs with spontaneous diffuse large B cell lymphoma.

2021

The work of **David E. Holt, BVSc**, involving near-infrared (NIR) intraoperative imaging of tumors, tumor margins, satellite lesions, and metastatic lymph nodes in dogs, helps lead to FDA approval of NIR for use in humans.

2022

Kotaro Sasaki, MD, PhD, and collaborators devise methodologies to coax stem cells in culture to differentiate and take on some of the functions of a human fetal adrenal gland, bringing the goal of adrenal cell replacement one step closer.

Katrin Hinrichs, DVM, PhD, and collaborators publish the first successful method for equine standard *in vitro* fertilization (IVF), culminating in the production of viable blastocysts and the birth of healthy foals.

2023

Dipti Pitta, MVSc, PhD, and collaborators find that low methane-emitting dairy cows showed a greater abundance of hydrogenotrophic bacteria, and a greater expression of genes related to propionate production, suggesting that targeting specific microbial pathways could offer promising avenues for mitigating methane emissions from dairy cows, a significant contributor to greenhouse gases.

Mary Beth Callan, VMD, and collaborators successfully perform AAV gene therapy in dogs with hemophilia, demonstrating lasting efficacy and improved quality of life.

2024

PENN VET PATENTS (1994—2024)

(alphabetical by Penn Vet inventors)

52 unique patents in 26 countries

INVENTOR(S)	PATENT	ISSUE DATE
Gregory Acland, **Gustavo Aguirre,** Jean Bennett, William Hauswirth, Samuel Jacobson, and Albert Maguire	Method of treating or retarding the development of blindness	4/3/2012
Gustavo Aguirre, William Beltran, Sanford Boye, Artur Cideciyan, Wen-Tao Deng, William Hauswirth, Samuel Jacobson, and Alfred Lewin	AAV-mediated gene therapy for RPGR X-linked retinal degeneration	1/5/2017
Gustavo Aguirre, William Beltran, Artur Cideciyan, William Hauswirth, Samuel Jacobson, Alfred Lewin, and Michael Massengill	AAV vectors for treatment of dominant retinitis pigmentosa	9/14/2021
Daniel Beiting, Ana Misic, and **Shelley Rankin**	Drug target for treating veterinary infections and methods of using same	9/15/2020
William Beltran, Leah Byrne, John Flannery, David Schaffer, and Mieke Visel	Adeno-associated virus virions with variant capsid and methods of use thereof	5/10/2022
Richard Behringer and **Ralph Brinster**	Synthesis of functional human hemoglobin and other proteins in erythroid tissues of transgenic animals	2/11/1997
Richard Behringer and **Ralph Brinster**	Nucleic acid vectors comprising DNase I hypersensitive sites	2/8/2000
Ralph Brinster and James Zimmerman	Repopulation of testicular seminiferous tubules with foreign cells	6/4/1997
Ralph Brinster and James Zimmerman	Techniques for freezing spermatogonia cells	10/6/1998
Ralph Brinster, Jay Degan, Richard Palmiter, and Eric Sandgren	Non-native liver generation in an animal with impaired native liver function by cell implantation	7/25/1997
Ina Dobrinski, Ali Hanaramooz, Stefan Schlatt, and **Hans Schöler**	Material and methods for the production of sperm and analysis thereof	8/8/2006
Gary Cohen, **Roselyn Eisenberg**, and Anthony Nicola	Herpes simplex virus glycoprotein D variants	8/5/1997
Gary Cohen, Gary Dubin, **Roselyn Eisenberg,** and Tao Peng	Soluble herpesvirus glycoprotein complex vaccine	12/5/2000

INVENTOR(S)	PATENT	ISSUE DATE
Lydia Aldaz-Carroll, Gary Cohen, **Roselyn Eisenberg,** Christina Fogg, Shlomo Lustig, Bernard Moss, and Charles Whitbeck	Compositions, methods and kits relating to poxvirus subunit vaccines	7/14/2009
Bruce Freedman, Ronald Harty, Michael Lee, H. Marie Loughran, Mark Olson, Allen Reitz, and Jay Wrobel	Antiviral compounds and methods using same	12/25/2018
Joan Hendricks, Leszek Kubin, Allan Pack, and Sigrid Veasey	Use of serotonin agonists to alleviate disordered breathing episodes in a mammal	5/14/2002
Christopher Hunter and Alejandro Villarino	Methods for modulating an inflammatory response	9/3/2009
Christopher Hunter and Jason Stumhofer	WSX-1/P28 as a target for anti-inflammatory responses	11/13/2012
Carolina López	Methods and compositions for stimulating immune response using potent immunostimulatory RNA motifs	4/21/2020
Nicola Mason	Single chain fragment variable antibody libraries and uses thereof	5/13/2014
Paulo Maciag, **Nicola Mason,** Yvonne Paterson, Matthew Seavey, Vafa Shahabi, and Anu Wallecha	Compositions and methods for prevention of escape mutation in the treatment of her2/neu over-expressing tumors	4/28/2015
Nicola Mason, Mohammed Panjwani, Daniel Powell, and Jenessa Smith	Treatment of a canine Cd20 positive disease or condition using a canine	9/14/2021
Nicholas Chester, **Nicola Mason,** and Donald Siegel	Canine monoclonal antibodies against canine cytotoxic T lymphocyte associated protein 4 (CTLA-4)	10/8/2024
Nicola Mason and Yvonne Paterson	Combination immuno therapy and radiotherapy for the treatment of HER2-positive cancers	7/10/2018
Ron Nash and **David Nunamaker**	External fixation device	11/26/1996
James Baumgardner and **Cynthia Otto**	Method and apparatus for measuring nitric oxide production and oxygen consumption in cultures of adherent cells	7/24/2007

PENN VET PATENTS (1994—2024) CONT'D.

INVENTOR(S)	PATENT	ISSUE DATE
Alan Charlie Johnson, Nicholas Kybert, **Cynthia Otto,** George Preti, Katharine Prokop-Prigge, and Janos Tanyi	Volatile organic compound-based diagnostic systems and methods	8/16/2022
Haig Aghajanian, Steven Albelda, Jonathan Epstein, and **Ellen Puré**	Methods for treating heart disease via redirected T cell immunotherapies	10/17/2023
Steven Albelda, Leslie Hopper, **Ellen Puré,** and John Scholler	Disrupting tumor tissues by targeting fibroblast activation protein (FAP)	10/15/2024
Patrick Reilly	Hydraulic crumb silicone and orthotics comprising same	10/24/2017
Bryan Hsu, Alexander Klibanov, **Thomas Schaer,** and Suzanne Stewart	Antibacterial coatings that inhibit biofilm formation on implants	7/28/2015
Jessica Gilbertie, **Thomas Schaer,** and Lauren Schnabel	Cationic platelet lysate compositions and related methods	4/9/2024
Josh Baxter, Megan Farrell, Michael Hast, Robert Mauck, Liane Miller, **Thomas Schaer,** David Steinberg, Brendan Stoeckl, and Hannah Zlotnick	Anatomic tissue-engineered osteochondral implant and method for fabrication thereof	4/25/2023
Karin Huebner and **Hans Schöler**	Compositions for the derivation of germ cells from stem cells and methods of use thereof	4/27/2010
Michele Boiani, Sigrid Eckardt, **John McLaughlin,** and **Hans Schöler**	Enhanced production of cloned mammals by zona pellucida-free homologous mammalian embryo aggregation	3/23/2010
Michele Boiani, Sigrid Eckardt, **John McLaughlin,** and **Hans Schöler**	Compositions and methods for the efficient and reproducible generation of clone animals of all developmental stages and methods of use thereof	7/17/2012
Phillip Scott and Giorgio Trinchieri	Compositions and methods for use of IL-12 as an adjuvant	11/5/1996
Mary Hazzard, **William Moyer,** and **Robert Sigafoos**	Protective covering for a horse's hoof and method of attaching	7/19/1994
Gail Smith	Method for assessing canine hip dysplasia	1/9/1996
Gail Smith	System and method for diagnosing onset of osteoarthritis	2/4/2014

INVENTOR(S)	PATENT	ISSUE DATE
Darko Stefanovski and Jian Tajbakhsh	Methods for single-cell prostate tissue classification and prediction of cancer progression	8/27/2024
Michael Dews, Joshua Mendell, **Andrei Thomas-Tikhonenko,** and Erik Wentzel	Compositions and methods for modulating angiogenesis	6/18/2013
Susan Volk	Collagen III composition and uses	5/14/2019
Wen Shieh, Shu Tai, and **Leon Weiss**	Methods and apparatus for biological treatment of aqueous waste	10/7/2003
Robert Whitlock	Method to reduce contamination when culturing mycobacteria	9/5/2006
Nigel Fraser and **John Wolfe**	Method of delivering genes to the central nervous system of a mammal	7/22/2008
Seung Kim, Evan Snyder, and **John Wolfe**	Engraftable human neural stem cells	9/28/1999
Qin Yu	Methods and pharmaceuticals compositions for treating coronary artery disease, ischemia,and vascular disease using angiopoietins	9/23/2008
Qin Yu	Compositions of angiopoietin, fragments, mutants and analogs thereof and uses of the same	8/4/2009
Yin Xu and **Qin Yu**	Methods of treating cancer, arthritis and/or diabetes with angiopoietins	7/31/2012
Yin Xu and **Qin Yu**	Composition comprising an angiopoietin-4 fragment	6/20/2017
Qin Yu	Function and regulation of ADAMTS-1	4/13/2010

PENN VET'S RESEARCH-FOCUSED ASSISTANT PROFESSORS WILL PLAY A PIVOTAL ROLE IN SHAPING THE FIELDS OF BIOMEDICAL AND VETERINARY SCIENCE. THEIR EXPERTISE IN CUTTING-EDGE TECHNOLOGIES, PASSION FOR DISCOVERY, AND COMMITMENT TO EXCELLENCE WILL DEFINE THE FUTURE OF RESEARCH AT PENN VET.

ASSISTANT PROFESSORS

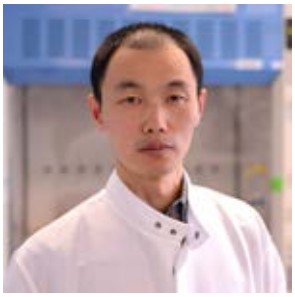
BIOMEDICAL SCIENCES

Timour Baslan, PhD

Cancer genetics and biology; sequencing based computational method development and analytics (ex: long and short read sequencing, statistics, unsupervised learning/ML); experimental perturbation approaches in cancer models systems (ex: shRNA, CRISPR, and chemical screens in in-vitro cell lines, organoids, and mouse models); copy number alterations (CNAs)

M. Andres Blanco, PhD

Mechanisms by which epigenetic information is encoded, interpreted, and propagated in normal and pathological (e.g., cancerous) cell identity programs



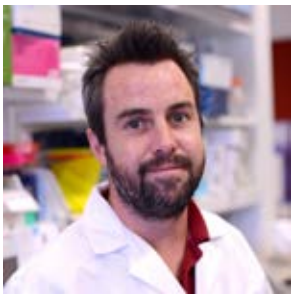
Ning Li, MD

Colorectal cancer initiation; colorectal cancer progression; multi-drug resistance; epithelial-mesenchymal interactions; engineered mouse models

Andrew J. Modzelewski, PhD

Retrotransposon activity; proteomics; genetics; bioinformatics/CRISPR-Cas9 genome editing technologies; CRISPR-EZ; transposons, endogenous retroviruses (ERV)

CLINICAL SCIENCES & ADVANCED MEDICINE

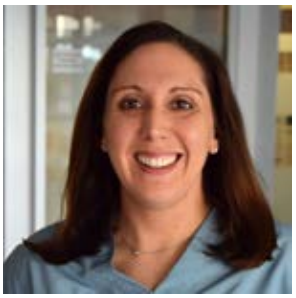


Matthew J. Atherton, BVSc, PhD, DECVIM-CA (Oncology)

Defining the prognostic and therapeutic role of T cells in hematologic neoplasms utilizing a multi-species comparative approach; identification and appraisal of novel targets for T cell-based immunotherapy in clinical trials of canine patients with spontaneous and aggressive tumors bearing translational importance for human oncology

Ana C. Castejon Gonzalez, DVM, PhD, DAVDC

Management of maxillofacial trauma; congenital and acquired palate defects



Dana L. Clarke, VMD, DACVECC

Interventional radiology; critical care

Rachel Clarkin-Breslin, DVM

Viscoelastic testing in small animals with clinical hypercoagulability; role of erythrocytes in clot formation; performance of viscoelastic testing both in the setting of comorbidities common to the small animal ICU as well as in spontaneous disease; immunothrombosis in viral and bacterial pneumonia; the relevance of point-of-care rheological testing in assessing microvascular health

Alessia Cordella, DVM, MSc, PhD, DECVDI (SA)

Use of advanced computed tomographic and ultrasonographic techniques, such as multiphase CT, contrast-enhanced US (CEUS) and elastosonography for the diagnosis of thoracic and abdominal diseases in small animals, with a particular focus on urinary, lymphatic and gastrointestinal systems

ASSISTANT PROFESSORS

CLINICAL SCIENCES & ADVANCED MEDICINE

Alexandra V. Crooks, VMD, DACVIM (Cardiology)

Heart failure management; arrhythmia diagnosis and treatment

Valérie L. Dufour, DVM, MSc, dip. ECVO

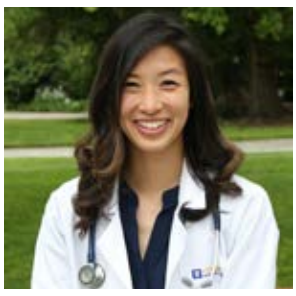
In vivo assessment of natural disease history in canine models of inherited retinal degeneration and preclinical assessment of retinal therapeutic strategies

Erin Gibson, DVM, DACVS (SA)

Minimally invasive surgery; laparoscopy/thoracoscopy and identifying/evaluating novel devices or approaches that may optimize minimally invasive surgical outcomes in patients; interventional radiology, including intravascular interventions, tumor embolization, interventional treatment of upper and lower urinary tract disease

Joshua G. Henry, DVM

Further defining the role of chemoradiation in clinical practice; molecular drivers of common feline cancers; felids' unique radiobiological properties; interplay of radiation medicine, the tumor microenvironment, and/or the immune system



Emmelyn Hsieh, DVM, DACVIM (SAIM)

Antimicrobial stewardship; antimicrobial resistance; antimicrobial use; lower urinary tract diseases

CLINICAL SCIENCES & ADVANCED MEDICINE

Thomas Lee, DVM, MVM, MS

Topical biologics to treat radiodermatitis; local immune response to radiation therapy and combined myeloid cell targeted therapy of head and neck carcinoma; establishing a translational sinonasal carcinomal model

Elizabeth M. Lennon, DVM, PhD

Elucidate the mechanism of the protective role of the mast cell in inflammatory bowel disease; gastrointestinal physiology; innate immunity; mast cells; large animal models; dog; cat; mucosal immunology

Jennifer Lenz, DVM, DACVIM (Oncology)

Generation of myeloid derived suppressor cells by tumor-derived extracellular vesicles

Anna M. Massie, DVM, DACVS (SA)

Regenerative medicine; bone quality assessment/biomechanics; implant selection; implant development

Wojciech K. Panek, DVM, DACVIM (Neurology)

Translational neuro-oncology and neuro-aging; regulatory signals that govern the immune system mobilization and/or exhaustion in patients suffering from central nervous system (CNS) tumors and canine cognitive dysfunction

ASSISTANT PROFESSORS

CLINICAL SCIENCES & ADVANCED MEDICINE

Tereza Stastny, DVM, DACVECC

Oxygenation indices in positive-pressure ventilation; optimal ventilator settings; assessing arterial-end-tidal PCO2 difference, driving pressure, and transpulmonary pressures

Raghavi Sudharsan, PhD

Mechanisms of disease for inherited retinal degenerations; identifying common degenerative pathways that can be targeted for developing gene-agnostic therapies; developing next generation promoters and AAV vectors; cell and molecular biology

CLINICAL STUDIES—NEW BOLTON CENTER

Leonardo F. Brito, DVM, MSc, MVSc, PhD, DACT

Neuroendocrine control of sexual development in males; genetic and environmental effects on sperm production in livestock; testicular thermoregulation; ultrasonography applied to andrology practice; semen preservation and use for artificial insemination and other ART's; abnormal spermatogenesis and sperm morphology; semen analysis automation and standardization; quality assurance in andrology laboratories

Kara A. Brown, VMD, DACVSMR

Equine rehabilitation; cervical and thoracolumbar spine pathology; sport horse performance

Hope Douglas, VMD, DACVS (LA), DACVAA

Optimization of anesthetic recovery; veterinary patient safety; and veterinary medical education

Alicia E. Long, DVM, DACVIM (LA), DACVECCS

Equine fecal microbiota and its relationship to colic; fecal microbiota transplant (FMT) in equine patients

Daniela Luethy, DVM, MPH, DACVIM (LAIM)

Investigation of novel diagnostics and treatments in large animal oncology; advancement of small ruminant medicine; large animal critical care; large animal infectious disease; veterinary epidemiology and public health

ASSISTANT PROFESSORS

CLINICAL STUDIES—NEW BOLTON CENTER

Monica Midon, DVM, MS

Effects of different anesthetic techniques on horse recovery; establishment of a validated score system to evaluate anesthetic horse recovery; use of isoprostanes as a biomarker of oxidative stress induced by anesthesia

Eduardo Rico, PhD, MS

Impacts of nutrition on nutrient metabolism and health in the dairy cow; identifying primary causes of metabolic dysfunction, particularly during the transition from gestation to lactation; the effects of hyperketonaemia and hyperlipidaemia on immune function and health; the investigation of the peripartal NAD+ metabolome in relation to energy utilization efficiency; the impact of dietary omega-3 fatty acids on dairy cow health and bovine milk quality

Mary A. Robinson, VMD, PhD, DACVCP

Veterinary pharmacology; equine drug testing; pharmacokinetics

Holly Stewart, VMD, PhD, DACVS (LA)

Understanding the relationship between subchondral bone and joint health; role of bone marrow lesions; development of translational experimental models; optimization of volumetric imaging for the detection of changes within the subchondral bone

Joy E. Tomlinson, DVM, PhD, DACVIM (LAIM)

Hepatology; viral liver disease in horses and donkeys; treatment of pleuropneumonia; transfusion medicine; equine primary hyperparathyroidism

PATHOBIOLOGY

Eman A. Anis, MS, PhD, PhD, DACVM

Development and improvement of conventional and molecular diagnostic methods for existing and emerging infectious disease; antivirals and vaccine development for domestic animals

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Charles-Antoine Assenmacher, DVM, DACVP (Anatomic Pathology)

Pathological evaluation of laboratory animals used in fundamental research and preclinical studies; assessment of effects and toxicities of immunotherapies and genetic engineering; image analysis on digital slides for data quantification and analysis; ocular pathology

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Stephen D. Cole, MS, VMD, DACVM

Understand and mitigate the spread of antibiotic resistant bacteria in companion animals via two major approaches: (1) To characterize the clinical and molecular epidemiology of extensively drug resistant bacteria (i.e., carbapenem-resistant Enterobacteriaceae) of dogs and cats; (2) To establish best educational practices in antimicrobial stewardship to promote proper use of these critical drugs

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Roderick B. Gagne, MSc, PhD

Disease ecology; wildlife ecology; molecular ecology

Michael J. Hogan, PhD

Non-classical MHC restriction; cryptic/unconventional T cell epitopes; protective immunity to viral infections; mRNA vaccine development

ASSISTANT PROFESSORS

PATHOBIOLOGY

Nicole D. Marino, PhD

The molecular mechanisms that bacteria use to block or interrupt phage infection and how phages overcome or evade these defenses; CRISPR-Cas

Louise H. Moncla, PhD

How viruses emerge in human populations and transmit between them; phylodynamics; virology; population genetics; cross-species transmission

Kathleen Mulka, DVM, PhD, DACVP

Diagnostic pathology; comparative pathogenesis of viral disease

Kevin Niedringhaus, BVetMed, PhD, DACVP

Diagnostic pathology in wildlife; improving the detection and recognition of infectious diseases in wildlife with an emphasis on emerging and parasitic diseases

PATHOBIOLOGY

Michael Povelones, PhD

Innate immune recognition and elimination of pathogens; the interaction between mosquitoes and the animal and human pathogens they transmit; host-pathogen interactions; vector biology; malaria; complement; innate immunity

Antonia Rotolo, MD, PhD

Immunobiology; comparative immuno-oncology; chimeric antigen receptor (CAR) immunotherapies; invariant natural killer T (iNKT) cell biology; adoptive cell therapies

Caroline Sobotyk de Oliveira, DVM, MSc, PhD

Improvement and development of conventional and molecular diagnostic techniques for detecting parasitic infections in domestic and wild animals

ABSTRACT NUMBERS

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

1. Matthew A. Adreance
2. Laura Anderson
3. Esha Banerjee
4. Nasreen Bano
5. Alyssa Chalmin Katz
6. Jinwen Chen
7. Tiffany Chen
8. Thomas Ede
9. Julie Engiles
10. Kristin L. Gardiner
11. Emma Gorenberg
12. Fuyu Guan
13. Agathe Guillemet
14. Ronald N. Harty
15. Joanne E. Haughan
16. Sarah Ibach
17. Juan M. Inclan-Rico
18. Lang Jiang
19. Zibin Jiang
20. Bethany Keen
21. Jennifer Lenz
22. Caitrin R. Lowndes
23. Leif K. McGoldrick
24. Jaclyn R. Missanelli
25. Leonardo Murgiano
26. Jessica K. Niggel
27. Sheridan O'Connor
28. Thomas D. Parsons
29. Brandon Peng
30. Rhiannon Ross
31. Marina M. Santos
32. Marina M. Santos
33. Louise Southwood
34. Raghavi Sudharsan
35. Kei Takahashi
36. Isabel Vergara
37. Amanda Watkins
38. Eoin C. Whelan
39. Youwen You

POSTER ABSTRACTS

Abstracts are ordered alphabetically by the presenting author's last name.

1. DETECTION, QUANTIFICATION, AND CONFIRMATION OF HYPOXIA-INDUCIBLE FACTOR INHIBITORS IN EQUINE PLASMA AND URINE BY LC-MS.

Fuyu Guan[1,2], **Matthew A. Adreance[1,2],** Savannah Fay[1,2], and Mary A. Robinson[1,2].

[1]Department of Clinical Studies—New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; [2]Pennsylvania Equine Toxicology and Research Laboratory, West Chester, PA.

Hypoxia-induced factors (HIFs) are a class of compounds being studied for use in treatments for cancer, metabolic disorders, and other diseases such as anemia. From this end and considering their pharmacology, HIFs have been used as doping agents in human sports and subsequently banned for the presumed advantage they provide. Based on this, HIFs have been appearing in equine specimens and thus become a potential new doping source and concern with the health and performance of both the equine and human racers who take part. Thus, this abstract's study assesses a method to detect, quantify, and confirm HIFs and associated compounds in mediums used to assess equine doping—plasma and urine—through LC-MS methodology. Fourteen HIF factors were obtained and combined into a 10 ug/mL stock solution in MeOH. Further serial dilutions were created spanning 10000 pg/mL down to 10 pg/mL to spike samples in both acidic liquid-liquid and neutral solid-phase extractions. All spiked samples run through LC-MS in both the positive and negative ionization modes under HESI conditions from a range of 133-2000 m/z. The method was validated in-house following the guidelines published by SWGTOX through accuracy, precision, stability, recovery, and potential matrix effects. Once validated, administration samples were obtained and the method created was shown to be viable in these, and thus also viable for use for other samples.

Research Grant: Pennsylvania Department of Agriculture State Horse Racing Commission

2. IDENTIFYING THE ROLE OF GLUTAMINE IN CANINE T CELLS.

Laura Anderson[1], Roddy O'Connor[2], and Nicola Mason[3].

[1]Biomedical Graduate Studies, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [2]Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [3]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

CAR T cell function is closely linked to their metabolic profile. Various strategies, including media conditioning and genetic modifications, have been developed to optimize fuel selection and accentuate mitochondrial metabolism, aiming for improved persistence, reduced exhaustion, and heightened anti-tumor efficacy. While human CAR T cells have achieved remarkable success in treating hematologic malignancies, canine CAR T cells have not demonstrated the same therapeutic effectiveness in clinical settings. This discrepancy suggests that species-specific barriers may be influencing their function. Canine cancer patients are increasingly used to evaluate next-generation adoptive T cell therapies to address barriers to effective treatment in humans. Understanding how activated canine T cells reprogram their metabolism is essential for comparative immunology and optimizing their therapeutic potential, particularly since current methods for culturing and generating these cells are based on human CAR T cell protocols. Mitochondrial function and oxidative phosphorylation (OXPHOS) are essential for sustaining T cell activity, with nutrient availability – particularly glutamine – playing a key role in regulating these metabolic processes. Increasing evidence suggests that plasma glutamine levels are two-fold higher in canines than in humans. Given that metabolite

levels are tightly regulated, we hypothesize that glutamine supports novel features of mitochondrial function in canine T cells. Preliminary findings from our lab show how glutamine "backfills" the TCA cycle via reductive metabolism in an IDH-1-dependent manner in human T cells. Since IDH enzymes are calcium-regulated, we are first investigating calcium signaling in canine T cells and assessing their ability to undergo reductive glutamine metabolism using 13C tracer technology. Complementary analysis via Seahorse Assays and electron microscopy will further delineate species-specific similarities and differences in functional competence. Determining the importance of glutamine in canine T cell function and persistence will allow us to condition canine CAR T cells for enhanced performance following adoptive transfer.

Support: Mason Cancer Research Fund

3. IMPACT OF DIFFERENT DECALCIFICATION APPROACHES ON GENE AND PROTEIN EXPRESSION ANALYSIS TECHNIQUES IN FORMALIN-FIXED AND PARAFFIN-EMBEDDED TISSUES (FFPE) MOUSE BONY SPECIMENS.

Esha Banerjee, Jill Verrelle, Ashley Forster, Giovanni Finesso, Charles-Antoine Assenmacher, and Enrico Radaelli.

Penn Vet Comparative Pathology Core, Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Decalcifying mineralized tissues, such as bones, is essential to obtaining high-quality sections from formalin-fixed, paraffin-embedded (FFPE) tissues while preserving their morphological features. The range of molecular analyses on FFPE samples has significantly expanded in recent years, emphasizing the need to understand better the impact of decalcification on molecular structures in these types of samples. This study aimed to assess the effects of EDTA-based and formic acid-based tissue decalcification methods on gene and protein expression analysis techniques (i.e., qPCR, IHC, ISH) in FFPE samples of the spine (including spinal cord) from mice with progressive toxoplasmosis and from naive controls. The genes *Arg1* and *Nos2*, which are both markedly upregulated during toxoplasma encephalomyelitis, were selected for this analysis. The results indicate that formic acid has a detrimental impact on total RNA concentration, purity, and transcript integrity, whereas EDTA-treated samples showed comparable values to non-decalcified ones. However, no difference in labeling intensity was observed when assessing *Arg1* and *Nos2* expression using ISH and IHC. In conclusion, the data suggests that decalcification of mouse specimens using EDTA is significantly better in terms of recovered nucleic acid quantity and quality compared to formic acid. Notably, no significant difference was found across the two tested decalcification methods and non-decalcified specimens for IHC and ISH analyses.

4. EXPLORING THE MECHANISM OF THE YY1 TF IN B-CELL LINEAGE COMMITMENT.

Nasreen Bano, Sulagna Sanyal, Sarah Naiyer, Suchita Hodawadekar, and Michael L. Atchison.

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

Immune cell development initiates in the bone marrow, where hematopoietic stem cells differentiate into lymphoid progenitors (B and T cells) and myeloid progenitors (such as macrophages and dendritic cells). This differentiation is controlled by transcription factors (TFs), chromatin remodelers, and the 3D architecture of chromatin, which directs lineage specification. To understand its role in regulating B cell lineage commitment, we explored the effects of a

conditional knockout (KO) of the transcription factor YY1 in pro-B cells. Transitioning from the pre-pro-B to the pro-B stage is crucial for B cell lineage commitment. Our study revealed that YY1 KO in pro-B cells, cultured on OP9 DL4 feeder cells that provide Notch signaling, resulted in a loss of B lineage commitment and the emergence of alternative lineages, including T cells, macrophages, dendritic cells, and monocytes. Using scATAC-seq, we observed increased chromatin accessibility in Fos and Jun motifs within YY1 KO pro-B cells compared to wild-type (WT) cells. Additionally, scATAC-seq peaks were reduced in some B-lineage genes (Ikzf3, Blk, and Ccr7) but elevated in alternative lineage genes (Ccr5 and Tmem51) in YY1 KO pro-B cells. scRNA-seq further demonstrated reduced expression of B lineage-specific genes such as Ikzf3, Blk, and Ccr7 in YY1 KO pro-B cells. Furthermore, Hi-C experiments indicated increased chromatin long-range interaction within alternative lineage genes (Ccr5 and Tmem51) in YY1 KO pro-B cells, while B-lineage genes (Ikzf3 and Ccr7) exhibited decreased interactions. Hi-C suggested that YY1 KO proB impairs long-range chromatin interactions that are vital for sustaining B cell lineage commitment. Overall, our findings highlight YY1's critical role in preserving B lineage identity.

5. SYSTEMIC CYTOKINE OR NEUROLOGICAL SYNDROMES FOLLOWING CHIMERIC ANTIGEN RECEPTOR (CAR) T-CELL IMMUNOTHERAPY IN A NON-HUMAN PRIMATE MODEL.

Alyssa Chalmin Katz[1], Imani Nicolis[1], Kristin Gardiner[1], Hannah Thomas[2], Jack Swain[2], Sara Sleiman[3], Tarek Araji[4], Vijay Bhoj[4], Saar Gill[3], and Raimon Duran-Struuck[1,2].

[1]University Laboratory Animal Resources, University of Pennsylvania, Philadelphia, PA; [2]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; [3]Center for Cellular Immunotherapies, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; [4]Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

Chimeric antigen receptor (CAR) T-cell therapy is a novel cellular immunotherapy currently being used for the treatment of lymphomas and other liquid and solid tumors. Autologous T cells are engineered to express receptors that target antigens on malignant cells. Side effects can be life-threatening and have been documented to be secondary to antigen load, cell dose, the type of CAR construct, the preparatory regimen, and off-target effects. We will discuss our experience with an anti-CD20, B-cell-specific CAR T cell (CD20CART), which resulted in different clinical and immunological outcomes in macaques. Two macaques in a cohort of four demonstrated cytokine-related toxicities following infusion of CAR T-cells. A 6-year-old male rhesus macaque experienced facial erythema and swelling one week after infusion. Another animal, a 5-year-old male cynomolgus macaque, experienced different clinical signs, including dull mentation and focal neurologic deficits four days following infusion. Diagnostics included complete blood count and serum chemistry panels, neurologic scoring, flow cytometry, and measurement of cytokine levels. Both conditions were associated with a paroxysmal expansion of CAR T-cells observed in white blood cell counts and on flow cytometry and B-cell aplasia. Differential diagnoses included hypersensitivity reaction for the rhesus macaque, and seizures, pain, or other neurologic events such as ischemia for the cynomolgus macaque; when considering the history and diagnostic results, however, these presentations were consistent with cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), respectively. Both cases were successfully managed with dexamethasone sodium phosphate (1-3 mg/kg intramuscularly), levetiracetam (18-25 mg/kg orally or subcutaneously), and/or tocilizumab (8 mg/kg intravenously), a monoclonal antibody that blocks interleukin-6 receptor, a potent inflammatory mediator. These studies reinforce the importance of macaques as models for immunotherapies, specifically for the assessment of novel CAR T constructs, prediction of potential toxicities, and their relevance as clinical-translational partners by increasing the predictive value gained from preliminary rodent studies.

6. TRANSFORMING GROWTH FACTOR β1 WAS UPREGULATED BY LIPOPOLYSACCHARIDE IN HORSES.

Jinwen Chen[1], Joanne E. Haughan[1], and Mary A. Robinson[1,2].

[1]Department of Clinical Studies—New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA; [2]Pennsylvania Equine Toxicology & Research Laboratory, West Chester, PA.

Transforming growth factor beta 1 (TGFβ1) regulates cell proliferation and differentiation. Lipopolysaccharide (LPS) acts on immune cells to trigger inflammation and sepsis. We proposed that TGFβ1 protein expression would change after LPS challenge in horses. To test this hypothesis, a dual antibody sandwich ELISA was validated according to standard criteria including linearity, specificity, precision and accuracy and used to determine equine plasma TGFβ1 levels following endotoxin challenge *in vivo* and *in vitro*. Since endogenous TGFβ1 binds to latency-associated peptide, plasma samples were treated with 1 N HCl to release TGFβ1 for detection. For the *in vitro* study, a subset of whole blood samples were challenged by LPS (n = 8) or calcium ionophore A23187 (CI, n = 6) for 24 h. For the *in vivo* study, six Thoroughbred horses were administered LPS intravenously at 125 ng/kg/h for 4 h and blood was collected at -31, -22, -20, 0, 2, 4 and 24 h. The results showed that the method did not exhibit significant cross-reactivity with other cytokines tested (< 1.5%) except TGFβ2. When the detection Ab was partially replaced with capture Ab at 25%, 50% and 75%, assay signals decreased by 19%, 39% and 67%, respectively. The inter- and intra-day precisions were ≤ 15.8% and ≤ 9.7%, respectively; the inter- and intra-day accuracies were ff116.4% and ff120.0%, respectively. Following *in vitro* stimulation of whole blood with LPS or CI, plasma TGFβ1 concentrations increased two-fold (n = 8, $p < 0.0001$) and one-fold (n = 6, $p < 0.05$) at 24 h, respectively. *In vivo* LPS challenge showed significant increases in plasma TGFβ1 concentrations occurring at 2 and 24 h post LPS administration to horses (n = 6, $p < 0.05$). The results showed that plasma TGFβ1 concentration increased significantly following LPS challenges both *in vitro* and *in vivo*. Plasma TGFβ1 increased following CI activation in whole blood.

7. COMPARISON OF NEEDLE ELECTRODES VERSUS GOLD CUP SKIN ELECTRODES FOR SCOTOPIC CLINICAL ELECTRORETINOGRAPHY IN THE DOG.

Tiffany Chen, Tian Tian Wu, Elaine Holt, and Mary Lassaline.

Department of Clinical Ophthalmology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

PURPOSE

Scotopic full-field flash electroretinography (ERG) is used to assess retinal function prior to cataract surgery. ERGs in canine patients are usually performed using needle reference and ground electrodes, and a corneal contact lens active electrode. Placement of needle electrodes may be poorly tolerated in small patients. Gold cup skin (GCS) electrodes could provide a humane alternative although their reliability has not been established in dogs. This study investigates the use of GCS electrodes as an alternative to needle electrodes for performing ERGs in the dog.

MATERIALS AND METHODS

Sixteen dogs with normal ophthalmic examinations had two scotopic full-field flash ERGs performed on both eyes, one using GCS electrodes and one using needle electrodes. Impedance, a- and b-wave amplitude, and a- and b-wave implicit times were recorded for each ERG and compared between electrode type. Pain scores for electrode placement were assessed using a modification of the Colorado State University Canine Acute Pain Scale.

RESULTS
Mean b-wave amplitude was 130uV with needle electrodes and 128uV with GCS electrodes, with no significant difference between them (p = 0.95). Pain scores were significantly higher for placement of needle electrodes than GCS electrodes (median = 2.5 for needle electrodes; all pain scores = 0 for GCS electrodes; z = -2.52).

CONCLUSIONS
Scotopic full-field flash ERG results are comparable between needle and GCS electrodes, with GCS electrodes eliciting significantly less pain. Thus, they are a viable and humane alternative to needle electrodes. Future studies may extend these findings to dogs with ophthalmic disease.

Research Grant: Vision For Animals Foundation

8. GILTS PREFER AN OPEN PEN TO A STALL.

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Stalls or crates are a very common type of housing used on pig farms that restrict an animal's movement. How this confinement impacts the animal's affective states is seldom investigated. We conducted a preference test over 7 days where trios of gilts (n = 10 trios, 27.4 ± 1.5 weeks old) had free access between individual self-locking stalls (~1.2 m^2) and a shared open area allowing 2.8 m^2 /animal (71% of total area). Gilts had access to *ad libitum* feed and water both inside the crates and in the open area. After 7 days, personality traits of the animals were assessed with open field (OF) and novel object (NO) tests. Principal Component Analysis (PCA) yielded two main components, which we defined as *Passivity* and *Engagement*. The median time spent outside the crate was 95.2% as 21/29 of the gilts exhibited a significant preference for pen over crate during the 7-day trial ($p < 0.05$). Passivity had no relationship with time spent in the open area, but engagement during OF/NO was associated with less use of the open area (OR = 0.39, 95CI = [0.25, 0.60]). Interestingly, gilts were likely to spend less time in the open area at nighttime compared to daytime (Odds Ratio = 0.49, 95CI= [0.40, 0.60]), as well as experimental days passed (OR = 0.70, 95CI = [0.66, 0.73]). During the first daytime and nighttime, 1/29 and 2/29 animals preferred the crate respectively, whereas by the last daytime and nighttime 5 and 9 gilts preferred the crate respectively ($p<0.05$). While both intrinsic (personality) and extrinsic (time of day, experimental day) factors appear to influence the gilt's housing preferences, most gilts significantly prefer an open area to a crate when free access is provided between the two. A smaller subpopulation of animals developed a preference for stalls but still utilize both the stall and the pen throughout the day.

9. LAMELLAR CELL DEATH AND PROLIFERATION ARE ASSOCIATED WITH RESTRICTED AMBULATION AND PREFERENTIAL WEIGHT BEARING IN A MODEL RELEVANT TO SUPPORTING-LIMB LAMINITIS.

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A non-painful *in vivo* experimental model using twelve healthy Standardbred horses housed in stocks with limb weight distribution logged continuously for 92h was performed to examine the effects of experimentally induced prolonged preferential weight bearing (PWB) and reduced ambulation (RA) on equine hoof lamellae. In the preferential weight bearing group (PWB; n=6), a platform shoe applied to the contralateral forelimb (CF) caused an increase in weight bearing load on the opposite forelimb (supporting limb [SL]) ~10% of bwt. The restricted ambulation group (RA; n=6) were continuously housed in stocks without further intervention. Archived tissues from healthy Standardbred horses (n=8) were used as controls (CON). Qualitative scoring, histomorphometry and staining for cell death (TUNEL, caspase-3) and proliferation (TPX-2) were performed. Results were analyzed using a mixed-effects linear regression model. Histologic lesions were present in multiple limbs from both the PWB and RA group, including elongation of secondary epidermal lamellae (SEL), cell death (mostly TUNEL positive, caspase-3 negative parabasal keratinocytes) focused near the keratinized axes of the primary epidermal lamellae (PEL) and epidermal basal cell proliferation (TPX-2 positive). Lesions were generally mild, but more severe in the PWB group SL where there were significant increases (vs control) in mean PEL length, SEL length, TUNEL count and TPX-2 count. The non-keratinized portion of the PEL was longer in all PWB and RA limbs vs control and both TUNEL and TPX-2 positive cell counts were increased in RA group forelimbs vs control. Restriction of normal ambulation, even in the absence of increased weight bearing, caused lamellar cell stress and death primarily affecting parabasal keratinocytes. This mechanism is likely to be important in the development of supporting-limb laminitis.

Research Grant: Grayson Jockey Club Research Foundation Grant

10. COMPARING THE SAFETY AND EFFICACY OF CONVENTIONAL AND CONVECTION-ENHANCED INJECTION OF VIRAL VECTORS FOR OPTOGENETICS THERAPY IN THE MACAQUE BRAIN.

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Optogenetics is a new technique used to modulate neuronal activity via cell transduction with opsin genes and stimulation with a light source. It is a promising technology that could provide relief from neurodegenerative diseases such as epilepsy, Parkinson's disease, and Alzheimer's disease, among others. Successful application of this technology in rodent neuronal disease models has been frequently reported, but far less commonly in non-human primate models. Here we assessed histologically the neuronal targeting ability and off-target effects of four optogenetic viral constructs at a range of titers (AAV1- or AAV9-hSyn-SwiChR++-TS-eYFP; AAV1- or AAV9-CaMKIIa-SwiChR++-TS-eYFP)

in the prefrontal, parietal and motor cortex of five adult rhesus macaques. Delivery occurred via surgical injection into the parenchyma using either conventional (low volume/speed) or convection-enhanced delivery (CED; high volume/speed). Volume of transduction, area of spread, expression cell-type specificity, and inflammation markers were analyzed using immunohistochemistry (IHC) and immunofluorescence (IF) at variable lengths of time post-surgery (8.5 weeks-2 years). Results indicated that expression was robust for all four constructs using both conventional and CED delivery. Cortical expression volume was consistent between viral vectors (AAV1 vs AAV9) and promoters (hSyn vs CaMKIIa) at matching viral titers and delivery methods. CED generated approximately 3x the expression volume in the cortex compared with conventional injection at comparable titers. Inflammatory microglia marker Iba1 increased as a function of titer, particularly in the white matter, with significant perivascular cuffing observed at higher titers. CED-induced inflammation was approximately 50-150% greater in the CED vs conventional samples when controlling for area of analysis (injection periphery vs core vs white matter). Results indicate that all four constructs effectively transduce cortical neurons, and that CED enhances volume of cortical transduction but increases localized inflammation in a titer-dependent but construct-independent manner. The results are concerning considering CED is already used in human patients at comparable titers and injection speed.

11. THE EFFECTS OF DOBUTAMINE ON HEMODYNAMIC AND OXYGENATION PARAMETERS IN STANDING AND ISOFLURANE-ANESTHETIZED HORSES.

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Dobutamine (DOB) is a first-line therapy for hypotension in anesthetized horses. This study investigates the effects of escalating doses of DOB on hemodynamics and oxygenation parameters in standing versus isoflurane-anesthetized horses.

Six healthy adult horses (415–525 kilogram bodyweight) were studied in a randomized prospective cross-over design including standing and isoflurane-anesthetized experiments. Heart rate (HR), mean arterial pressure (MAP), central venous pressure (CVP), pulmonary arterial pressure (PAP), and thermodilution cardiac output (CO) were measured, as well as mixed venous and arterial blood gases in anesthetized horses. Measurements were obtained at baseline and following fifteen minutes of escalating DOB continuous rate infusion at 0.5, 1, and 2 $\mu g\ kg^{-1}\ minute^{-1}$. Oxygen delivery ($DO_2$), oxygen extraction ratio (O_2ER), A-a gradient, fShunt, and PaO_2/F_IO_2 were calculated for anesthetized horses. Following a week of washout, each horse underwent the second experiment. After confirming normal distribution variables were compared to baseline and between groups using two-factorial ANOVA (alpha = 5%).

MAP, CVP, PAP, and CO increased significantly with increasing DOB infusions in both groups. At similar DOB infusion rates, MAP (increase of 24 – 31 mmHg, $p < 0.001$) and CO (increase of 16 – 28 liters, $p < 0.001$) were significantly higher in standing versus anesthetized horses. For anesthetized horses DO_2 significantly increased ($p < 0.01$) and O_2ER significantly decreased ($p < 0.001$) between every timepoint. DOB produced a significant increase in PaO_2/F_IO_2 (398 versus 491 mmHg, $p = 0.021$), and significant decreases in fShunt (15.6 versus 11.2, $p = 0.016$) and AaO_2 (205 versus 141 mmHg, $p = 0.031$).

DOB administration produces dose-dependent improvements in CO and perfusion parameters and improves indicators of peripheral oxygenation at higher doses in horses under isoflurane anesthesia. DOB dosages of 2 $\mu g\ kg^{-1}\ minute^{-1}$ are needed to reach standing baseline MAP and CO.

Research Grant: Raymond Firestone Trust, Raker-Tulleners Fund, and Tamworth Fund: University of Pennsylvania New Bolton Center

12. IDENTIFICATION OF RECOMBINANT HUMAN ERYTHROPOIETIN AND RECOMBINANT HUMAN GRANULOCYTE COLONY-STIMULATING FACTOR IN CONFISCATED ITEMS FROM RACETRACKS.

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INTRODUCTION

Recombinant human erythropoietin and recombinant human granulocyte colony-stimulating factor are prohibited substances in horse racing. Despite the banning of these substances by horse racing authorities, a few individuals still try to illicitly use them in horse racing. As an effort to enforce the banning of prohibited substances, field investigations confiscate suspect items. Among the confiscated items received by our laboratory, there were glass bottles (5 mL) and syringes labeled with recombinant human erythropoietin alfa injection and recombinant human granulocyte-colony stimulating factor (rHu G-CSF) injection. Here, we report the identification results for those bottles and syringes.

EXPERIMENTAL

The powders in the bottles were dissolved in 50 mM ammonium bicarbonate. The liquid in the syringes was buffer exchanged to 50 mM ammonium bicarbonate. Aliquots of the samples in solution were digested with trypsin. The digests were analyzed by capillary-column liquid chromatography coupled to a high-resolution Q Exactive Plus mass spectrometer. The acquired LC-MS raw data with high-resolution full MS scans and data-dependent MS/MS scans were processed with Proteome Discoverer (CD) software for protein identification.

RESULTS

Recombinant human erythropoietin (rhEPO) was identified for the bottles and syringe, and rHu G-CSF was identified in another syringe. Specifically, CD automatically identified several tryptic peptides for each of the two proteins, by matching the measured product ions of a peptide with predicted ones. CD also automatically validated the protein identification results using Percolator software. Additionally, extracted ion chromatograms using the theoretical masses of the identified peptides were manually generated to verify the protein identification results. The results confirmed the presence of the prohibited substances in the contents of the vials and syringes as labeled.

13. COMPARISON OF TWO VENTILATION SYSTEMS ON PULMONARY MECHANICS IN A PORCINE MODEL OF THORACIC INSUFFICIENCY.

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Thoracic Insufficiency Syndrome (TIS) in children with spinal deformities is associated with altered pulmonary development and impaired lung function. Severe cases require surgical correction with multiple anesthetic events being particularly challenging for the anesthesiologist. In a porcine model of acute respiratory distress syndrome, FLEX reduced ventilation-induced lung damage, decreased focal inflammation, increased dynamic compliance, and improved ventilation. Furthermore, FLEX homogenized ventilation-perfusion matching by increasing ventilation in the dorsal/

dependent lung regions in both lung-injured and lung-healthy patients.

At 28 months post-tethering and with no surgical correction of the deformity, Cobb angle on the coronal plane decreased to θS=13.2° (25° at 17 months). In the TA the MLV (1586 cm^3) and TV (290 mL) were lower compared to AMC (MLV=1912 cm^3, TV 485 mL). Cdyn was lower in the TA (32mL/cmH_2O) compared to the AMC (55mL/cmH2O) during CPV and improved during FLEX ventilation by 68% in the TA and by 19% in the AMC. During FLEX ventilation the PaO_2/FiO_2 ratio increased from 390 mmHg to 468 mmHg and dead space decreased from 18% to 3%, indicating improved ventilation-perfusion matching.

Despite the lack of data on the incidence of respiratory failure as a factor of mortality and morbidity in patients with TIS, it is well known that hypoxemia and impaired pulmonary perfusion are common complications in these patients. Using our large animal untreated scoliosis model, we have shown that pulmonary function remains highly affected by chest deformity likely due to decreased lung volumes and reduced chest compliance. FLEX ventilation considerably improves lung function and dynamics by homogenizing distribution of ventilation as well as gas and blood flow during expiration. The modulating and linearization of the expiratory phase further reduces the degree of atelectasis formation during the second half of the expiratory period.

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14. INTERSECTION OF THE HOST SIGNALING PATHWAYS HIPPO & CASA WITH THE EBOLA VIRUS LIFECYCLE.

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EBOV and MARV are emerging viruses that cause acute hemorrhagic fever in humans. As current therapeutic treatments are limited, effective antivirals are urgently needed to curb these deadly infections. The PPxY motifs conserved in the VP40 matrix proteins of EBOV and MARV drive egress of infectious virions by interacting with select host WW-domain proteins that either positively or negatively regulate filovirus egress and spread. We demonstrated that the mTORC1/CASA axis regulates EBOV egress, as suppression of mTORC1 signaling inhibits viral egress through selective autophagic degradation of VP40 via the BAG3-mediated CASA complex. We recently identified the Hippo pathway effectors YAP/TAZ, as WW-domain interactors with the PPxY motifs of EBOV and MARV VP40. Hippo signaling is a pivotal host pathway that controls cell physiology and is itself regulated by PPxY/WW interactions. Specifically, the core Hippo kinases LATS1/2 bear PPxY motifs that interact with YAP/TAZ and the ensuing phosphorylation prevents shuttling of YAP/TAZ from the cytoplasm (Hippo ON) into the nucleus where they function as transcriptional co-activators (Hippo OFF). Thus, the potential competitive PPxY/WW interplay is likely to impact both the filovirus lifecycle and host signaling pathways and cellular processes.

15. MEASURING OXYCODONE METABOLITES IN PLASMA AND URINE ENHANCES DETECTION OF ORAL OXYCODONE ADMINISTRATION TO HORSES.

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Oxycodone is rapidly absorbed and eliminated by horses resulting in short detection times. We hypothesized oxycodone metabolites would achieve higher plasma and urine concentrations enabling longer detection of oxycodone administration.

Oxycodone (5 mg) was administered orally to 12 healthy horses. Plasma and urine samples were collected up to 96 hours. Concentrations of oxycodone, 6-α-oxycodol, 6-β-oxycodol, oxymorphone, 6-α-oxymorphol, 6-β-oxymorphol, noroxycodone, 6-α-noroxycodol and 6-β-noroxycodol were measured using LC-MS/MS with a lower limit of quantification (LLOQ) of 5, 5, 5, 25, 25, 25, 25, 50 and 50 pg/ml respectively. Plasma and urine C_{max} and T_{last} >LLOQ were determined. Plasma $AUC_{0\text{-}inf}$ and $t_{1/2}$ were calculated using non-compartmental analysis. Metabolite values were compared to oxycodone using Wilcoxon matched-pairs signed-rank test with significance $P<0.05$. Values are presented as median (range). 6-α- and 6-β-noroxycodol plasma concentrations were insufficient for analysis. Plasma C_{max} was significantly higher for oxymorphone (3,635 pg/ml (7,550)), $P=0.002$) than oxycodone (526 pg/mL (645)). $AUC_{0\text{-}inf}$ was significantly higher for oxymorphone (16,620 h*pg/ml (18,183), $P = 0.002$), 6-β-oxymorphol (3,600 h*pg/ml (3,234), $P=0.002$) and 6-α-oxymorphol (1,545 h*pg/ml (2,561), $P=0.028$) than oxycodone (756 h*pg/ml (837)). Metabolite $t_{1/2}$ ranged from 1.96 – 5.52h and was significantly longer for all metabolites ($P>0.05$) than oxycodone: 1.79h (3.19). Plasma T_{last} was significantly longer for oxymorphone (20h (8), $P=0.002$), 6-β-oxymorphol (20h, (8), $P=0.002$), 6-α-oxycodol (16h (16), $P=0.002$) and 6-β-oxycodol (18h (16), $P=0.003$) than oxycodone (8h (10)). Urine C_{max} was significantly higher for oxymorphone (320,500 pg/ml (2,259,700), $P=0.002$), 6-β-oxymorphol (44,350 pg/ml (1,790,000), $P=0.002$), 6-β-oxycodol (8,700 pg/ml (49,400), $P=0.004$) and noroxycodone (8,125 pg/ml (44,530), $P=0.002$) than oxycodone (4,485 pg/ml (14,880)). Urine T_{last} was significantly longer for oxymorphone (72h (72), $P=0.003$) and 6-β-oxymorphol (66h (72), $P=0.031$) compared to oxycodone (20h (88)). Measuring oxycodone metabolites in plasma and urine will enhance detection of illicit oxycodone administration to horses. Further investigation of individual variation is warranted.

Research Grant: Pennsylvania Horse Racing Commission

16. DISSECTING THE *HOW* FROM THE *WHAT*: AN INVESTIGATION INTO THE QUALITATIVE BEHAVIOR ASSESSMENT OF SOWS.

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Qualitative Behavior Assessment (QBA) is a welfare assessment tool valued for its holistic approach to assessing an animal's emotional state. However, the method has been criticized due to its subjective method of capturing data. The objectives of this study were two-fold: 1.) To determine whether veterinary students could assess sow welfare

using QBA similarly to swine experts and 2.) To investigate how welfare is assessed through the comparison of QBA and behavioral outcomes. A video library of sows was scored using QBA with predetermined emotional descriptors by five swine experts and 14 veterinary students. Responses from experts and students were analyzed separately using principle component analysis. Two main principal components (PCs) were identified as the valence and arousal of each descriptor. 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). A significant correlation was found between student and expert PC1 ($r=0.927$, $p<0.001$) and PC2 ($r=0.958$, $p<0.001$), indicating the two groups perceived observed animals similarly. However, descriptors that contributed significantly to each PC differed between the two groups, indicating that although animals may have been perceived in similar emotional states, each group selected different descriptors to describe these animals. For the second aim, the same videos were coded using six behaviors. The predictive effect of performed behaviors on QBA outcomes was investigated with linear mixed models. 18 of the 20 descriptors were significantly predicted by at least one event behavior ($p<0.05$), suggesting the performance of certain behaviors may be more likely to invoke the use of certain QBA descriptors. Further investigation is needed to better elucidate the specifics of these relationships, however, results speak to the robustness of QBA for assessing sow welfare.

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

17. MrgprA3 NEURONS DRIVE CUTANEOUS IMMUNITY AGAINST HELMINTHS THROUGH SELECTIVE CONTROL OF MYELOID-DERIVED IL-33.

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Skin employs interdependent cellular networks for barrier integrity and host immunity, but most underlying mechanisms remain obscure. Herein, we demonstrate that the human parasitic helminth, *Schistosoma mansoni,* inhibited pruritus evoked by itch-sensing afferents bearing the Mas-related G protein-coupled receptor A3 (MrgprA3) in mice. MrgprA3 neurons controlled IL-17+ γδ T cell expansion, epidermal hyperplasia, and host resistance against *S. mansoni* through shaping cytokine expression in cutaneous antigen-presenting cells (APCs). MrgprA3 neuron activation downregulated interleukin 33 (IL-33) but induced IL-1β and TNF in macrophages and cDC2s partially through the neuropeptide calcitonin gene-related peptide (CGRP). Macrophages exposed to MrgprA3-derived secretions or bearing cell-intrinsic IL-33 deletion showed increased chromatin accessibility at multiple inflammatory cytokine loci, promoting IL-17/23-dependent changes to the epidermis and anti-helminth resistance. This study reveals a previously unrecognized intercellular communication mechanism wherein itch-inducing MrgprA3 neurons initiate host immunity against skin-invasive parasites by directing cytokine expression patterns in myeloid APC subsets.

18. PROFILING THE IMMUNE TUMOR MICROENVIRONMENT IN CANINE CUTANEOUS MAST CELL DISEASE.

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Canine cutaneous mast cell tumors (MCTs) are a common, yet clinically challenging tumor type given their variable biological behavior. Although patients with low-grade MCTs can often be effectively managed with surgery alone, most dogs with high-grade MCTs succumb to their disease despite multimodal therapy. An improved understanding of the immune tumor microenvironment (TME) may help identify novel prognostic and therapeutic targets. In this study we interrogated the immune transcriptional profiles of the TME in low- and high-grade MCTs, and quantified intratumoral T cells. Twelve client-owned dogs with MCTs (6 Kiupel low-grade with clinically benign behavior and 6 Kiupel high-grade with clinically aggressive behavior) that underwent curative-intent surgery were identified. Tumor grade was confirmed by a single veterinary pathologist. RNA was extracted from all tumors followed by immune transcriptional profiling utilizing the Nanostring Canine IO panel and analysis using the ROSALIND platform. T cell density was determined by immunohistochemical staining for CD3 and quantified using ImageScope software (Leica Biosystems) following digital slide capture. Immune transcriptional profiling identified 9 differentially expressed genes between low- and high-grade MCTs (p-adj < 0.05). Programmed cell death protein 1 (*PDCD1*) and inducible T-cell costimulator ligand (*ICOSLG*) gene expression were significantly higher in a subset of high-grade MCTs. *ICOSLG* expression positively correlated with T cell transcripts (r_s = 0.6434, p = 0.0278). Although the mean T cell density was not significantly different between low- (mean of 76.42 CD3+/mm^2, SD 12 CD3+/mm^2) and high-grade MCTs (mean of 129.1 CD3+/mm^2, SD 96.06 CD3+/mm^2), greater variation of T cell densities was observed across high-grade MCTs compared to low-grade. Our data revealed significant differences in the immune TME of low- and high-grade MCTs and provides early, pre-clinical rationale for targeting a subset of high-grade MCTs with immune checkpoint blockade.

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19. DESIGNING AND EVALUATING PRIMERS FOR PCR TO DISTINGUISH AAV2-MEDIATED GENE DOPING FROM WILD TYPE AAV2 INFECTION.

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Gene therapy is currently prohibited in human and equine athletes and novel analytical methods are needed to detect abuse, known as gene doping. Most *in vivo* products deliver "transgenes" into cells, where they are transcribed and translated into functional proteins using non-integrating, recombinant viral vectors derived primarily from adeno-associated virus serotype 2 (AAV2). In a previous study, a PCR screening test targeting the ITR sequences of recombinant AAV2 (rAAV2) was developed to detect rAAV2 administration. To rule out the presence of wildtype (naturally occurring) AAV2 virus (wtAAV2) which contains the identical ITR region, 13 pairs of primers targeting AAV2 sequences unique to

wtAAV2 in the REP and CAP regions, were designed. qPCR was performed using each primer set and three plasmid vectors containing rAAV DNA sequences as templates. The pAAV2/2 vector contained the AAV2 ITR sequence, as well as REP and CAP region sequences, the rAAV2 vector contained AAV2 ITR sequence only, and the pAAV2/5 vector contained the AAV2 ITR sequence and the AAV serotype 5 (AAV5) CAP and REP region sequences. The AAV5 serotype was chosen as it is the most common wtAAV found in the horse. Melt curve, gel electrophoresis and cross-reaction were used to evaluate all of the primers. The qPCR results showed that only primer set 13 amplified pAAV2/2, but not pAAV2/5 or rAAV2. Melt curve analysis showed a single peak for primer set 13, demonstrating primer set 13 was best suited to distinguish AAV2-mediated Gene Doping from wtAAV2 virus with no cross-reaction to AAV5. Plasma samples from 30 thoroughbred racehorses were analyzed for presence of rAAV2 using a previously validate primer set and for wtAAV2 using primer set 13. No rAAV2 or wtAAV2 DNA was amplified from any of the samples.

20. UNTARGETED METABOLOMICS FOR IMPROVED DETECTION OF BISPHOSPHONATE USE IN EQUINE ATHLETES.

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Metabolomics has been proposed as a complementary technique for current anti-doping efforts due to better detection of the effects of a doping agent that may be difficult to detect through mass spectrometry or have a short detection window in the testing matrix. The equine racing regulatory community is concerned about the use of bisphosphonates in racehorses as they inhibit normal bone turnover and remodeling which may lead to risk of fracture. Bisphosphonates' effects on bone is their main pharmacological effect, but other known side effects include analgesia, anti-inflammatory, and anti-angiogenic effects. Any performance altering agent is prohibited within horse racing and ensuring detection of these compounds is essential for maintaining the integrity of the sport and athlete welfare. Plasma samples were collected from a 16-horse administration study involving 8 bisphosphonate treated and 8 placebo treated horses. A protein precipitation method using 0.1% formic acid in acetonitrile extracted samples for the reverse-phase and normal-phase metabolomics analyses. The lipidomic method used the organic fraction of a liquid-liquid extraction using MeOH/MTBE/H2O (17:60:20) to identify compounds of interest. Samples were randomized and analyzed using 3 liquid chromatography-high resolution mass spectrometry methods in both ionization modes to obtain good coverage of the metabolome. These methods were optimized for the detection and coverage of standard and internal standard mixtures of common metabolites and lipids. Compound Discoverer was used to detect features within the data and identify the identity of potential biomarkers using library searching and the AcquireX generated MS/MS data. Batch effects were accounted for through normalization to the pooled plasma QC samples and sample randomization during analysis. Data quality control was assessed through retention time monitoring, box plots and the QC correction plot. Comparisons between the samples from bisphosphonate treated and control groups allowed for qualitative assessment of distinguishing biomarkers using partial least squares-discriminant analysis.

Research Grant: Racing Medication and Testing Consortium Post-Doctoral Fellowship Program

21. UTILIZING MINIMALLY INVASIVE SAMPLING FOR TRANSCRIPTOMIC PROFILING OF THE IMMUNE TUMOR MICROENVIRONMENT IN CANINE B-CELL LYMPHOMA.

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B-cell lymphoma is a frequently encountered canine neoplasm. Following good initial responses to traditional cytotoxic chemotherapy, relapse is common with the majority of canine patients succumbing to their disease within a year. However, a subset of patients exhibit prolonged remissions. To investigate potential biomarkers associated with remission duration, we prospectively collected specimens from 48 canine lymphoma patients treated at Penn Vet following cytologic diagnosis and immunophenotyping supporting a diagnosis of intermediate-to-large cell lymphoma. Patient plasma, whole blood, and cells obtained from aspirates of neoplastic nodal tissues were acquired prior to treatment with CHOP chemotherapy (vincristine, cyclophosphamide, doxorubicin, prednisone). Blood and plasma samples were cryopreserved and cells from tumor aspirates were lysed in TRIzol and stored at -80°C. RNA isolation was subsequently performed on batches of lysed cells and purified RNA was stored at -80°C. Treatment protocol, remission duration, and outcome were recorded for each patient, establishing a minimally invasive biobank with linked cytologic, immunophenotypic, and clinical metadata. Immune transcriptional profiling was undertaken on a subset of 15 representative B-cell lymphoma patients with comprehensive clinical follow-up data utilizing the NanoString nCounter Canine IO panel, and data was analyzed on the ROSALIND platform. Median time to progression (TTP) was 316 days, with 5 patients retaining remission over 2 years. Immune transcriptional profiling identified 21 differentially expressed genes ($p < 0.05$) between patients with TTP ≤ 316 days (n=8) compared to TTP > 316 days (n=7). MSigDB pathway analyses revealed an enrichment in genes associated with angiogenesis (p-Adj = 0.04397) primarily in the short-term responders, and enrichment in genes associated with IL-12 signaling (p-Adj = 0.0261) in the long-term responders. Our data revealed significant differences in the immune microenvironment of canine B-cell lymphoma patients stratified by remission duration, and provides early, pre-clinical rationale of potential predictive biomarkers and therapeutic targets for this disease.

Research Grant: Institute for Immunology, University of Pennsylvania, NIH/NCI K08CA252619

22. EFFECTS OF TWO DIFFERENT PIOGLITAZONE DOSAGE REGIMENS ON INSULIN DYNAMICS IN SEVERELY HYPERINSULINEMIC EQUIDS.

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Pioglitazone previously decreased insulin concentrations in response to oral sugar test (OST) in horses with mild insulin dysregulation over 1 month. The objective of the current study was to characterize the effects of pioglitazone on insulin dynamics in equids with severe, persistent hyperinsulinemia over a prolonged (70 day) study period. We hypothesized that pioglitazone at 2mg/kg twice a day (PIO-BID) would normalize insulin more rapidly than once daily treatment (PIO-SID).

Seventeen client-owned equids with severe hyperinsulinemia (resting insulin >100 μIU/mL) were included in a prospective cohort study. Equids were assigned to PIO-SID or PIO-BID on an alternating basis at time of enrollment. Physical exam and gait evaluation were performed, and blood samples obtained for basal insulin, total and high molecular weight (HMW) adiponectin, liver enzymes and serum drug levels. OSTs were performed on days 0 and 28. Results were analyzed using a mixed effects linear regression model.

Basal insulin decreased from day 0 (PIO-SID mean [95% confidence interval] 191.2 [10.1-372.1], PIO-BID 113.0 [46.9–179.2]) to day 70 (PIO-SID 183.4 [-44.6–411.4] p=0.002; PIO-BID 66.7 [28.8–104.5] p=0.004). Insulin in response to OST did not significantly decrease in either group. There was a significant increase in both total and HMW adiponectin in the PIO-BID group (p<0.001), but not in the PIO-SID group. The mean serum concentration of pioglitazone at day 70 was higher than the lower end of the therapeutic range reported for humans (617 ng/mL) in PIO-BID (739.7 [453.0–1026.3]) but not in PIO SID (312.3 [0.7–624.0]).

Pioglitazone failed to normalize resting insulin in equids with severe hyperinsulinemia and is therefore not suitable as a sole treatment at these dose rates. Increases in adiponectin may reflect improved metabolic health and reduced laminitis risk warranting further investigation.

23. DETECTION OF PERFLUOROCARBONS IN EQUINE BLOOD VIA HEADSPACE GC-MS/MS.

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INTRODUCTION

Perfluorocarbons are used in multiple disciplines for their beneficial characteristics. Cooking and clothing for its non-stick properties and medicine and athletics for the ability to efficiently dissolve gases, notably acting as powerful oxygen carriers in blood during times of stress. Parimutuel racing is no exception, as perfluorocarbons are banned as performance enhancing compounds in the sport. It is important to have various screening methods for these compounds in racing laboratories to promote the health and safety of equine athletes and to promote a fair and balanced racing environment. Presented here is a headspace gas chromatography tandem mass spectrometry method for detecting perfluorodecalin, a commonly used perfluorocarbon for oxygen carrying, in a low volume of equine plasma in under 7-minutes.

METHODS

This methodology utilized a Thermo Trace1310 GC with a TSQ9000 MS. The column was an Agilent DB-ALC2 30m, 0.32mm, 1.8μm. To prepare the sample for analysis, 100 μL of equine plasma was added to a 10 mL headspace vial, capped, and put onto the autosampler rack for analysis. The GC used an isothermal temperature at 40°C, a run time of 6.8 minutes with a flow rate of 1.7 mL/min. The inlet was 200°C, with a split ratio of 15. The syringe was at 50°C, the incubation time was 5 minutes at 80°C, and the injection volume was 0.3 mL. The MS transfer line was at 200°C, the ion source was at 230°C. Five SRM transitions were chosen for each isomer.

PRELIMINARY DATA

The *trans-* isomer elutes first at 1.73 minutes and then the *cis-* isomer at 1.86 minutes. There were no interfering

peaks for either isomer in any of the SRM transitions in water samples (LCMS grade water), which are used as blanks, or negative plasma samples (using six unique lots of equine plasma). The LOD for this method was 15 ng/mL for *cis*- and for *trans*- isomers. Both isomers have stable linearity that was measured up to 20 μg/mL with a weighting of $1/x^2$. Considering the column used for this experiment is also generally used in equine racing for the detection of gases in blood, usually total content of CO_2, it was also examined if the combination of the is possible, to be able to simultaneously detect CO_2 and perfluorodecalin. Although it is possible, the to perfluorodecalin spiked into water cause a reduction in peak area up to 65%, especially at lower concentrations of perfluorodecalin at and below 500 ng/mL.

24. QUANTIFICATION OF SIROLIMUS IN EQUINE WHOLE BLOOD BY UHPLC-MS/MS.

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Sirolimus is an FDA-approved immunosuppressant used for antirejection post organ transplant in humans. In horses, sirolimus can suppress insulin production and has potential therapeutic applications for insulin-dysregulated (ID) horses. To understand the pharmacokinetics of sirolimus in horses, a quantitative method to accurately determine the concentration of sirolimus in horse whole blood samples is needed. In this study, a method was developed and validated for sirolimus using ultra high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). The analyte was recovered by protein precipitation followed by liquid-liquid extraction with water and methyl *tert*-butyl ether. The limits of detection (LOD) and quantification (LLOQ) were 0.025 and 0.25 ng/mL, respectively. Excellent linearity of calibration curves was observed with a linear dynamic range from 0.25 to 75 ng/mL by employing linear regression with a weighting factor of $1/x^2$. The method was validated according to the acceptance criteria of the U.S. Food and Drug Administration Guidelines and was successfully applied to analyze >250 pharmacokinetic study samples.

25. FRAMESHIFT VARIANT IN CIRNECO DELL'ETNA WITH SYNDROMIC RETINOPATHY AND TREMORS.

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While the manifestations of many inherited retinal disorders are limited to loss of vision, others are part of a syndrome that affects multiple tissues, often the nervous system. Most syndromic retinal disorders are thought to be recessively inherited. Two male dogs from a litter of Cirneco dell' Etna, a rare breed of Italian Pharaoh-hound type dogs, showed

tremors and signs described as either atypical seizures or paroxysmal dyskinesias. The other two male littermates were clinically normal (CSF taps were unremarkable), and so were the parents. A genetic etiology was suspected for this oculo-neurological syndrome, which we have named CONS (Cirneco Oculo-Neurological Syndrome). The aim of this study was to further characterize the phenotype and identify the putative genetic basis of this syndrome. MRI of the brain and cervical spinal cord, followed by cerebrospinal fluid analysis was performed alongside clinical examinations. Homozygosity mapping was carried out using Illumina 220k SNP-chip. Whole genome sequencing was performed for the two cases, searching into the candidate regions. The Dog10k canine variants database was used to filter out non-exclusive genetic variants. MRI showed reduced cerebral white matter volume and in one case a T2 hyperintensity. We detected a 1-bp deletion in CFA6 predicted to cause a frameshift and premature stop codon within the canine AMPD2 gene, which encodes adenosine monophosphate deaminase, an enzyme that converts adenosine 5'-monophosphate (AMP) to inosine 5'-monophosphate (IMP) and is known to cause. Genotyping of the available Cirneco population suggests perfect segregation between cases and controls for the variant. Population studies suggest the variant is limited to the breed. The AMPD2 genetic variant we identified in canines presents with novel retinal manifestations, adding to the spectrum of neurological manifestations associated with *AMPD2* variants in humans, which are primarily pontocerebellar hypoplasia (PCH) and spastic paraplegia.

Supported by: NIH grants: EY06855, Van Sloun Fund for Canine Genetic Research

26. DNA EXTRACTION AND AMPLIFICATION FROM OSMICATED EPON-EMBEDDED RETINAL AND CORNEAL TISSUES.

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PURPOSE

In the pre-molecular era, clinical mammalian samples were embedded in epoxy resin blocks, such as EPON™ or Poly/Bed®, for future evaluation by electron microscopy. However, use of these archival specimens for more modern genotyping studies can be challenging. The aim of this study was to determine if genomic DNA could be extracted from archival epoxy-embedded tissues to a quality suitable for short-amplicon PCR amplification.

METHODS

We selected nine archived EPON and Poly/Bed embedded blocks of mammalian retinal and corneal tissue that were ~10mm in length, embedded in the 1970s-1990s, and had an extensive phenotypic description. Tissues were fixed in 2.5% glutaraldehyde/2% osmium prior to embedding. The blocks were shaved of excess resin, fragmented, and digested using an epoxy resin removal solution. The softened plastic was cut with a scalpel, washed, drained, and incubated overnight in a tissue lysis solution containing Proteinase K. TRIZOL® was added to the samples, which were further mechanically homogenized. Chloroform was added, and the samples were centrifuged. Upon phase separation, the upper clear phase was removed and 95% EtOH was added. The mix was filtered through a mini genomic DNA extraction column, washed twice, and DNA was eluted with 10mM Tris-HCL. Following final removal of phenol contamination using water-saturated ether, the purified DNA was quantified and used for PCR amplification.

RESULTS

The extraction success was tested by targeted PCR amplification using primers that were 90-260bps in length and

targeted genes relevant for inherited eye studies (*PRCD, RHO, GUSB1*), plus additional control genes (*REEP*). All but one of the epoxy-embedded eye samples were successfully amplified. Sanger sequencing confirmed the gene identity of amplified products.

CONCLUSIONS
By identifying methods to extract DNA from EPON and Poly/Bed -embedded mammalian eye samples, our results identify a valuable resource for determining the genetic basis of inherited diseases and to retroactively confirm molecular diagnosis based on microscopic analysis.

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27. NECK MASS IN *TUPAIA BELANGERI* (NORTHERN TREE SHREW).

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One eight-year-old adult male, singly housed, northern tree shrew presented with slow onset of a range of clinical signs within a 6-month period, including patchy haircoat, alopecia, weight loss, and hyperactivity. Additionally, an approximately 2 mm in diameter, raised, subcutaneous, skin-colored mass was visualized on the midline of the ventral neck. Top differentials included abscess, hyperthyroidism, granuloma, neoplasia (lymphoma, lipoma, adenocarcinoma). Diagnostics included point-of-care blood glucose monitoring, which was within normal limits, and serial weight checks. A full diagnostic workup was offered to the lab and declined due to the advanced age of the animal. This animal was humanely euthanized due to declining body condition and acute onset of decreased activity. A necropsy was performed, with the most notable finding consisting of a 7 x 7 x 3 mm soft mass localized to the right thyroid gland, which was approximately 10 times the size of the left thyroid gland. Histopathological examination of this tissue revealed 90% effacement and replacement of normal right thyroid tissue with a malignant epithelial neoplasm. Additionally, though the left thyroid gland appeared grossly atrophied, histopathology revealed 50% replacement with the same epithelial neoplasm. Both thyroid glands revealed neoplastic cell infiltration into the capsule and vascular invasion, which confirmed the diagnosis of follicular-compact thyroid carcinoma, without evidence of metastasis. This is the first report of a spontaneously occurring thyroid carcinoma in the northern tree shrew.

28. IMPACT OF CONFINEMENT ON GILTS ACTIVITY LEVELS.

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Common husbandry practices on farms today have the possibility to compromise animal welfare. Confinement can inhibit the expression of species-specific behavior and challenge natural homeostatic activities. Many breeding females on pig farms remain confined in individual crates, serving to restrict the animal's social, explorative, and maternal behavior and likely induce stress. Here, we studied how the activity levels in naïve gilts were impacted by the housing system.

Twelve replacement gilts were enrolled in a within-subject repeated-measure designed study. Gilts were housed in a collective pen for three weeks (W1-W3) and then transferred to individual crates for four weeks (W4-W7). Three 2-minute videos taken during each observation session (totaling 12 minutes/animal/day) were coded. The behaviors observed to classify Activity Levels were walking in the collective pen and pseudo-walking (moving back and forth) in the crates. The duration of each behavior was divided by the number of events (time/events). Linear mixed models were used to analyze outcome variables with 95% confidence, using the time/events of each behavior as the dependent variable, the week as a fixed effect, and individuals as a random factor.

The Activity Levels on W1-W3 were consistently higher than W4-W7 ($p < 0.05$). Additionally, W1 was higher than W3. The behavioral drive for species-specific behavior, such as walking, was affected by the housing conditions, starting with the first week of crating. The activity level differences between W1 and W3 may be due to the establishment of their social structure, which has been reported to happen within the first weeks after mixing. The crate confinement thwarts the realization of prolonged walking, compromising their welfare.

29. DEVELOPING NEXT-GENERATION TANDEM CAR T THERAPY FOR CANINE LYMPHOMA.

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Despite initial favorable responses with cytotoxic drugs, most dogs with high grade B-cell lymphoma succumb to this disease, emphasizing the need for novel therapeutics. Chimeric antigen receptor (CAR) T cell therapy has revolutionized the treatment of human B cell malignancies. Although the treatment of canine B cell lymphoma patients with anti-CD20 CAR T cells is feasible, it is impeded by antigen editing of CD20. To mitigate this, we designed tandem anti-CD19/CD20 CAR T (TCAR) constructs that simultaneously target two antigens. Here, we report the results of initial *in vitro* testing of four TCAR candidates and confirm the expression of CD19 and CD20 in clinical samples from dogs diagnosed with B cell lymphoma. To investigate CAR trafficking to the cell surface, Jurkat cells were transduced with γ-retroviruses encoding the 4 TCARs, and CAR expression was determined by flow cytometry. Preliminary TCAR signaling was tested using Jurkat NFAT GFP reporter cells that were transduced with TCARs and subsequently co-cultured with cells expressing target antigens. CAR signaling was detected via GFP expression. A custom-designed anti-CD19 and -CD20 antibody panel was designed to assess antigen expression in canine B cells. Both TCAR1 and TCAR2 show significantly higher surface CAR expression compared to TCAR4, with minimal surface expression noted for TCAR3. TCAR2 and TCAR4 showed signaling in the presence of their cognate antigens *in vitro*. CD19 and CD20 expression was documented using flow cytometry in 6 canine patients with B cell lymphoma. Collectively, we show that our novel TCARs can traffic to the cell surface with promising preliminary CAR signaling data, and that our selected target antigens are present in canine B cell lymphoma patients. Future studies including more comprehensive signaling experiments and transduction of primary T cells have been designed to identify the most promising CAR construct to move into veterinary clinical trials.

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30. TREATMENT OUTCOMES FOLLOWING INCREASE IN FREQUENCY OR CONCENTRATION OF TOPICAL TACROLIMUS IN DOGS WITH REFRACTORY KCS.

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PURPOSE

To evaluate response to increased frequency or concentration of topical tacrolimus in dogs with refractory immune-mediated keratoconjunctivitis sicca (KCS).

METHOD

Retrospective review of dogs diagnosed with immune-mediated KCS between 2011-2023. Inclusion criteria included: (1) initial Schirmer Tear Test 1 (STT) < 15mm/min; (2) clinical signs of KCS; (3) treatment with tacrolimus 0.03% aqueous solution q12h; (4) follow-up of at least four weeks from initiation of treatment. STT and clinical signs were recorded at each examination. Eyes were classified, as responders (>/= 5mm/min increase) or non-responders (< 5mm/min increase), based on the difference between the initial STT and the average STT after treatment. Responders were considered treatment successes if the STT >/=15mm/min and clinical signs of KCS were mild to absent, Tacrolimus 0.03% frequency (q8h) or concentration (0.5%) was increased for non-responders for at least an additional four weeks; dogs were classified as second-chance responders (>/= 5 mm/min increase) or non-responders (</=5mm/min increase).

RESULTS

One hundred seventy-two eyes of 120 dogs were included; 72% (124/172 eyes) were responders, 63% (108/124 eyes) were treatment successes. Follow up was available for 33% (16/48 eyes) of non-responders; 44% (7/16 eyes) responded to a change in treatment, these were considered second chance responders (p>0.05); three eyes to an increase in concentration of tacrolimus 0.03%, four to an increase in frequency. Average STT increase for second chance responders was 5.4 mm/min; 6/16 (38%) dogs were considered second chance successes (p>0.05).

CONCLUSIONS

Increasing frequency and/or concentration of tacrolimus in dogs with refractory KCS is associated with treatment success in almost one-half of dogs.

31. MICROFRACTURE AFFECTS BONE STRUCTURE DURING BONE-MARROW STIMULATION FOR CARTILAGE REPAIR IN A RAT MODEL.

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Microfracture surgery (MF) technique is a reconstructive treatment that aim to stimulate cartilage repair in response to cartilage defects. This technique is widely performed, however MF surgery produces a fibrocartilage repair tissue that is suboptimal, and several reports have demonstrated that this procedure promotes significant bone changes that compromise joint integrity. This study aimed to assess the effect of MF surgery on bone integrity and cartilage

regeneration in young, older, and aged male and female rats. With IACUC approval, male and female Brown Norway rats allocated into middle aged (18-month-old), older (30 month-old) and male and female (n=3-6/group) to receive MF following a chondral defect on the medial femur condyle in the right knee. Rats receiving chondral defects alone (no MF) were used as controls to assess the effects of MF on cartilage regeneration (n=6/group). Skeletally mature Sprague Dawley rats (7-8-months-old) that received MF surgery were used as young controls. Contralateral limbs from all groups were used as non-surgery controls. Rats were sacrificed at 8 weeks post-surgery and the sectioned limbs underwent microCT, histopathological and immunohistochemical analysis. Chondral defect and MF treatment led to profound bone destruction. This effect was age-associated and was more pronounced in aged rats (30 months-of-age) when compared to 18-month and 8-month-old rats. Proteoglycan content of the fibrocartilage tissues low, as evidenced by weak toluidine blue staining. Remodeled fibrocartilage tissues were evident in both male and female rats of all ages. Our findings demonstrate that creating a chondral defect with or without MF treatment results in periarticular bone destruction in male and female rats, the severity of which increases with age. Stem cell induced formation of chondral tissues as a result of MF treatment were fibrocartilaginous with low proteoglycan and aggrecan content. Strategies aimed at mitigating bone destruction and rejuvenating old resident stem cell function are warranted.

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32. APPLICATION OF *IN-VIVO* RAMAN SPECTROSCOPY FOR THE EVALUATION OF MICROFRACTURE SURGERY IN EQUINE CARTILAGE DEFECTS.

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Raman spectroscopy is an inelastic light scattering technique that reflects the vibrational modes of the biochemical building blocks of key cartilage extracellular matrix (ECM) specific biomarkers such as glycosaminoglycan (GAG), type-II collagen (COL) and H2O. In this study, we used a novel Raman spectroscopy needle probe and real-time spectral analysis platform capable of performing both *ex-vivo* and *in-vivo* measurement of ECM-specific compositional biomarkers for cartilage to assess the formation and maturation of neocartilage formed in an osteochondral defect of the equine stifle joint treated by microfracture. With IACUC approval, two (proximal and distal) 15mm diameter chondral defects were created on the lateral trochlear facet of two 5-year-old thoroughbred horses which was then repaired by microfracture. Raman measurements were performed using a custom Raman probe (In Photonics) comprised of a threaded needle tip (Ø2.75mm) with a distal Ø2mm sapphire ball lens was fiber-coupled to a battery powered 785nm laser (100mW output; IPS) and spectrometer (Eagle; Ibsen). Spectra were acquired for 10 seconds at multiple sites along the lateral trochlear facet within each defect and the surrounding native cartilage. Sequential *in-vivo* assessments at each site were performed before creating the defect, and 3, 6, and 12 months after microfracture. The most obvious Raman spectra changes were elevated bone signals in the neocartilage with little change in the surrounding native cartilage over time, reflecting that the repair tissue was thinner. At both the microfracture site and surrounding native cartilage the GAG increased at 3 months for horse 1 at the proximal defect and for horse 2 at both defects, signifying an overall anabolic response stimulated by microfracture. This study establishes the capability of our Raman spectroscopic probe platform

to perform *in vivo* real time assessments of the composition of emerging neocartilage repair tissue allowing for serial monitoring of cartilage regeneration.

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33. CHANGES IN THE MICROBIOTA IN HORSES RECOVERING FROM COLIC.

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Complications occur during the management of horses with colic. A possible association between gut dysbiosis and complications has been suggested, and such an association has been described in human patients. The objective of this study was to further investigate the change of the microbiota during recovery from colic and its association with complications.

Hospital admission and discharge fecal samples were obtained from horses presenting for colic (2020 and 2021). Complications were classified as present or absent, and then as gastrointestinal- or infection- related. Recurrent colic before or after hospitalization was recorded. Fecal samples from 51 horses presented for colic at admission to the hospital and at discharge were collected, extracted for DNA and amplified for V1-V2 region of the 16S rRNA bacterial gene and sequenced on an Illumina MiSeq platform. The state of the microbiota was described using alpha diversity indices, beta diversity (weighted and unweighted UniFrac analysis), and relative abundance of bacteria.

Horses with an uncomplicated recovery had higher alpha diversity indices at the time of hospital admission compared to hospital discharge, and compared to horses with a complicated recovery. Differences in bacterial communities in horses with a complicated recovery was noted only in unweighted PCoA, indicating that less abundant bacterial populations are affected. The progression in relative abundance of specific bacterial taxa from admission (disease state) to discharge (recovered state) enabled us to characterize the microbiota as eubiotic, minor, moderate, or severe risk of dysbiosis, and as dysbiotic.

Fecal microbial dysbiosis was primarily observed at the time of hospital admission and progressed to a more eubiotic state at the time of hospital discharge. There was evidence of an association between fecal microbial dysbiosis and complications, warranting ongoing investigation.

Research Grant: Friesian Horse Association of North America

34. IDENTIFICATION OF PHOTORECEPTOR-SPECIFIC PROMOTERS FOR GENE THERAPY AT ADVANCED STAGES OF RETINAL DEGENERATION.

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Photoreceptor (PR)-specific promoters used in current retinal gene therapies are typically validated in normal retinas, driving efficient transgene expression early in retinal degeneration (RD). However, their performance at advanced stages of RD is often variable. This study aimed to develop novel PR-specific promoters for efficient transgene expression during advanced stages of inherited RDs. Using retinal transcriptomic data from two non-allelic canine RD models (Sudharsan et al., 2017), we identified genes with sustained transcriptional activity in PRs even at advanced RD stages (>50% PR loss). Laser capture microdissection, RNA isolation, qPCR, and RNA *in situ* hybridization (RNA-ISH) validated PR-specific expression of six candidate genes. Minimal promoters and upstream regulatory elements of these genes were identified through interspecies sequence alignment (CLUSTALW) and regulatory motif discovery (MEME, GPMiner). Promoter sequences were cloned and their transcriptional activity assessed *in vitro* using dual-luciferase assays in Y79 and 661W cells, with HEK293 cells serving as controls. Equivalent human promoter sequences were also analyzed. Promoters with high *in vitro* activity were cloned into barcoded GFP reporter constructs and packaged into AAV5 vectors. Promoter activity was compared to existing PR-specific promoters (RHO, GRK1, IRBP, PR2.1) in normal and mutant canine retinas at advanced RD stages using a massively parallel reporter assay. We identified three canine (IMPG2, GNGT2, PDE6H) and two human (GNGT2, TPH1) promoters under 1000 bps that efficiently drive PR-specific transgene expression in rods and/or cones at advanced RD stages. These findings provide new PR-specific promoters that offer enhanced transgene expression, particularly in cones, representing an improvement over current gene therapy strategies.

35. CHARACTERIZATION OF THE CANINE PHOTORECEPTOR SENSORY CILIUM BY ULTRASTRUCTURE EXPANSION MICROSCOPY.

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Photoreceptors are highly polarized sensory neurons, possessing a unique ciliary structure known as the photoreceptor sensory cilium. The photoreceptor sensory cilium is composed of the outer segment, where highly elaborated membrane discs are packed, and a nine-fold microtubule axoneme that originates from the basal body in the inner segment, transitions through the connecting cilium, and extends into the outer segment. Vertebrates possess two subtypes of photoreceptors: rods, which are responsible for night vision, and cones, which mediate daylight vision, color perception, and visual acuity. Despite the identification of functional and morphological differences between these photoreceptor subtypes, ultrastructural analysis of the ciliary molecular architecture between rods and cones are still lacking due to the limited resolution and detection capabilities of conventional imaging techniques. In this study, we employed ultrastructure expansion microscopy (U-ExM) to characterize the molecular architecture of the photoreceptor sensory cilium in the normal adult canine retina. We demonstrated that U-ExM is applicable to both

fresh and long-term cryopreserved canine retinal tissues that have undergone standard paraformaldehyde fixation. Using this validated U-ExM protocol, we mapped the precise molecular localization of several retinal ciliopathy-related proteins across different compartments of the cilium in canine photoreceptors. Furthermore, we identified significant architectural differences in each compartment of the photoreceptor sensory cilium between canine rods and cones, suggesting their potential involvement in the heterogeneity of ciliopathy-associated phenotypes between these subtypes. These findings pave the way for a better understanding of alterations in the molecular architecture of the photoreceptor sensory cilium in canine models of retinal ciliopathies.

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36. THE HEALING POST-ARTHROTOMY JOINT MAINTAINS AN ALKALINE pH UNDER NORMAL AND SEPTIC CONDITIONS.

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During periprosthetic joint infections (PJI), the joint environment is assumed to be acidic which has driven the development of various therapies and devices. We previously validated pH to be equivalent between sheep undergoing joint replacement and a large coxofemoral arthrotomy so to minimize animal numbers and suffering. Here we report pH measurements in the immediate post-operative wound environment in a sheep arthrotomy model, compared with human wound/synovial fluid collected from arthroplasty patients. There were 2 phases in this study, non-infected (Phase 1) and infected (Phase 2). Sheep were placed in 3 cohorts for each phase, control (no antibiotic), systemic antibiotic (Ceftiofur Sodium) and local antibiotic (Ceftiofur Sodium through JP drain). With IACUC approval, sheep underwent a coxofemoral joint arthrotomy and a JP drain was placed for fluid collection. Samples were taken at time 0, every 6 hours for 48 hours, then every 12 hours until 192 hours and immediately analyzed for pH using a digitally-calibrated pH meter. At 192 h, the JP drain was removed, and sheep underwent a healing and wash out period. Four weeks later, a second surgery was then performed on the contralateral hip with contamination of $1x10^5$CFU/mL *Staphylococcus aureus* ATCC 25923 in 1mL PBS through the JP drain (t=0) followed by fluid collection for 192 h. For humans, synovial fluid samples were collected and frozen at -80°C until thawed for pH measurement. Results showed that the starting pH across all ovine cohorts ranged from 7.89-8.09 and generally increased to pH 8.5 or above, remaining for 8 days. Since the human samples had been stored frozen, the effect of freezing postoperative wound/synovial fluid from the sheep was evaluated showing a uniformly decrease in pH. Thawed human wound/synovial samples, mean pH, was alkaline and centered at pH 7.9. Published literature reports pH ranges between acidic and alkaline for the wound/synovial environment. In contrast, our studies showed that pH of the postoperative joint environment started out at an alkaline pH (~7.9) and remained alkaline over post-operative time. The initiation of local antimicrobial therapy had a slight acidifying impact on pH in the infected ovine cohort, however the pH remained alkaline and did not drop below pH 7.7. Wound/synovial fluid samples from human patients seen for suspected PJI further support the finding that post arthroplasty environment in both acute and chronic wound/synovial fluid is alkaline across septic and aseptic conditions.

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37. ESTABLISHMENT OF A MODEL OF GINGIVITIS IN YUCATAN MINIPIGS.

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Oral biofilms cause gingivitis and periodontal disease, conditions that affects approximately 743 million people worldwide. Gingivitis is inflammation of the gingiva and, if left untreated, will progress into periodontal disease, which results ultimately in tooth loss. Dysbiosis in the oral microbiome has detrimental effects ranging far beyond the oral cavity with Alzheimer's Disease, colorectal cancer, and periprosthetic infection, being linked to oral pathogens. Therefore, there is an increasing need for new treatment technology for gingivitis and periodontal disease. Yucatan Minipigs are an excellent translational model for humans due to their similar craniofacial and dental anatomy and porcine periodontal disease models are described. Periodontal disease has passed beyond the point of being easily resolved due to bone resorption. Our interest was in a less severe model that could be used to test anti-biofilm therapies that would respond to treatment. By placing a dental ligature around the fourth mandibular premolar 1-2 mm above the gingival margin that was left *in situ* for 21 days we were able to cause gingivitis to occur over the study period. Gingivitis was assessed using a plaque score, periodontal pocket depth, and gingival bleeding which showed evidence of gingivitis in the affected location. Gingival biopsies were obtained at baseline and day 21, which showed increased immune cell infiltration at day 21. Plaque samples were collected from the ligature area at day 0 and 21 for shotgun sequencing which showed a shift in the composition of microbes before and after gingivitis. Two days following ligature removal the pigs were assessed a final time, and the clinical evidence of gingivitis had improved significantly back to baseline. Based on these findings we have demonstrated that our methodologies result in a reversible porcine model of gingivitis that is appropriate for testing response to treatment following use of oral biofilm removal technologies.

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38. SINGLE-CELL MULTIOMIC COMPARISON OF MOUSE AND RAT SPERMATOGENESIS REVEAL GENE REGULATORY NETWORKS CONSERVED FOR OVER 20 MILLION YEARS.

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Spermatogenesis is driven by dramatic temporal and spatial changes in chromatin, transcription and protein expression, but how these processes are coordinated remains largely unclear. Our goal was to assess the mechanistic bases for these developmental changes at a single-cell resolution. We analyzed mouse and rat germ cells by single-nuclear and single-cell RNA-sequencing (sn/scRNA-seq) jointly with assays for transposase-accessible chromatin (snATAC-seq) or cellular indexing of transcriptomes and epitopes (CITE-seq) to quantify select protein expression in adult rodent testis cells alongside their transcriptomes. We matched RNA and protein expression of cell-type-specific genes to cell types

via *in situ* hybridization and immunochemistry. We systematically investigated the correlation between chromatin structure, mRNA levels and protein expression in cell types within and between mouse and rat spermatogenesis. In both species, promoter accessibility was greatest during meiosis, as well as most correlated with the corresponding genes, underscoring the tight control of chromatin structure and transcription during meiosis. For example, *Cd9* chromatin states correlated with CD9 protein abundance, allowing for the isolation of distinct germ cell types with improved resolution. To understand the regulation of spermogenic transcriptional programs, we employed a gene regulatory network (GRN) model to investigate the expression of transcription factors (TFs) and their downstream targets across the process of spermatogenesis. Our model identified 40 key regulons conserved between mouse and rat germ cells, i.e., regulatory units consisting of TFs expressed in association with specific motif-containing peaks of open chromatin regions that, in turn, were linked to downstream gene activity. Our single-cell multiomic dual species approach captured conserved regulators of gene networks across spermatogenesis. Such a degree of conservation highlights the relevance of chromatin-related factors in regulating the transcription and translation of key genes across spermatogenesis. Our findings provide the foundation for novel, gene-targeted male contraceptives.

39. EQUINE DOPING CONTROL ANALYSIS WITH NEGATIVE ION ELECTROSPRAY HIGH-RESOLUTION MASS SPECTROMETRY.

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To maintain integrity in equine sports, hundreds of potential performance-enhancing substances are banned by the Association of Racing Commissioners International (ARCI) and the Fédération Équestre Internationale (FEI). A very challenging task faced by equine anti-doping laboratories is to develop efficient methods to screen for a wide range of doping substances in a short period of time. High-resolution mass spectrometry operating in negative ion mode offers enhanced sensitivity and specificity, enabling screening of a wide spectrum of equine doping agents.

A high-resolution mass spectrometry (HRMS) method was developed using a Q-Exactive Plus mass spectrometry instrument in negative ion mode to screen for different classes of doping substances, such as nonsteroidal anti-inflammatory drugs, HIF (Hypoxia-Inducible Factor) prolyl-hydroxylase inhibitors, and barbiturates in equine plasma. Substances were recovered from plasma by liquid-liquid extraction (LLE) extraction and separated by a C18 column. HRMS/MS data was acquired by a Full scan/*dd*-MS2 method. Accurate mass, retention time, isotopic pattern, product ions, and MS/MS spectra were used as screening criteria.

The LLE showed good extraction efficiency for the studied substances. The liquid chromatography (LC) method separated substances within 7.5 minutes and yielded sharp and symmetrical peaks, indicating robust separation and high resolution. The retention times were stable from run to run. By using an internal lock mass of N-butyl benzenesulfonamide, less than 2 ppm mass accuracy was observed for 5 days without mass calibration. An HRMS/MS compound database and spectra library were built for each substance at an optimized collision energy to improve the method specificity and sensitivity. The screening and identification criteria included accurate mass, retention time, isotopic pattern, fragment ions and MS/MS spectra. The limit of detection for each analyte was validated. The acquired full-scan HRMS data allows for non-targeted retrospective analysis, enabling the detection of substances not previously known during sample analysis.

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NOTES

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