Laminitis—a crippling equine disease  Andrew van Eps, BVSc, PhD, graduated from the University of Queensland School of Veterinary Science in Australia. He remained there to carry out his PhD studies, which primarily focused on the effects of therapeutic hypothermia on the development of laminitis. After completing a large animal medicine residency at New Bolton Center, he returned to the University of Queensland as a faculty member for seven years, before returning to New Bolton Center (NBC) in 2017 as an associate professor of musculoskeletal research in the Department of Clinical Studies (NBC). Dr. van Eps’ research goal is to identify the key pathophysiological events that lead to different forms of laminitis in order to develop clinically applicable means of preventing this crippling equine disease.  Continued on page 2
Laminitis: an important equine foot condition with diverse clinical causes—Laminitis is a major cause of morbidity and mortality in horses worldwide. In a normal hoof, connecting tissues called lamellae attach to the coffin bone, suspending it within the hoof capsule. In laminitis, failure of the lamellar attachment leads to a largely unrecoverable loss of this suspensory function, resulting in chronic and often progressive lameness and dysfunction that may ultimately require euthanasia. Laminitis occurs secondary to a diverse array of primary disease states, which primarily fit into 3 main categories: 1) sepsis-related laminitis (SRL); 2) endocrinopathic laminitis (associated with insulin dysregulation/hyperinsulinemia) and 3) supporting limb laminitis (SLL).

Over the last decade, we have recognized key differences (and some similarities) in the initial events that lead to these three types of laminitis. A focus on these early events is leading to a better understanding of why laminitis occurs in different clinical situations and is helping to identify therapeutic targets.

What causes lamellar attachment failure in different forms of laminitis?—In each form of laminitis, a combination of epithelial cell adhesion loss and epithelial cell stretch weakens the lamellar attachment (Figure 1). Endocrinopathic laminitis is dominated by cell stretch, whereas SRL and SLL are dominated by adhesion loss. A question of vital importance to our understanding is whether this attachment failure occurs as a result of 1) a specific cellular signaling event that is common to all forms of laminitis; or 2) insult-specific alterations in epithelial cell homeostasis that lead to a common endpoint due to the unique and immense physical stresses on the lamellar epithelium. The van Eps laboratory is taking a multidisciplinary approach to examining structural, molecular and physiologic events during laminitis development to answer this fundamental question.

Cellular energy failure a likely candidate but is it a consistent feature?—The maintenance of lamellar epithelial adhesion and cytoskeletal dynamics required to withstand the immense mechanical stress within the equine hoof is an energy consuming process. Using our recently developed tissue microdialysis system capable of assessing lamellar perfusion and energy balance *in vivo*, we have demonstrated a relatively high rate of glucose consumption and lactate production within the lamellae[1]. As lamellar epithelial cells are highly dependent on glucose as an energy substrate[2] and there is no means for local storage of glycogen in the lamellar tissue, lamellar epidermal glucose uptake must be matched by constant delivery via the blood to maintain function. However, our microdialysis urea clearance data demonstrate that even the normal lamellar dermis is relatively poorly perfused compared with the adjacent sublamellar dermis or skin [1], making it inherently prone to energy imbalances secondary to
perfusion or metabolic derangement. The van Eps lab has therefore focused on determining whether this contributes to the development of different forms of laminitis.

![Figure 1. H&E stained histological sections of mid-dorsal lamellae from control (A, B) and acute laminitis (C,D) feet.](image)

Lengthening and attenuation of secondary epidermal lamellae with epidermal cell stretch and loss of normal nuclear position/orientation indicative of cytoskeletal collapse (D) is a typical change particularly with laminitis due to excess insulin, however loss of epithelial intercellular and cell-matrix adhesion can lead to more severe lamellar failure and separation regardless of inciting cause (C).

**Evidence for ischemia only in supporting-limb laminitis**—While perfusion derangements have long been implicated in different forms of laminitis, direct evidence has been lacking. Using the lamellar tissue microdialysis technique described above, we have demonstrated a unique relationship between limb load cycling and lamellar perfusion [3]. Our new data reveal lamellar ischemia in a preferential weight bearing model relevant to SLL, characterized by an increased lamellar interstitial lactate:pyruvate (L:P) ratio coupled with reduced urea clearance (Figure 2A) providing the first evidence of ischemia/perfusion failure in the development of SLL. Notably, our microdialysis studies suggest ischemia is not a primary event in the development of SRL (oligofructose (OF) model) [4] or endocrinopathic laminitis (hyperinsulinemic clamp model, unpublished data). The Van Eps lab is now focusing on methods to prevent perfusion failure specifically in SLL.

**What about non-ischemic energy failure?**—Disturbances of energy metabolism despite adequate blood perfusion have been increasingly recognized in the pathophysiology of human sepsis-related organ failure [5] and after traumatic brain injury [6]. Although lamellar ischemia was not a feature of SRL (OF model), we have new evidence of non-ischemic oxidative energy failure later in laminitis development in this model (Figure 2B) [7], characterized by an increase in microdialysate L:P ratio (without decreased pyruvate or urea clearance), consistent with non-ischemic energy failure. Controlling this may help to prevent progression in SRL.

Continued on page 4
Therapeutic hypothermia prevents lamellar attachment failure, but why?—The van Eps laboratory has demonstrated the protective effect of continuous digital hypothermia in several experimental studies of SRL and it has translated into an effective clinical preventative. In their early studies, they found hypothermia dramatically inhibited transcription of inflammatory mediators in lamellar tissue when applied prophylactically [8]. However, while protective effects were still observed when hypothermia was applied later in the SRL model, inhibition of inflammation was no longer observed [9]. Furthermore, while the Van Eps laboratory found that hypothermia protected against the non-ischemic energy dysregulation noted in the SRL model, it also has a remarkable protective effect in the hyperinsulinemic clamp model of endocrinopathic laminitis, where there is no evidence of energy dysregulation. Thus, continued studies evaluating the specific effects of hypothermia are required to help unlock the important mechanistic pathways in SRL and endocrinopathic laminitis.

The Holy Grail- discovery of a common pathway leading to all types of laminitis?—Together with collaborators at the Ohio State University, Dr. van Eps and coworkers have generated new evidence demonstrating that lamellar signaling in all 3 models laminitis converges at the level of the mTORC1 signaling pathway, which can trigger cytoskeletal rearrangement and cell adhesion dissolution. While the exact pathways leading to mTORC1 signaling have not been fully elucidated, its induction could occur via insulin activation of IGF1-R in endocrinopathic laminitis, via IL-6 in SRL and secondary to hypoxia/ischemia in SLL. Importantly, our data indicates that mTORC1 signaling is specifically inhibited by hypothermia in SRL and endocrinopathic laminitis models, supporting this as a pharmacological target that could potentially prevent lamellar failure in different clinical forms of laminitis.

Continued on page 8
Student Summer Research at Penn Vet

Students from various programs found their way to a Penn research laboratory during the summer of 2018. Some of the students were participants in the NIH/Boehringer Ingelheim Veterinary Scholars’ program. Others were part of Penn’s Summer Undergraduate Internship Program (SUIP), designed to provide an intense research experience to students interested in graduate study in the biomedical sciences. Undergraduates from a variety of universities as well as motivated high school students found a laboratory and a mentor where they experienced the world of laboratory and clinical research. Students have a vast selection of laboratories and research topics available to them in the Penn biomedical community. Credit goes to faculty mentors in the summer research program and some are listed here: Montserrat Anguera, Daniel Beiting, Rumela Chakrabarti, Hannah Gallantino-Homer, Oliver Garden, Urs Giger, Beatrice Hahn, De’Broski Herbert, Martha Jordan, Christopher Lengner, Nicola Mason, Michael May, Erica Miller, Keiko Miyadera, Kyla Ortved, Cynthia Otto, Tom Parsons, Meghann Pierdon, James Perry, Michael Povelones, Jennifer Punt, Ellen Puré, Boris Striepen, Andrew Vaughan, and Susan Volk.

Whether they are veterinary or medical students, undergraduates or high school students—these students were inspired to investigate a career in research—basic, clinical or translational medicine. Shown here are some of the student participants.

Vet student Katherine Neupauer-Geating worked with Dr. Andrew Vaughan on vascular regeneration after influenza involving multiple progenitor cells.

Eric Rodriguez López, a Penn undergrad in the SUIP program, worked with Dr. Igor Brodsky to understand the role of CARD19 in regulated cell death.

Hemma Murali, Bryn Mawr College, studied Elf5-mediated interferon Gamma signaling in triple negative breast cancer in the laboratory of Dr. Rumela Chakrabarti.

Emma Hunter, Strath Haven High School, worked with Jennifer Dumaine of the Striepen laboratory making transgenic Cryptosporidium.

Vet students in Swine Research—Matt Herber (sow behavior during lactation in confinement); Emily Nogay (impact of shoulder lesions on sow behavior); and Justin Schumacher (lesions responsible for lameness).

Mentors: Drs. Tom Parsons and Meghann Pierdon.

Fabio Alvarado, UPENN undergrad, worked on the impact of intravenous anesthetic agents on canine NK cells in Dr. Oliver Garden’s laboratory.
Undergrad Andrew Pham (UPENN), in Dr. Oliver Garden's Laboratory, worked on myasthenia gravis antigen-specific vaccine development.

Natalia Aponte-Borges, an undergrad in the SUJP program, worked with Dr. Montserrat Anguera on kinetics for Xist RNA return to the inactive X (Xi) during CD8+ T cell activation.

Nora Okwara, undergrad student from Columbia University interned at the De'Broski Herbert Laboratory and worked on examining LINGO and Trefoil factor proteins interactions using immuno-fluorescence staining and proximity ligation assays.

Vet Student Maya Sekhar, NIH/BI summer scholars program, worked with Dr. Ellen Puré on delineating the enzymatic versus non-enzymatic roles of fibroblast activation protein.

Vet student Lauren Therriault worked with Drs Paula Henthorn and Urs Giger on genomic analysis of the SLC3A1/SLC7A9 renal transporter in canine cystinuria (NIH/BI Scholars Program).

Evie Oliver, Lower Merion High School student, worked with Dr Michael Povelones on transmission of heartworm and malaria in mosquito vectors.

Vet Student Mariel Covo (NIH/BI summer research program) worked in Dr Nicola Mason's lab on optimization of canine CAR T cell production for re-directed T cell therapy.

Vet student Elizabeth Collins worked on studying the early stages of skin immunity against a soil transmitted helminth (NIH/BI Veterinary Scholars Program), in Dr. Herbert's laboratory.

Vet Student John Cain worked with Dr. Michael Povelones on molecular dissection of mosquito resistance to heartworm infection (Morris Animal FDN Summer Scholar awardee).
Vet student Alexandra O’Donnell, (NIH/BI program) worked with Dr. James Perry on characterizing the immunomodulatory effects of canine histiocytic sarcoma on peripheral T cells. Dr. Jennifer Punt mentored Anjali Gupta (UPENN undergrad) and Trevor Edgar Esilu (Haverford College undergrad) who worked on the the role of IGF1 in canine immunity.

Ryan Xu, high school student in the Institute for Regenerative Medicine Summer Internship Program, worked with Dr. Andrew Vaughan on assessing the paracrine effects of endothelial-derived BMP6 on epithelial repair after influenza.

Dr. Jennifer Punt mentoring Anjali Gupta and Trevor Edgar Esilu in their research on canine histiocytic sarcoma.

Jimmy Ferrara V’20 along with two other Penn Graduate Students received the Provost’s Fellowship for Interdisciplinary Innovation and will partner with the Center of Molecular Dynamics Nepal (CMDN) to conduct baseline research on Campylobacter, a bacteria found in unpasteurized milk and animals’ digestive systems. The team carried out a pilot research project this past summer in Nepal. Dr. Jennifer Punt is his mentor.

Vet Student Kimmy Hildreth worked with Dr. Andrew van Eps on protein chaperone expression resulting from endoplasmic reticulum stress in an HI model of laminitis pathogenesis. NIH/BI Summer Scholars Program.

Vet Student Kimmy Hildreth working with Dr. Andrew van Eps.
Dr. van Eps’ research is supported by the Grayson Jockey Club Research Foundation. His office may be found at Room 122, Myrin Building.

References


A newly installed walkway called Hendricks Walk at New Bolton Center is in honor of former Dean Joan Hendricks.

Awards and Honors......

Dr. De’Broski Herbert, an associate professor of immunology, Department of Pathobiology, regaled a Wednesday Afternoon Lecture (WALS) audience with stories of his research on worms. Herbert shared his enthusiasm for nematodes with the NIH audience on June 21, 2018, when he delivered his lecture entitled “LINGO Proteins: A New Language for the Mucosal Barrier.” He told the story of how his laboratory used a nematode model to discover the LINGO-2 protein. A direct URL for the article is: https://irp.nih.gov/catalyst/v26i5/for-the-love-of-worms

NIH P50 Center Grant Award to Jeremy Wang

The Penn Center for the Study of Epigenetics in Reproduction (M. Bartolomei, C. Coutifaris, and J Wang) will elucidate epigenetic mechanisms that govern male and female reproduction, contribute to male infertility and impact development of mouse and human concepti conceived through assisted reproductive technologies (ART). The centerpiece of the Penn Center is three integrated, innovative research projects, spearheaded by experienced leaders in the areas of epigenetics and reproduction. The Center also features an outreach program; the Penn Academy of Reproductive Sciences uses hands on laboratory experiences and interactive lectures to educate high school students, largely from the Philadelphia area schools, in the reproductive sciences. P50 grants support research program projects and centers in the full range of research and development from very basic to clinical studies. Dr. Wang is a professor in the Department of Biomedical Sciences.NIH P50 HD068157 $4,775,101 7/23/18—3/31/23

The Leslie B Brent Award

Awards (direct costs)

**Monserrat Anguera**
DOD—Role for Abnormal Gene Expression From the Inactive X in Female-Biased Lupus Disease  9/15/18—9/14/21  $322,817

**Leszek Kubin**
Cohen Veterans Bioscience
Model of individual differences in response to social defeat in rats. ($500K award to CHOP; subcontract to Dr. Kubin)  2/1/18—1/31.2020  $20,000

**Raimon Duran-Struuck**
NIH/NIAID Collaboration w/ Univ of Michigan & Temple Univ.
S-Nitrosothiol-Based Rinse/Aerosol Solutions for Treatment/Prevention of Rhinosinusitis (Phase II)  07/2018-07/2019  $220,367

**Raimon Duran-Struuck**
Helmisley Charitable Trust
with Jim Riley and Ali Naj PSOM
Durable Islets Transplant by co-administrating engineered regulatory T cells  01/2018-12/2020  $1,633,997

**Dan Beiting with Lisa Murphy**
PA state, Dept of Agriculture
Development of a Rapid, Stall-Side Diagnostic Assay for Equine Respiratory Pathogens  01/01/18—06/30/19  $62,000

**Molly Church**
NIH Science Education Partnership Award (SEPA)
with Purdue College of Veterinary Med
This Is How We Role: Inspiring Future Researchers through Veterinary Medicine  06/01/18—04/30/19  $4,629.63

**Julie Ellis**
US Fish and Wildlife Service
Northeast Wildlife Disease Cooperative: diagnostic, field, and training assistance services. 9/1/18—8/31/19  $10,000

**Chris Hunter**
NIH/T32 Parasitology: Modern approaches  9/1/18—8/31/23  $1,138,100

**Chris Hunter**
Abramson Cancer Center Emerson Grant
IL-27 in metastatic melanoma  7/1/18—6/30/20  $100,000

**Donna Kelly**
PA Dept of Agriculture
Evaluation of integrated pest management systems in aquaponics production for Salmonella species and Aeromonas hydrophila contamination of harvested product  1/1/18—6/30/19  $5000

**Susan Volk**
NIH/NIGMS—The regulatory roles of type III collagen in cutaneous wound healing  9/21/18—8/31/23  $1,332,796

**Susan Volk**
NIH/NCI Type III Collagen as a suppressor of breast cancer progression and metastasis  7/1/18—5/31/20  $239,250

**Michael Atchison**
NIH/ NIAID T32
VMD-PhD Training in Infectious Disease-Related Research  7/1/18—6/30/19  $1,051,361

**Michael Atchison**
NIH Office of the Director T35
Short term training for students in health professional schools  4/19/18—3/31/23  $616,120

**Carolina López**
NIH— Defective Viral genomes in RSV pathogenesis  05/03/18—4/30/22  $1,249,447

**Carolina López**
NIH—Mechanisms of DDO Adjuvancy  8/15/18—7/31/23  $1,490,585

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Nicola Mason, in the Department of Clinical Sciences and Advanced Medicine, in collaboration with Aimee Payne, Perelman School of Medicine, has received an NIH Transformative Research Award. This award is given for an exceptionally innovative and unconventional research project. By this award she joins the ranks of accomplished high-risk, high-reward researchers. The project is entitled: Translating cellular immunotherapies for autoimmunity to canine clinical trials. Direct costs: $2,853,761 for the period of 11/1/18—10/31/23

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The Penn Vet Research Newsletter is distributed quarterly. Suggestions, comments, requests and story ideas may be directed to: resnews@vet.upenn.edu

**Phillip Scott, PhD**
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